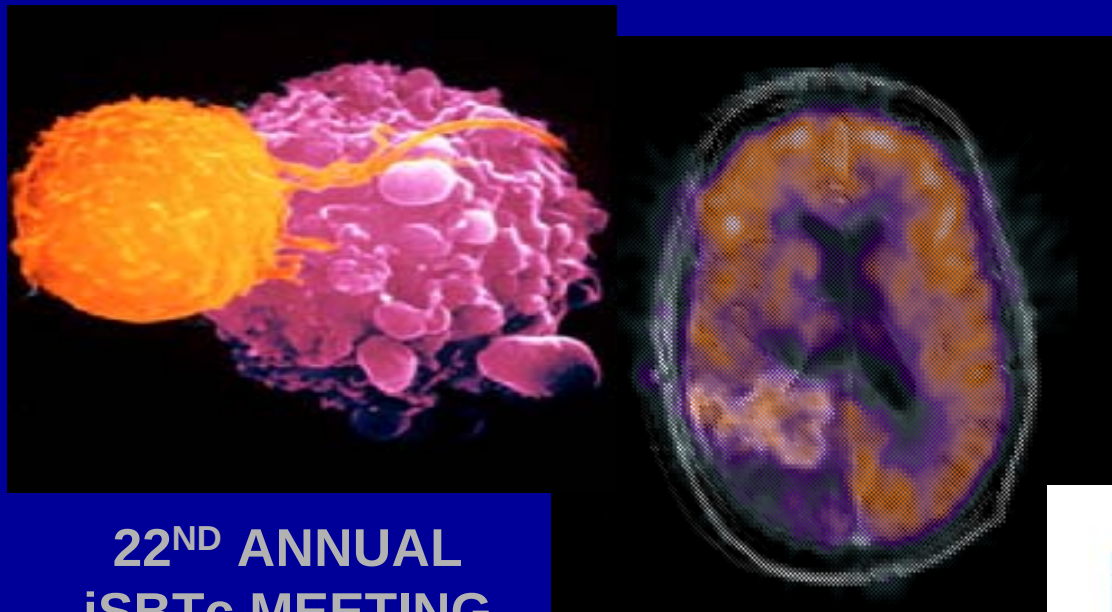


# ENGINEERING CYTOLYTIC EFFECTOR CELLS FOR GLIOMA IMMUNOTHERAPY USING GENE INSERTION AND ZINC FINGER NUCLEASE GENOMIC EDITING

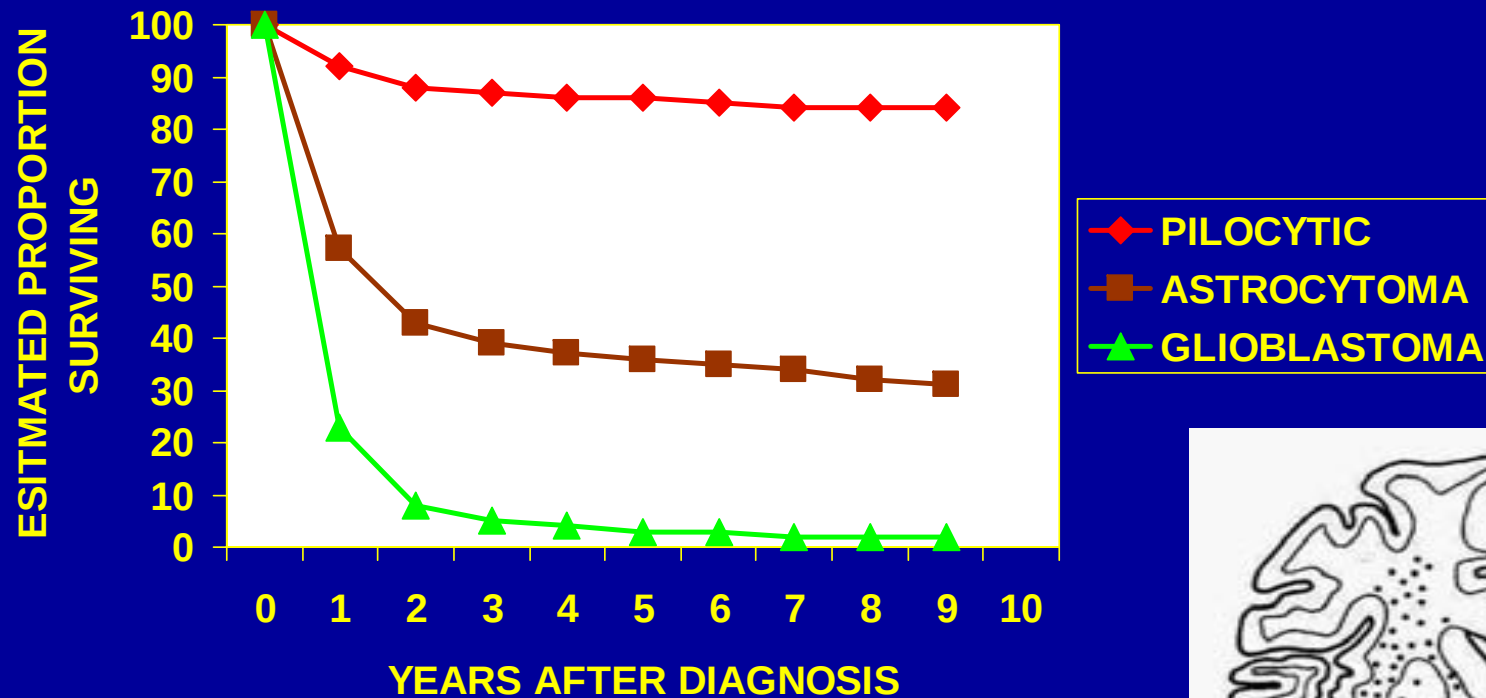


22<sup>ND</sup> ANNUAL  
iSBTc MEETING  
BOSTON



DIVISION OF CANCER IMMUNOTHERAPEUTICS & TUMOR IMMUNOLOGY  
BECKMAN RESEARCH INSTITUTE

# TARGET POPULATION:



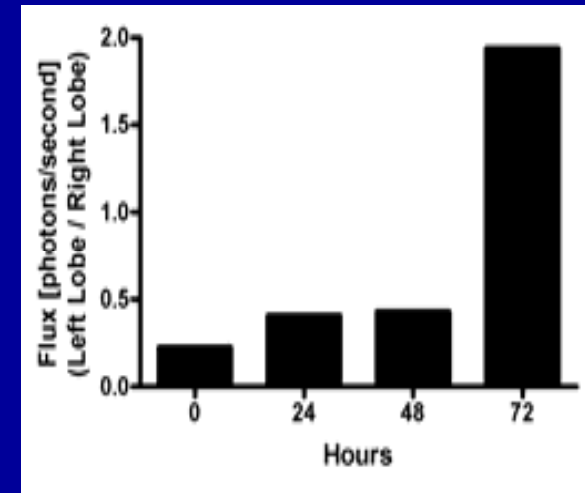
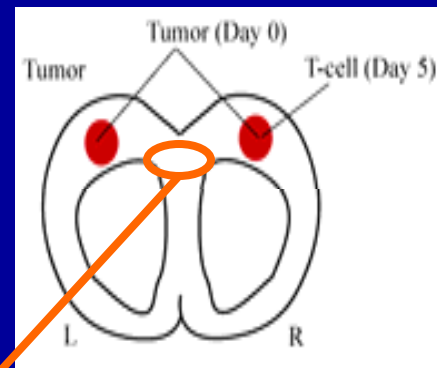
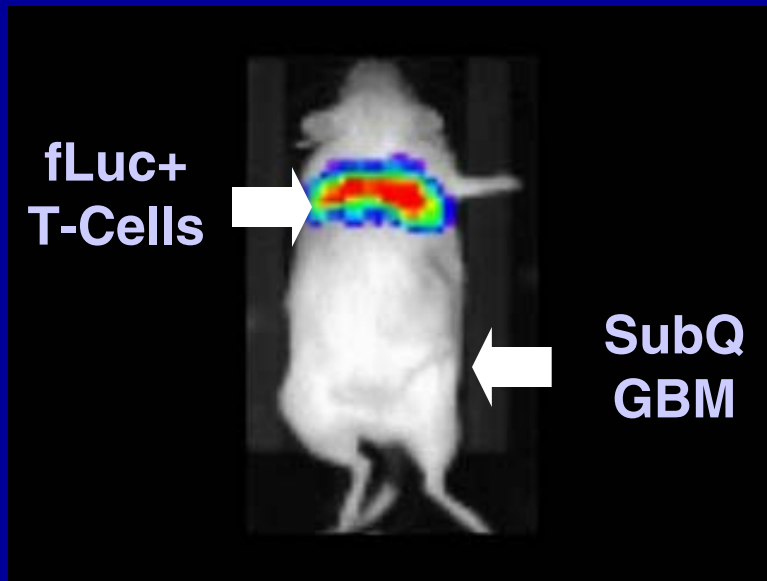
SEER DATA REPORTED BY DAVIS *et al*  
J. Neurosurg 88:1-10, 1998

- Median survival duration following GBM relapse is 36-weeks

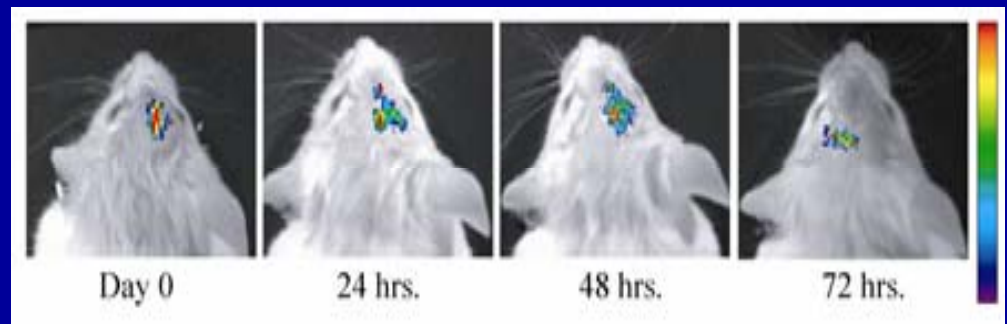
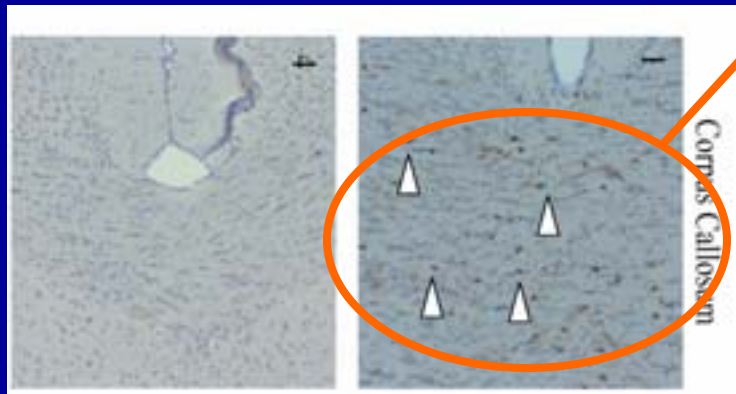


# GLIOMA TROPISM OF ADOPTIVELY TRANSFERRED EX VIVO PROPAGATED T-CELLS:

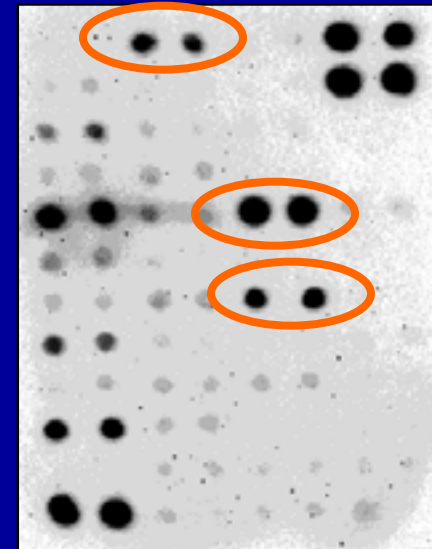
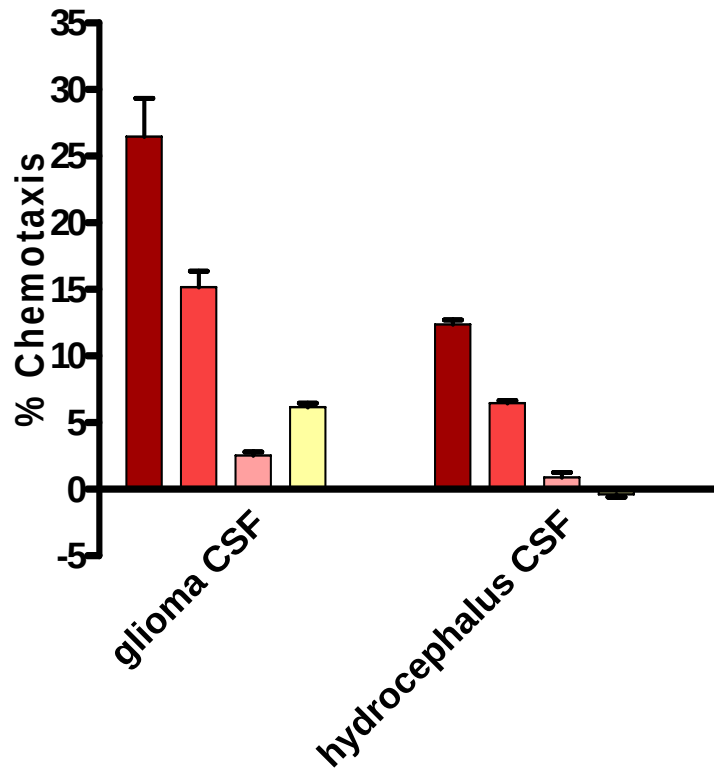
## INTRAPARENCHYMAL HOMING OF T-CELLS TO ORTHOTOPIC CNS GLIOMA XENOGRAFTS



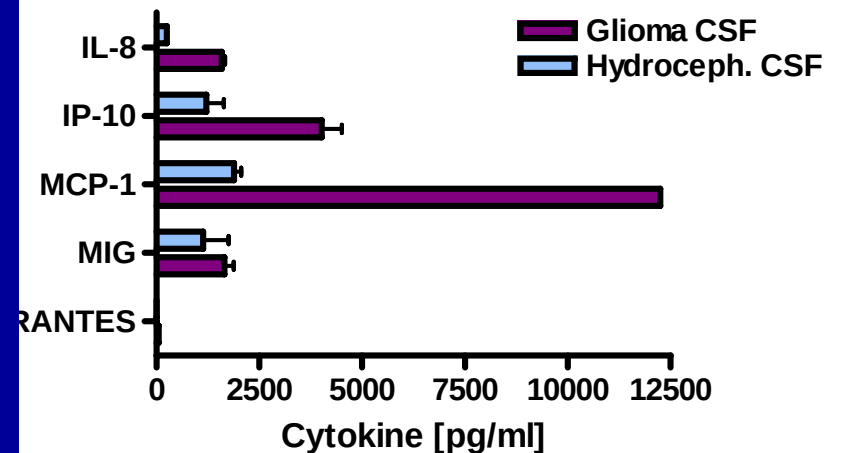
T-CELLS USE WHITE MATTER TRACKS



# PATIENT-DERIVED RESECTION CAVITY CSF CHEMOKINE CONTENT AND CHEMOTACTIC BIOACTIVITY

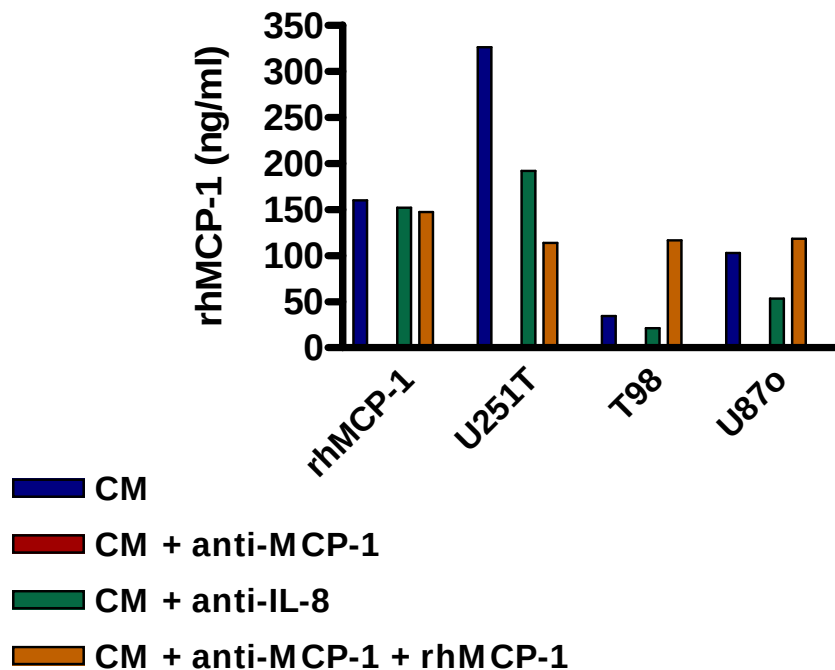


|               |                |               |               |
|---------------|----------------|---------------|---------------|
| TNF- $\alpha$ | MCP-1          | IL-2          | Pos           |
| TNF- $\beta$  | MCP-2          | IL-3          | Pos           |
| EGF           | MCP-3          | IL-4          | Neg           |
| IGF-I         | MCSF           | IL-5          | Neg           |
| Ang           | MDC            | IL-6          | EMA-78        |
| OSM           | MIG            | IL-7          | GCSF          |
| Tpo           | MIP-1 $\delta$ | IL-8          | GM-CSF        |
| VEGF          | RANTE          | IL-10         | GRO           |
| PDGF- $\beta$ | SCF            | IL-12         | GRO- $\alpha$ |
| Leptin        | SDF-1 $\delta$ | IL-13         | I-309         |
| Neg           | TARC           | IL-15         | IL-1a         |
| Pos           | TGF- $\beta$   | IFN- $\gamma$ | IL-1b         |

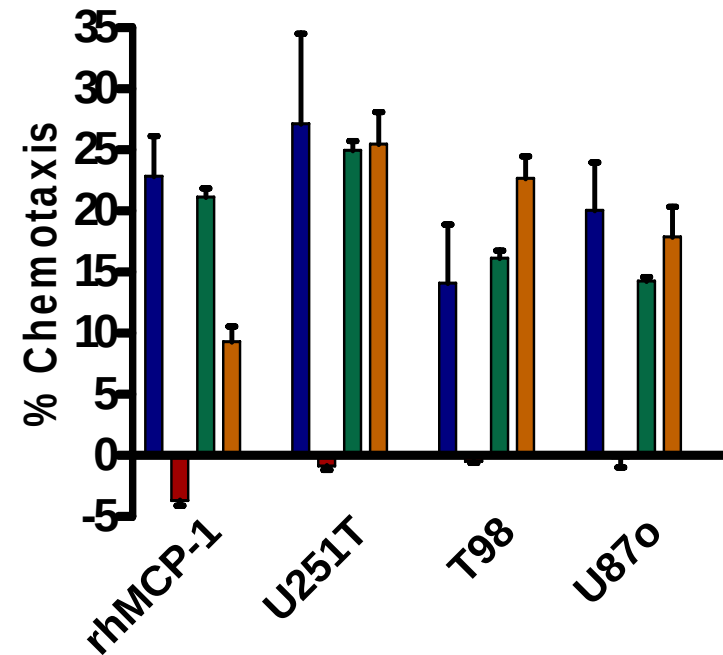


# GLIOMA-DERIVED CCL2/MCP-1 DRIVES T-CELL TUMOR TROPISM

## MCP-1 Quantitation (CBA Analysis)



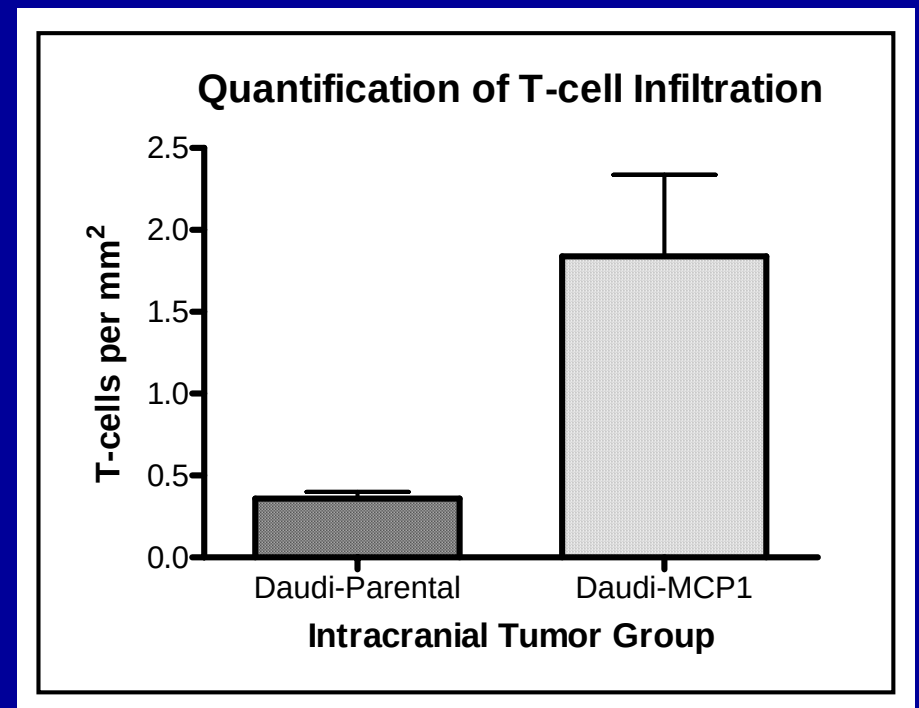
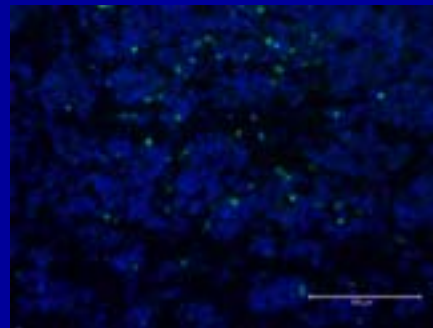
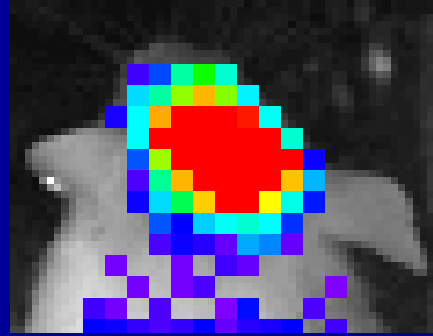
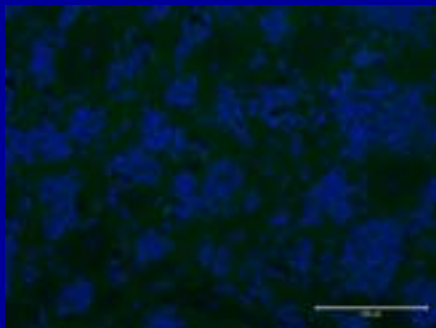
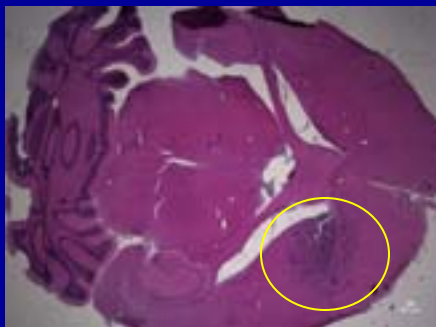
## Chemotaxis Assay



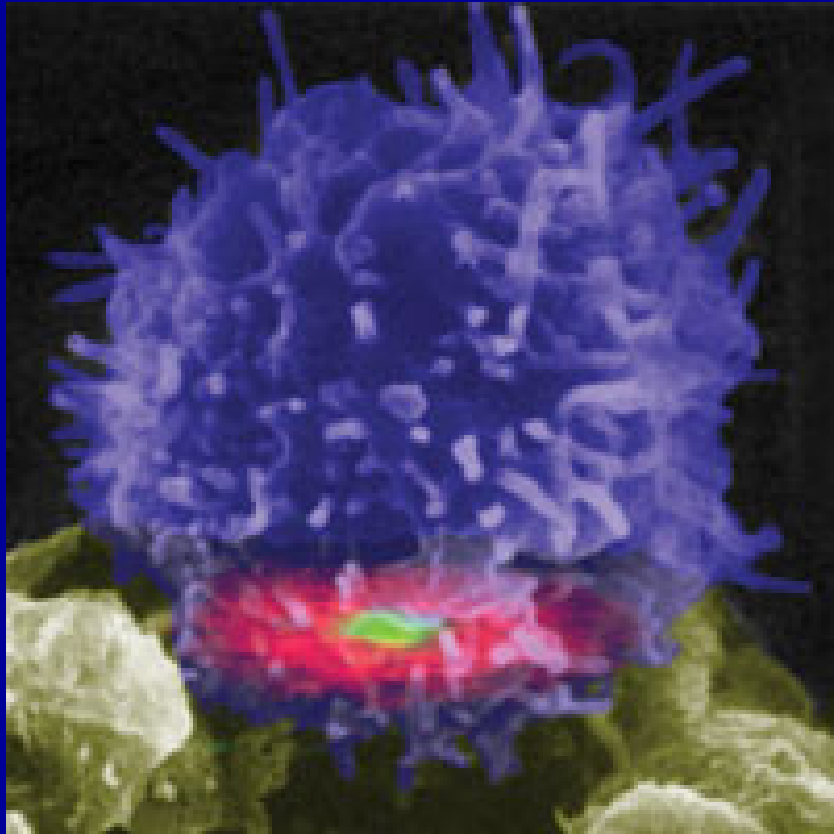
# TUMOR SECRETED MCP-1 IS SUFFICIENT FOR *IN VIVO* HOMING OF ADOPTIVELY TRANSFERRED T-CELLS

DAUDI-p

DAUDI-MCP1



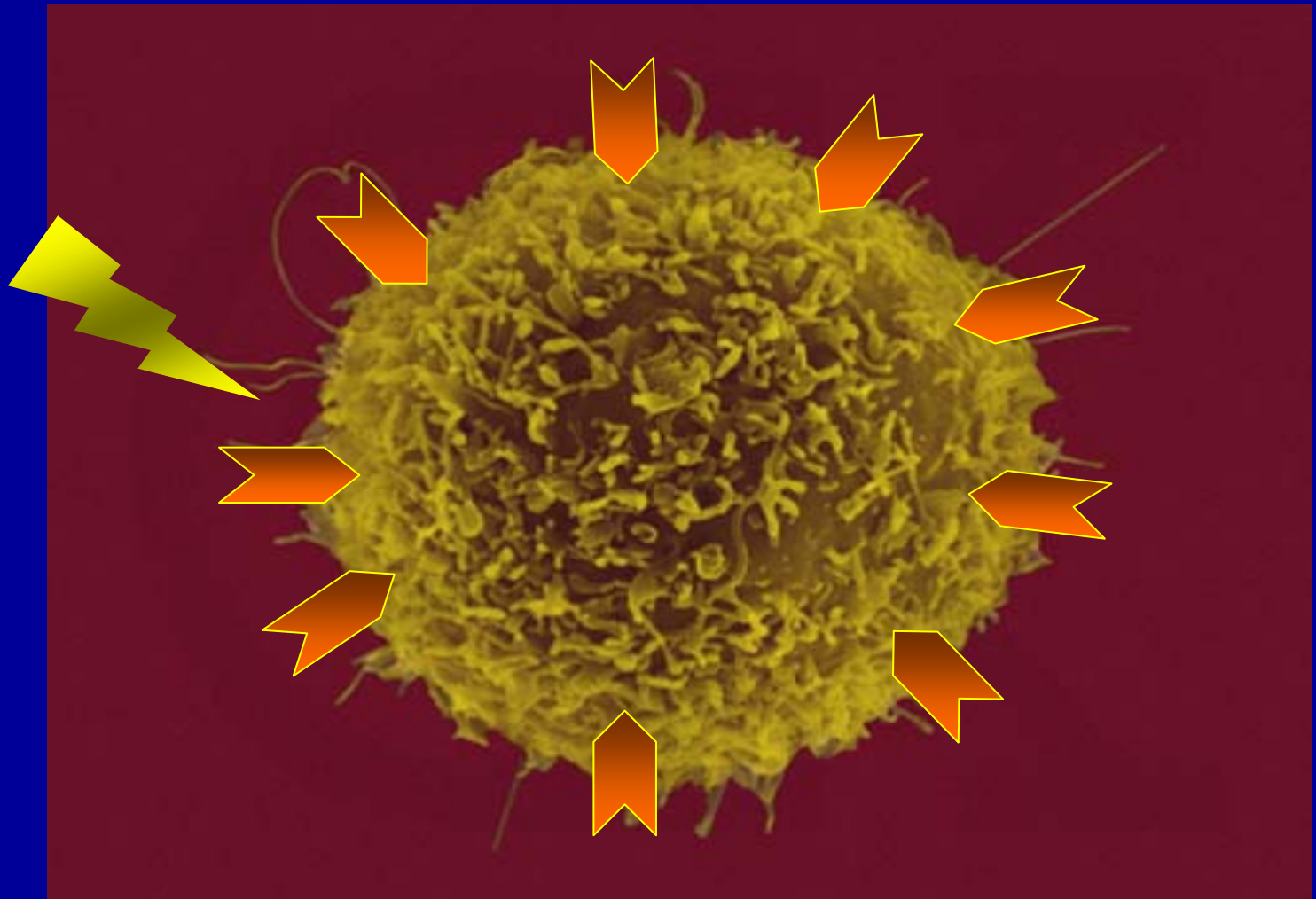
# TUMOR HOMING CYTOLYTIC T-CELLS: NANOSURGEONS IN THE BRAIN



**CYTOTOXIC T-LYMPHOCYTE:**  
A specialized white blood cell responsible for eliminating unwanted body cells (e.g. cancer) is killing a cell infected with the influenza virus

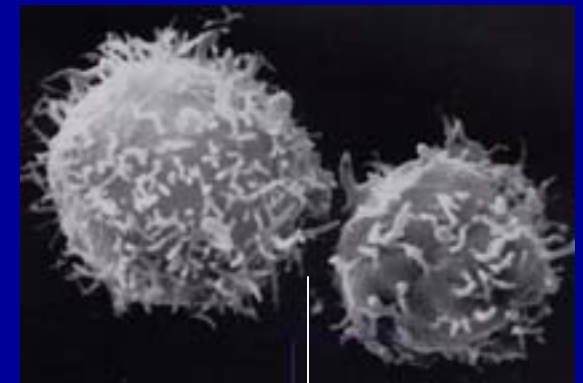
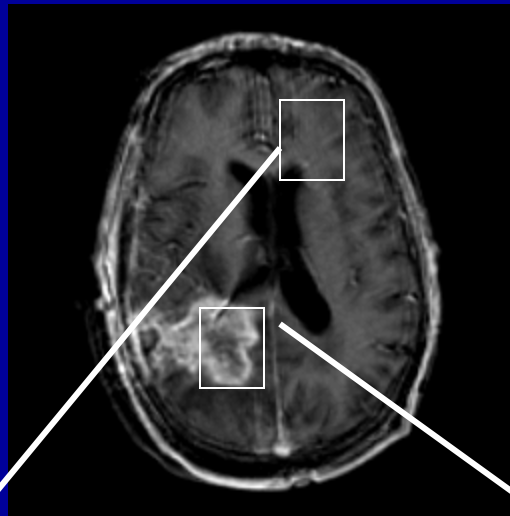


# EQUIPPING CTLs FOR TUMOR RECOGNITION BY GENE INSERTION





# SELECTION OF A GLIOMA-SPECIFIC TARGET ANTIGEN



TARGET: NORMAL BRAIN GLIOMA T-CELLS

|                   |     |             |     |
|-------------------|-----|-------------|-----|
| TnfR              | +/- | +++         | +++ |
| EGFR              | ++  | ++++        | -   |
| EGFRvIII          | -   | ++++ (<30%) | -   |
| IL-13R $\alpha$ 2 | -   | ++++ (>90%) | -   |

# THE IL13-ZETAKINE CHIMERIC ANTIGEN RECEPTOR FOR RE-DIRECTING CTL EFFECTOR FUNCTION TO GBM

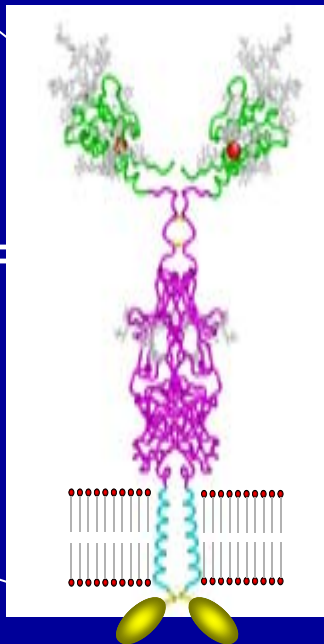
## IL13-ZETAKINE CHIMERIC ANTIGEN RECEPTOR

huIL-13(E13Y)

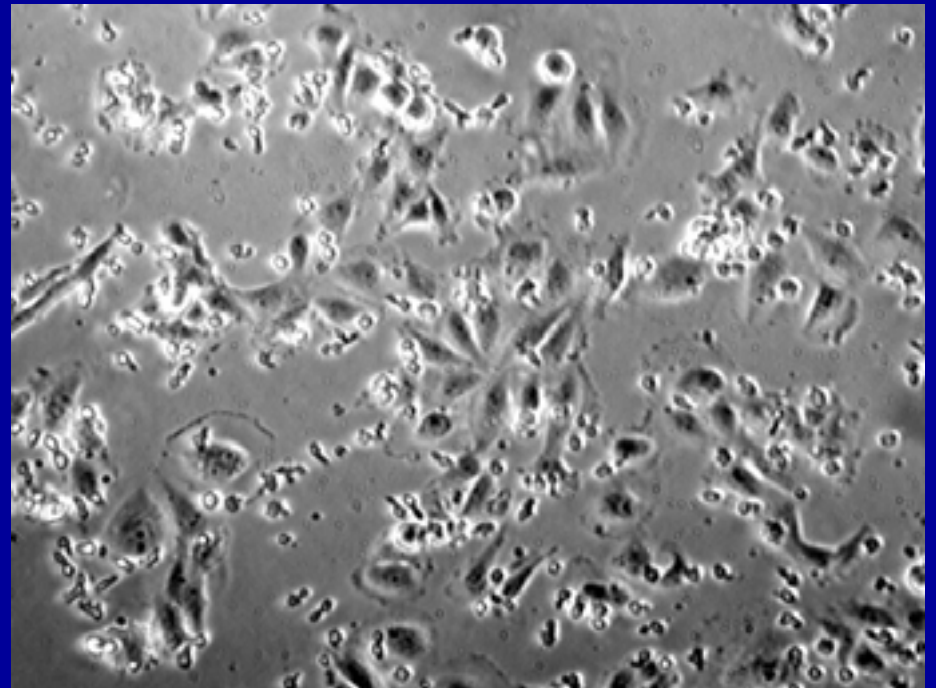
huIgG4 hinge-F

CD4<sub>TM</sub>

huCD3- $\zeta$ <sub>cyto</sub>

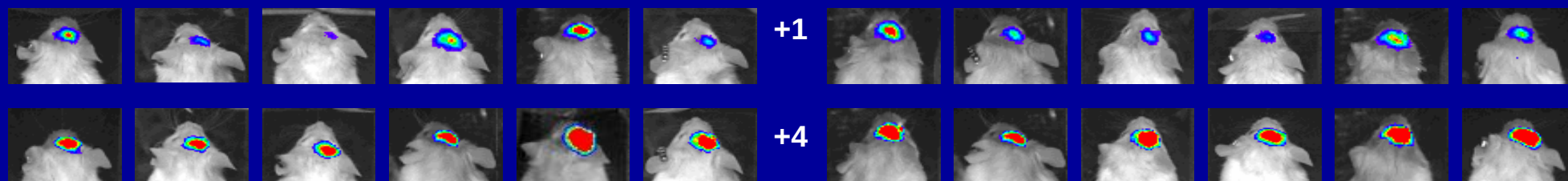


## RE-DIRECTED HLA UNRESTRICTED ZETAKINE-REGULATED GLIOMA KILLING



# U87 GLIOBLASTOMA (ffLucZeo/IL-2)

DAY 0:  $2 \times 10^5$  tumor cells i.c.

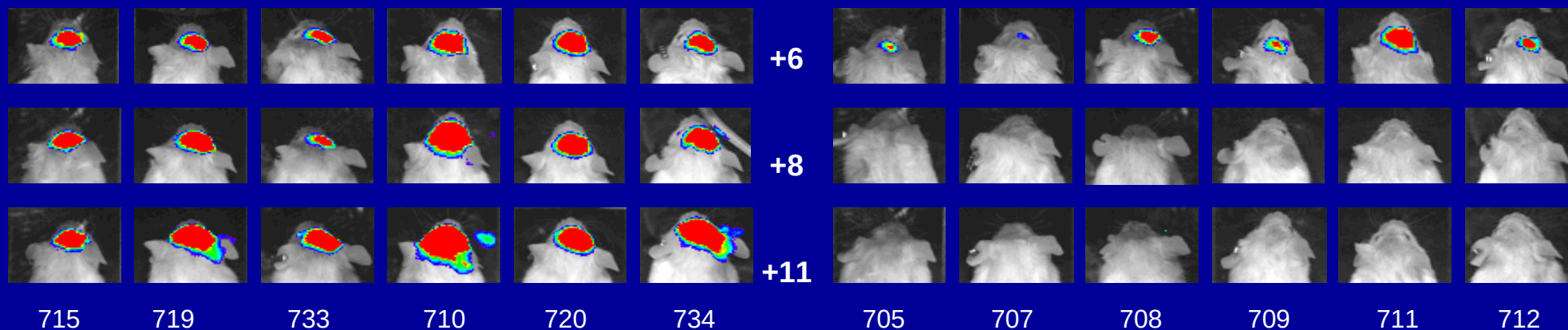
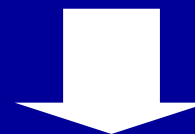


AntiCD19-CAR<sup>+</sup> CD8<sup>+</sup>  
CTL Clone E8

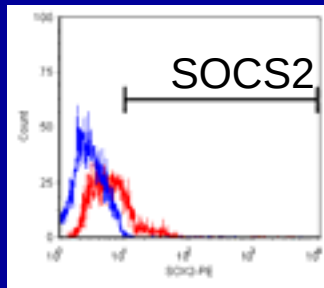
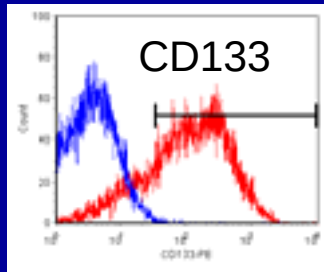


DAY +5:  
 $10 \times 10^5$  CD8<sup>+</sup> CTL i.c.

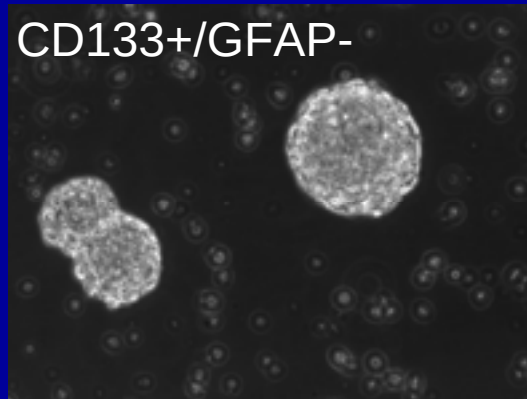
IL13-zetakine<sup>+</sup> CD8<sup>+</sup>  
CTL Clone 2D7



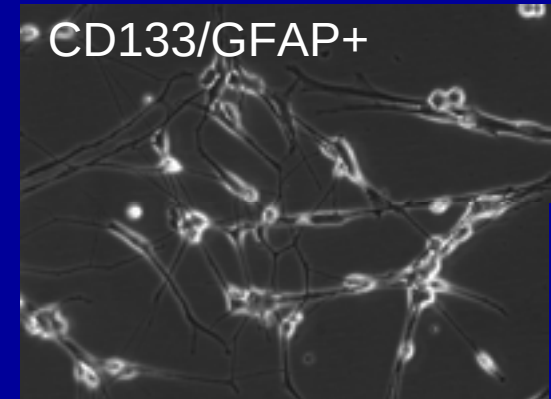
# GLIOMA STEM-LIKE TUMOR PROGENITOR CELLS



CD133+/GFAP-

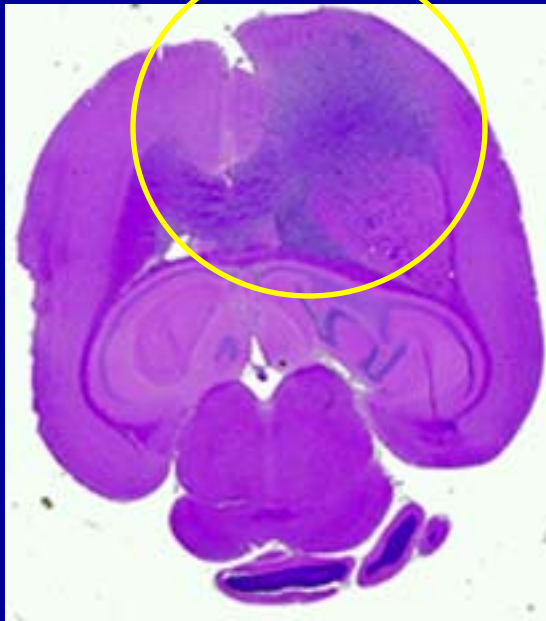


CD133/GFAP+



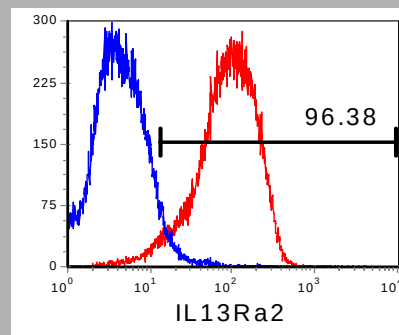
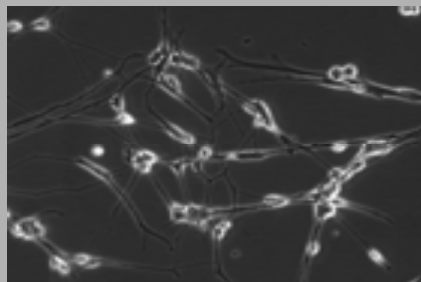
EGF/FGF/LIF/NO SERUM

DMEM 10%FCS

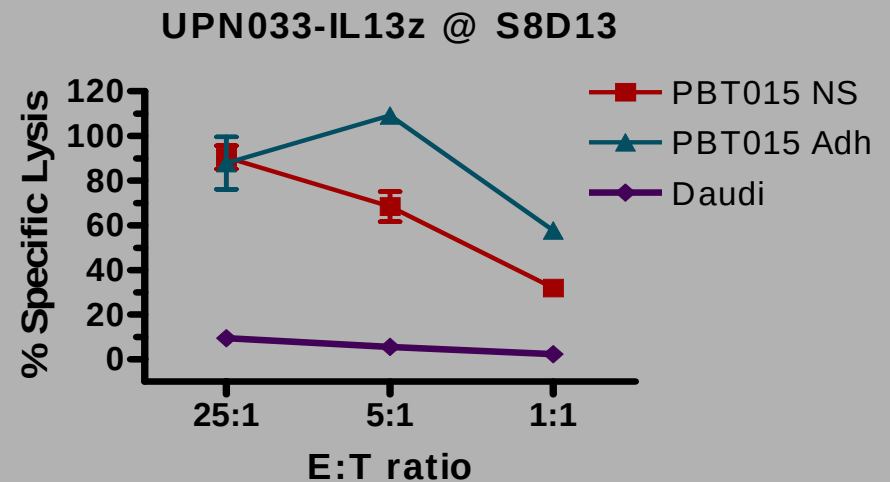
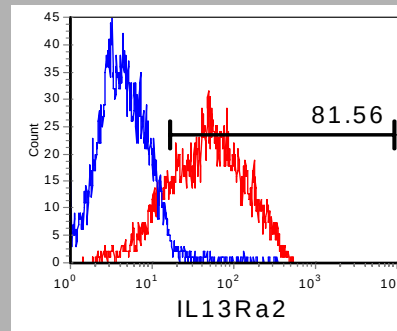
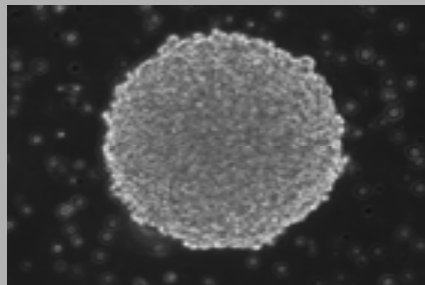


# PROTOCOL 01020 UPN-033: TARGET VALIDATION/T-CELL KILLING OF AUTOLOGOUS TUMOR/TUMOR STEM CELLS

## GFAP<sup>+</sup>/CD133<sup>-</sup> DIFFERENTIATED TUMOR



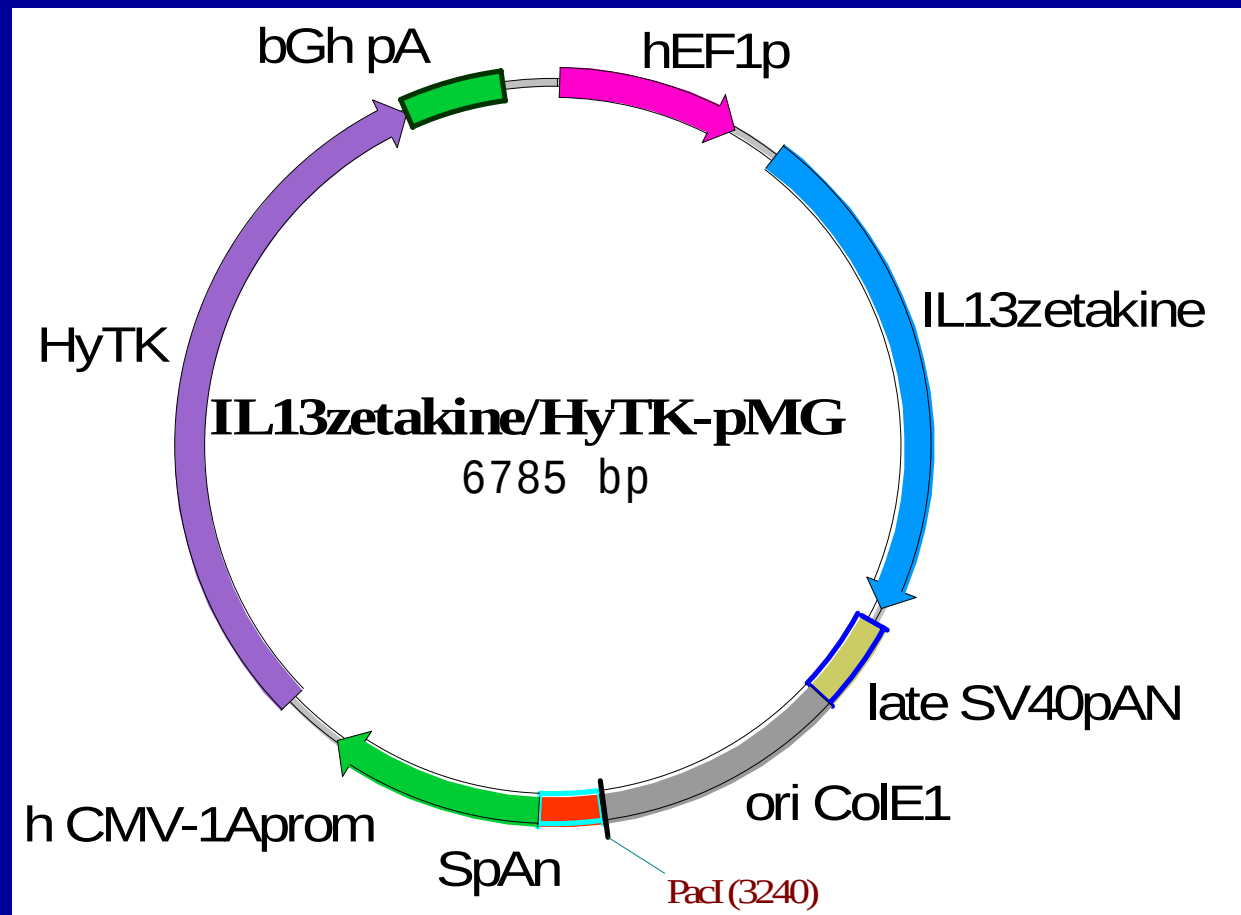
## GFAP<sup>-</sup>/CD133<sup>+</sup> TUMOR PROGENITORS



# CLINICAL APPLICATIONS

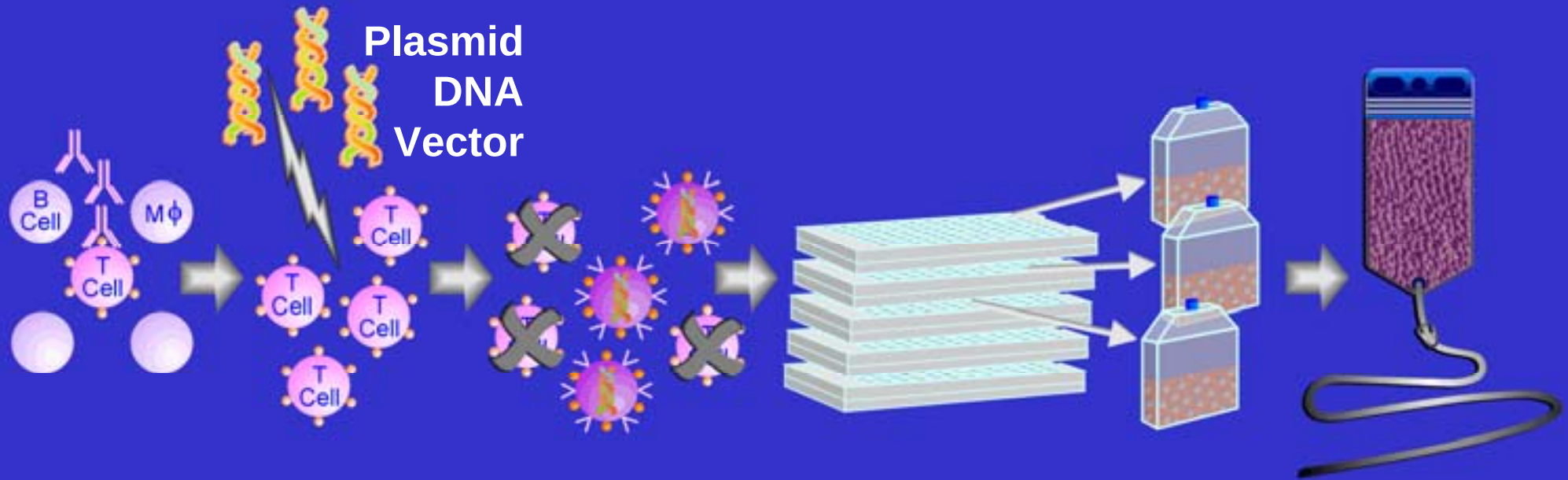


# PLASMID EXPRESSION VECTOR IL13 ZETAKINE/HyTK-pMG





# SCHEMA FOR T-CELL GENETIC MODIFICATION, CLONING, AND EX VIVO EXPANSION



**Day 0**  
Polyclonal  
Activation  
of T Cells  
with OKT3

**Day 3**  
Electroporation  
of OKT3-  
Activated  
PBMC

**Day 5**  
Addition of  
Selection  
Drug

**Day 28**  
Cloning of  
Surviving  
T Cells

**Day 42**  
Expansion  
of Drug-  
Resistant  
Clones

**Day 56**  
Large Scale  
Expansion  
of Zetakine+  
Clones for  
Re-Infusion

# PROCESS DEVELOPMENT



**T-CELL BIOREACTORS  
CLOSED SYSTEM CELL PROCESSING  
IMMUNOMAGNETIC SELECTION  
ENGINEERED FEEDER CELLS  
OPTIMIZED CYTOKINE COMBINATIONS**



# THE CENTER FOR BIOMEDICINE AND GENETICS AT CITY OF HOPE



**cGMP-MANUFACTURING FACILITY:**

**PLASMID DNA,  
VIRAL VECTORS,  
MONOCLONAL ANTIBODIES,  
AUTOLOGOUS CELL PRODUCTS**



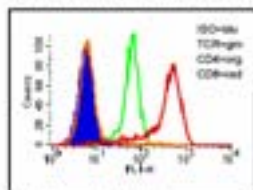
KCRB-3008 T-CELL THERAPEUTICS PRODUCTION SUITE  
CITY OF HOPE NATIONAL MEDICAL CENTER

# CERTIFICATE OF ANALYSIS CRYOPRESERVED AUTOLOGOUS T-CELL PRODUCT WORKING CELL BANK

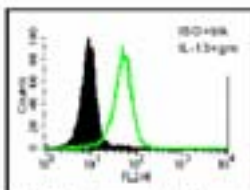
THE USE OF THIS PRODUCT IS RESTRICTED TO RESEARCH PURPOSES ONLY

|                    |                |
|--------------------|----------------|
| COH PROTOCOL IRB # | 01020          |
| UPN DESIGNATION    | 027            |
| CLONE DESIGNATION  | 1C9            |
| WCB DESIGNATION    | WCB-027-1C9-01 |

## I. FLOW CYTOMETRY:

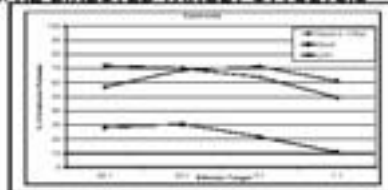


ACQ DATE: 12/26/02  
DATA LOCATION: MBKCRB 3008



ACQ DATE: 12/26/02  
DATA LOCATION: MBKCRB 3008

## II. RE-DIRECTED TARGET CYTOLYSIS



ACQ DATE: 11/19/02  
DATA LOCATION: MBKCRB 3008

## III. VECTOR-SPECIFIC SOUTHERN



ACQ DATE: 11/26/02  
DATA LOCATION: MBKCRB 3008

## IV. ANTI-ZETA WESTERN



ACQ DATE: 11/26/02  
DATA LOCATION: MBKCRB 3008

## V. AG-INDEPENDENT PROLIF.

|                       |        |
|-----------------------|--------|
| CLONE CPM             | 323    |
| JURKAT CPM            | 138126 |
| CLONE % OF JURKAT CPM | 0.2%   |

ACQ DATE: 12/3/02  
DATA LOCATION: MBKCRB 3008

## VI. VIABILITY FOLLOWING GANCICLOVIR

| CX. COND. | % VIABILITY |
|-----------|-------------|
| GANCIC.   | 9%          |
| MEDIA     | 90%         |

ACQ DATE: 11/22/02

## VII. STAGE I STERILITY

| TEST TYPE | RESULT | DATE/ACCESSION #     |
|-----------|--------|----------------------|
| U.S.P.    | NEG    | 12/26/02 F25147      |
| FUNGAL CX | NEG    | 12/26/02 F25147      |
| MYCO      | NEG    | 12/26/02 MBKCRB 3008 |

ACQ DATE: 12/26/02

## VIII. VIABILITY/ POST-THAW EXPANSION

|                |                              |
|----------------|------------------------------|
| VIABILITY      | 90%                          |
| FOLD EXPANSION | 86x (R74019)<br>75x (R74440) |

DATA LOCATION: MBKCRB 3008

DATA LOCATION: MBKCRB 3008

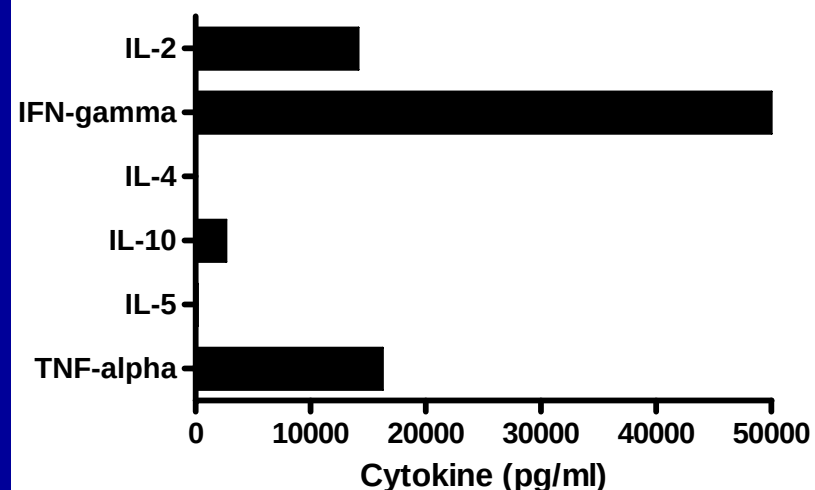
ALL QUALITY CONTROL PARAMETERS MEET SPECIFICATION. THIS CERTIFICATE OF ANALYSIS HAS BEEN REVIEWED AND AUTHENTICATED BY:

MICHAEL C. JENSEN, M.D.

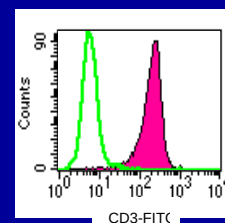
DATE

ANDREW RAUBITSCHKE, M.D. DATE

## Glioma-Stimulated Cytokine Production

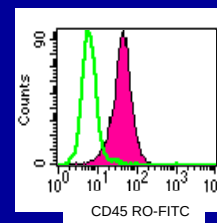


## CD3



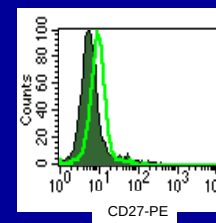
CD3-FITC

## CD45RO



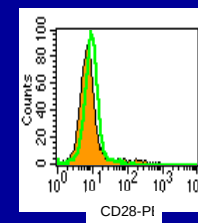
CD45 RO-FITC

## CD27



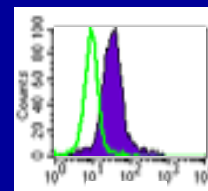
CD27-PE

## CD28



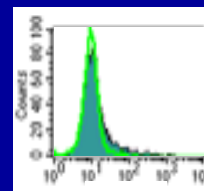
CD28-PI

## CXCR3



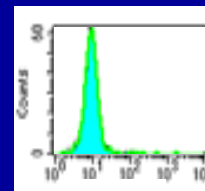
CXCR3-P

## CXCR4



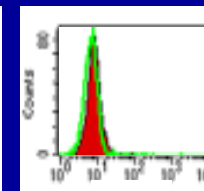
CXCR4-P

## CCR2



CCR2-PE

## CCR5



CCR5-PE

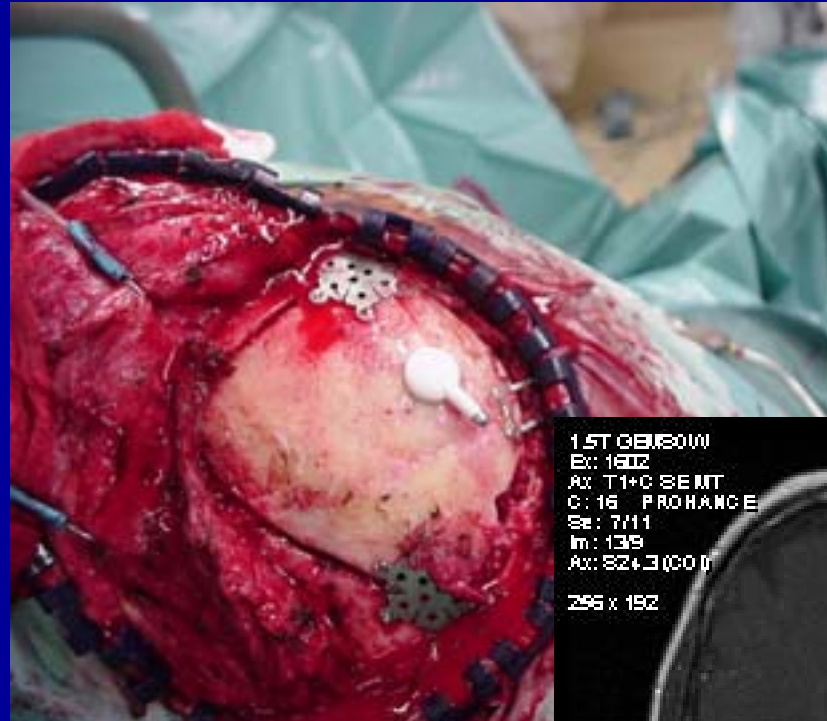
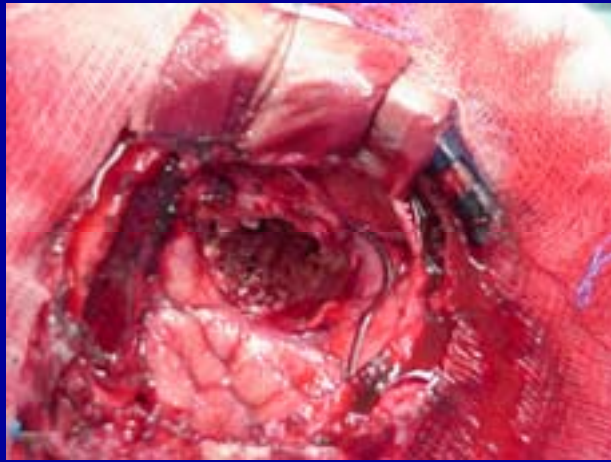
**COHNMC PROTOCOL IRB#01020:  
(BB-IND #10109)**

**A PILOT FEASIBILITY/SAFETY STUDY OF CELLULAR  
IMMUNOTHERAPY FOR RECURRENT/REFRACTORY  
MALIGNANT GLIOMA USING GENETICALLY MODIFIED  
AUTOLOGOUS CD8<sup>+</sup> T-CELL CLONES**

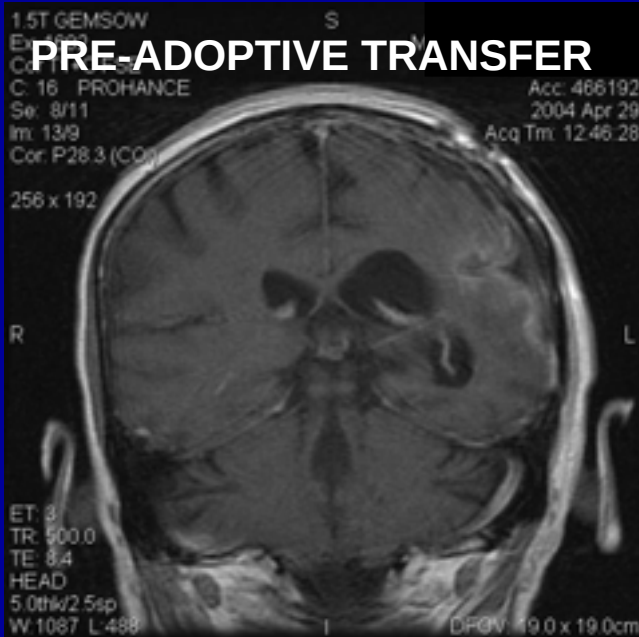
**PROTOCOL #01020  
CELL DOSING SCHEDULE**

|                  |              |                | INTRACAVITARY     |                 |
|------------------|--------------|----------------|-------------------|-----------------|
| <u>CYCLE:</u>    | <u>WEEK:</u> | <u>DAY:</u>    | <u>CELL DOSE:</u> |                 |
| L020<br>SCHEDULE | 1            | 1              | 10 <sup>7</sup>   |                 |
|                  |              | 3              | 5x10 <sup>7</sup> |                 |
|                  |              | 5              | 10 <sup>8</sup>   |                 |
|                  | 2            | 2              | 1                 | 10 <sup>8</sup> |
|                  |              |                | 3                 | 10 <sup>8</sup> |
|                  |              |                | 5                 | 10 <sup>8</sup> |
|                  | 3            | 3              | REST/RESTAGING    |                 |
|                  |              | 4              | 1                 | 10 <sup>8</sup> |
|                  |              |                | 3                 | 10 <sup>8</sup> |
|                  | 5            |                | 10 <sup>8</sup>   |                 |
|                  | 4            | 5              | 1                 | 10 <sup>8</sup> |
|                  |              |                | 3                 | 10 <sup>8</sup> |
| 5                |              |                | 10 <sup>8</sup>   |                 |
| 5                | 6            | REST/RESTAGING |                   |                 |

# PLACEMENT OF RICKHAM SHUNT VAD IN RESECTION CAVITY







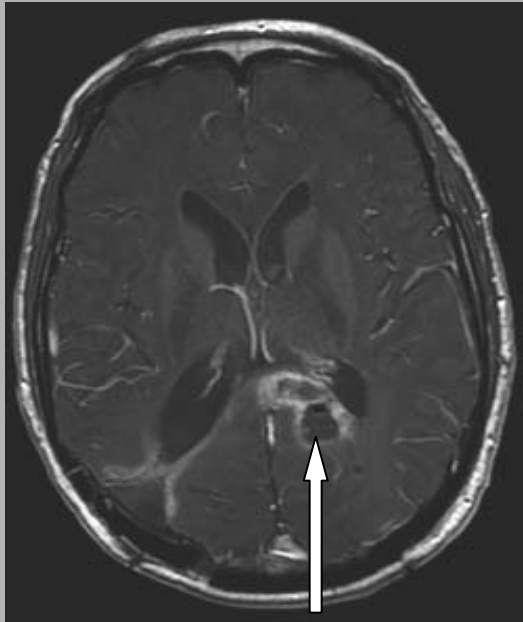
**T1-GADOLINIUM MRI PULSE SEQUENCE**

# PROTOCOL 01020 SAFETY DATA

| <u>Cell Dose Level</u>                                               | <u>Number of Doses</u> | <u>Observed Side-Effects Grade 3 or higher Attributable to Cell Doses</u> |
|----------------------------------------------------------------------|------------------------|---------------------------------------------------------------------------|
| <b>•Intra-cavitary</b>                                               |                        |                                                                           |
| 10 <sup>7</sup>                                                      | 2                      | None                                                                      |
| 5x10 <sup>7</sup>                                                    | 2                      | None                                                                      |
| 10 <sup>8</sup>                                                      | 19                     | Headache 2x – only on UPN 028                                             |
| <b>•Intra-parenchyma</b>                                             |                        |                                                                           |
| 25 x 10 <sup>6</sup>                                                 | 1                      | None                                                                      |
| 50 x 10 <sup>6</sup>                                                 | 1                      | WBC, Headache, Pain – localized to scalp/catheter site                    |
| 10 <sup>8</sup>                                                      | 3                      | Hypoxia, Headache (3x), Fatigue                                           |
| <b>•Cumulative Cell Dose</b>                                         |                        |                                                                           |
| UPN-028 - 9.6 x 10 <sup>8</sup> cells over 21-days (Intra-cavitary)  |                        |                                                                           |
| UPN-033 - 10.6 x 10 <sup>8</sup> cells over 21-days (Intra-cavitary) |                        |                                                                           |
| - 3.75 x 10 <sup>8</sup> cells over 5-days (Intra-parenchymal)       |                        |                                                                           |
| <b>•Best Response to therapy</b>                                     |                        |                                                                           |
| UPN-028 Radiographic CR                                              |                        | Survival Duration post Relapse: 12 months                                 |
| UPN-033 Radiographic CR                                              |                        | Survival Duration post Relapse: 11 Months                                 |

# **PROTOCOL 01020 UPN-033: RADIOGRAPHIC COMPLETE RESPONSE FOLLOWING ADOPTIVE THERAPY**

**PRE-RX**

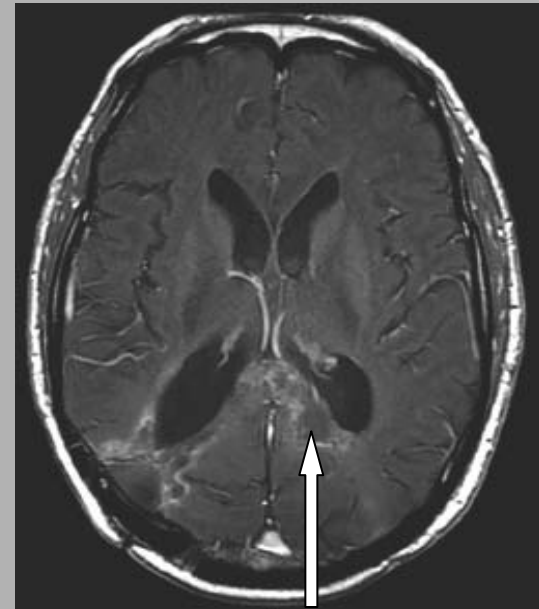


**Bx. Proven Progressive GBM**

**5 T-CELL  
DOSES @  
10e8**



**14-WEEKS POST-RX**



**Spectroscopy=Necrosis  
FDG PET = Cold**

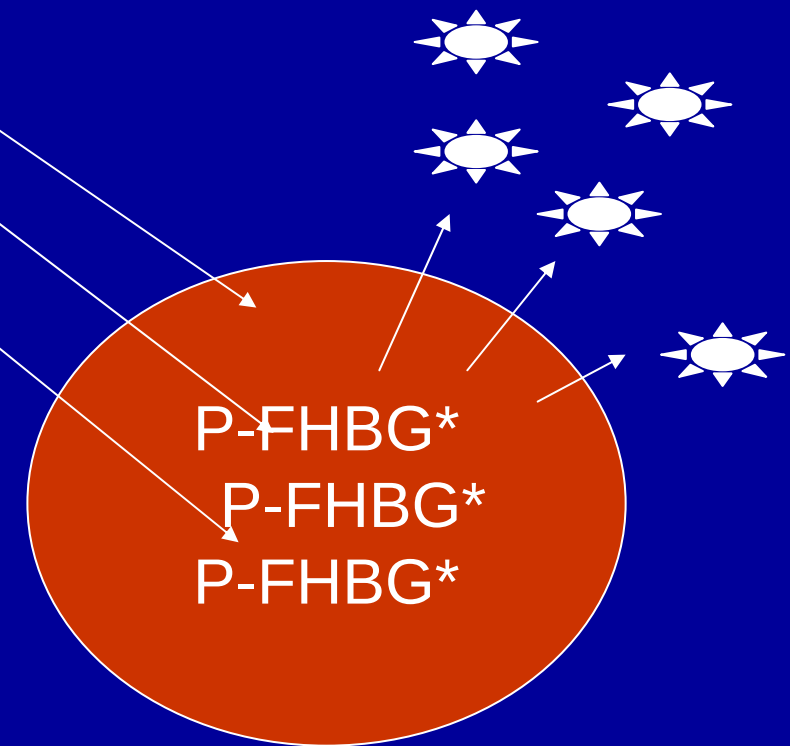
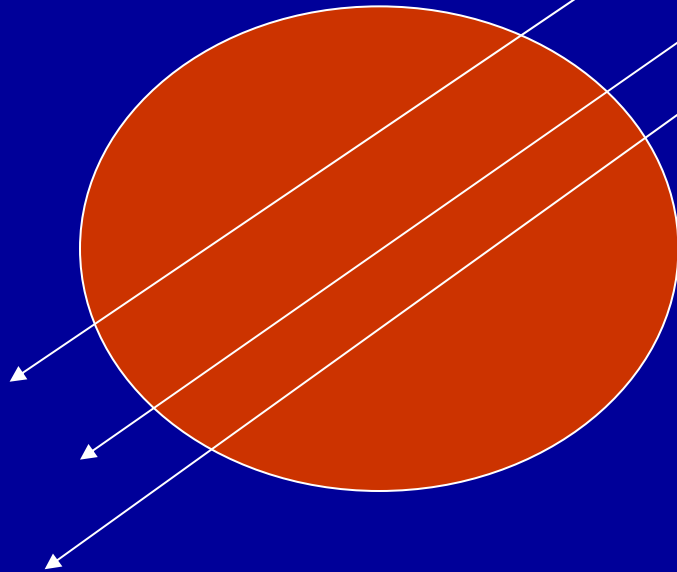
**INITIAL CLINICAL IMAGING EXPERIENCE  
USING [ $^{18}\text{F}$ ]-FHBG PET ON COHNMC  
PROTOCOL #01020**

# PET-BASED MOLECULAR IMAGING OF HyTK EXPRESSING T-CELLS

HSV TK-

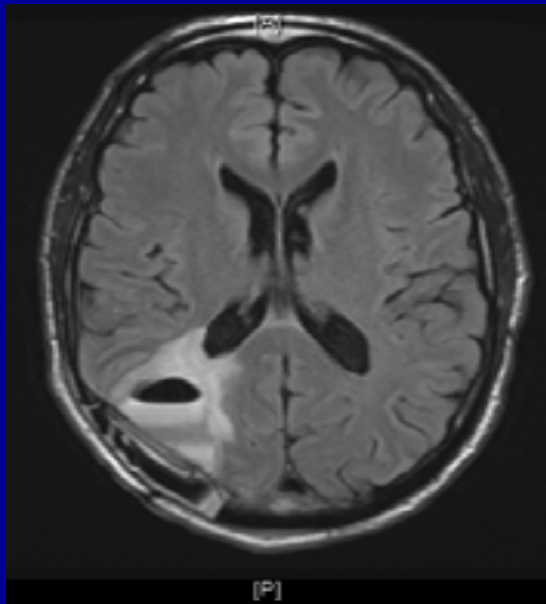
HSV TK+

$[^{18}\text{F}] \text{ FHBG}^*$

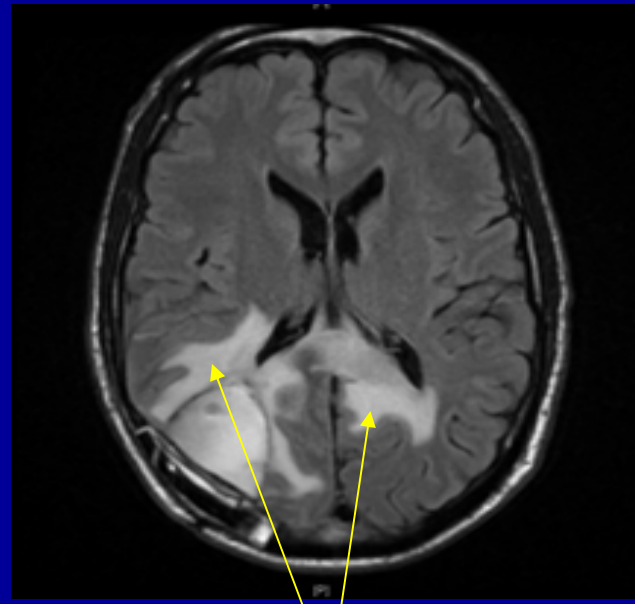


# UPN-033: Correlative Imaging of CNS Changes Observed Following Intracranial Adoptive Transfer Of Autologous IL13-zetakine<sup>+</sup>/HyTK<sup>+</sup> CD8<sup>+</sup> CTL Clone

Pre-Adoptive Rx  
Ax Flair irFSE

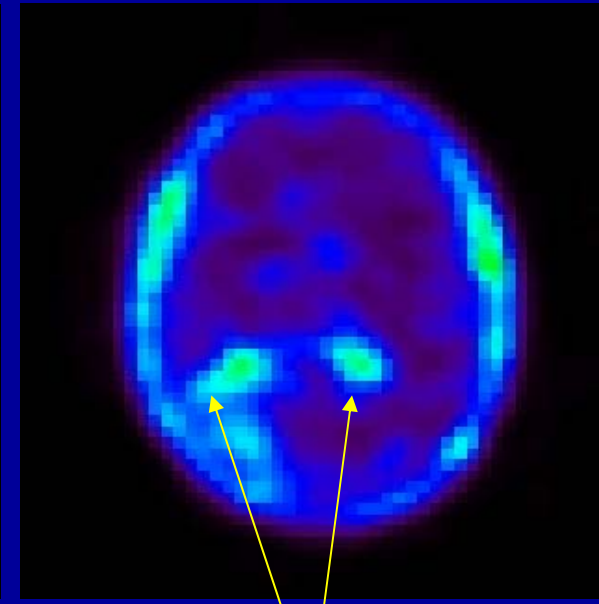


Post-Adoptive Rx  
Ax Flair irFSE



Tumor Progression  
Versus T-Cell Mediated  
Inflammation

<sup>18</sup>FHBG PET

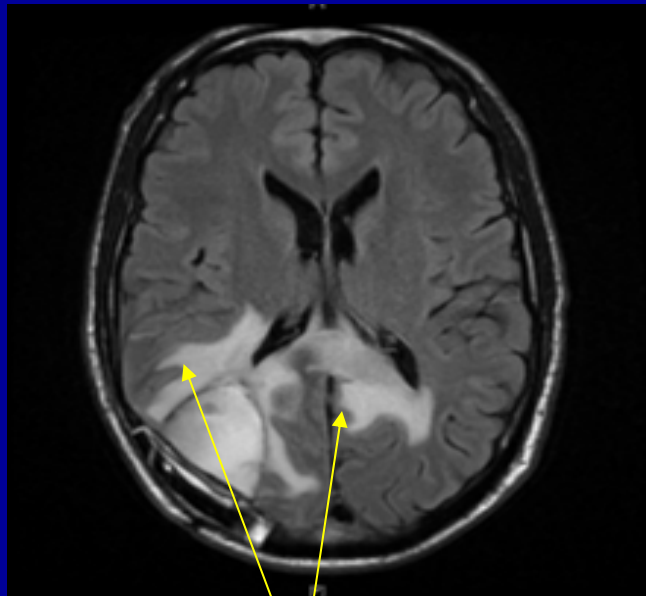


FHBG Signal  
From TK Reporter  
Expressed in T-Cells



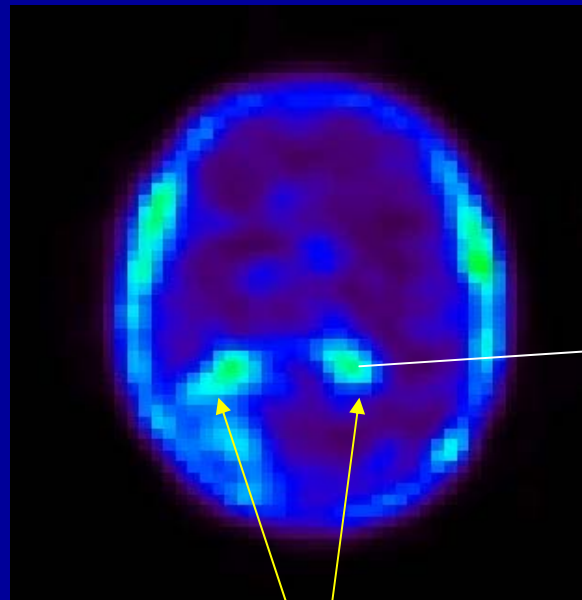
# UPN-033: Correlative Imaging of CNS Changes Observed Following Intracranial Adoptive Transfer Of Autologous IL13-zetakine<sup>+</sup>/HyTK<sup>+</sup> CD8<sup>+</sup> CTL Clone

Ax Flair irFSE

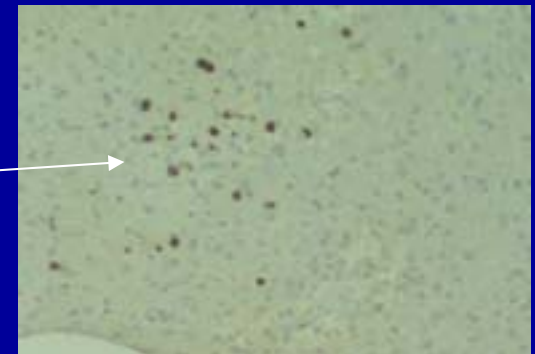


Tumor Progression  
Versus T-Cell Mediated  
Inflammation

<sup>18</sup>FHBG PET



FHBG  
PET SIGNAL



CD8+ T CELL  
INFILTRATE

# **CHALLENGES TO BROADER CLINICAL UTILITY:**

- **PATIENTS FREQUENTLY NEED TO TAKE  
GLUCOCORTICOIDS (DEXAMETHASONE) TO  
MANAGE CERBRAL EDEMA**
- **TIME TO PRODUCE AUTOLOGOUS PRODUCT  
NOT COMPATIBLE WITH RECURRENT  
DISEASE PROGRESSION KINETICS**

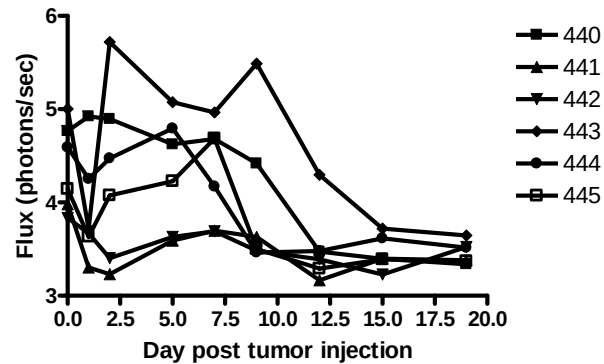
# Potential Advantages of Steroid-Resistant Alloclone:

- READY-TO-USE PRODUCT AVAILABLE AT TIME OF NEED
- FUNCTIONAL IN PATIENTS RECEIVING DEXAMETHASONE
- CONCURRENT USE OF DEXAMETHASONE LIMITS CNS INFLAMMATORY REACTION TO ADOPTIVE THERAPY/  
PROLONGS SURVIVAL OF T-CELL ALLOGRAFT BY INHIBITION OF REJECTION RESPONSE

# INTRAPARENCHYMAL CNS LYMPHOID ALLOGRAFTS ARE NOT REJECTED

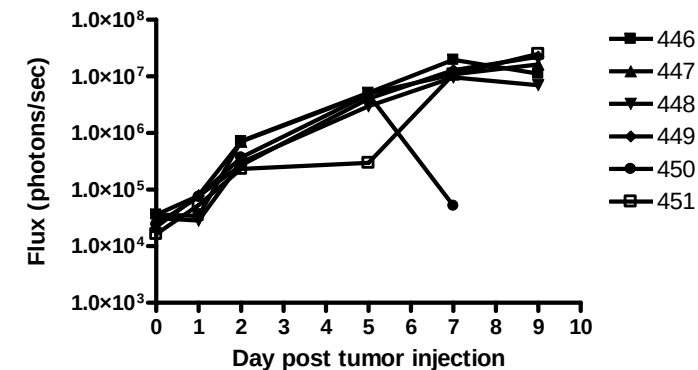
FLANK

Grp.A  $10^6$  NSO IL2(2)\_ffluc-Hytk-pMG,GFP-DHFRdm-pEK s.c.



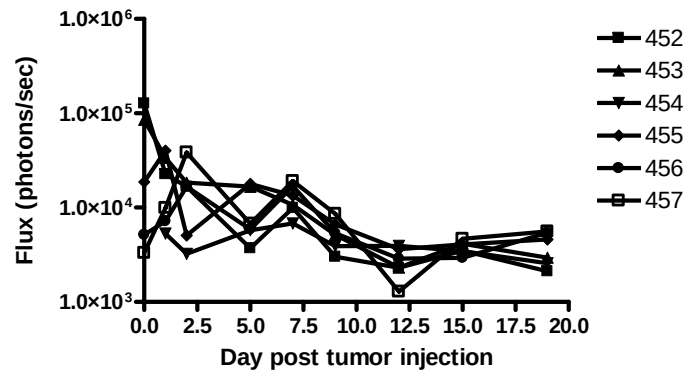
BRAIN

Grp.B 25,000 cells NSO IL2(2)\_ffluc-Hytk-pMG,GFP-DHFRdm-pEK i.c.



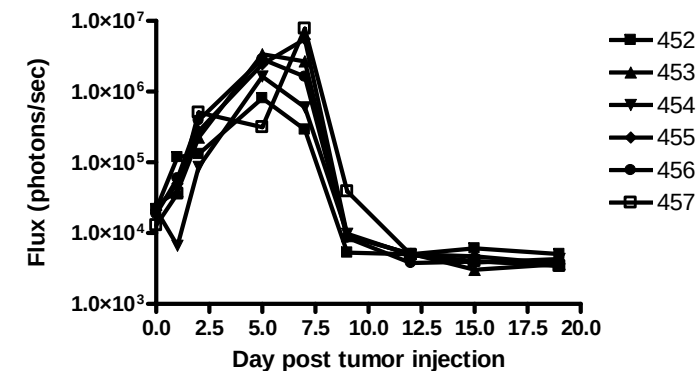
FLANK

Grp.C 25,000 cells NSO IL2(2)\_ffluc-Hytk-pMG,GFP-DHFRdm-pEK i.c. &  $1 \times 10^6$  s.c. - Flank only



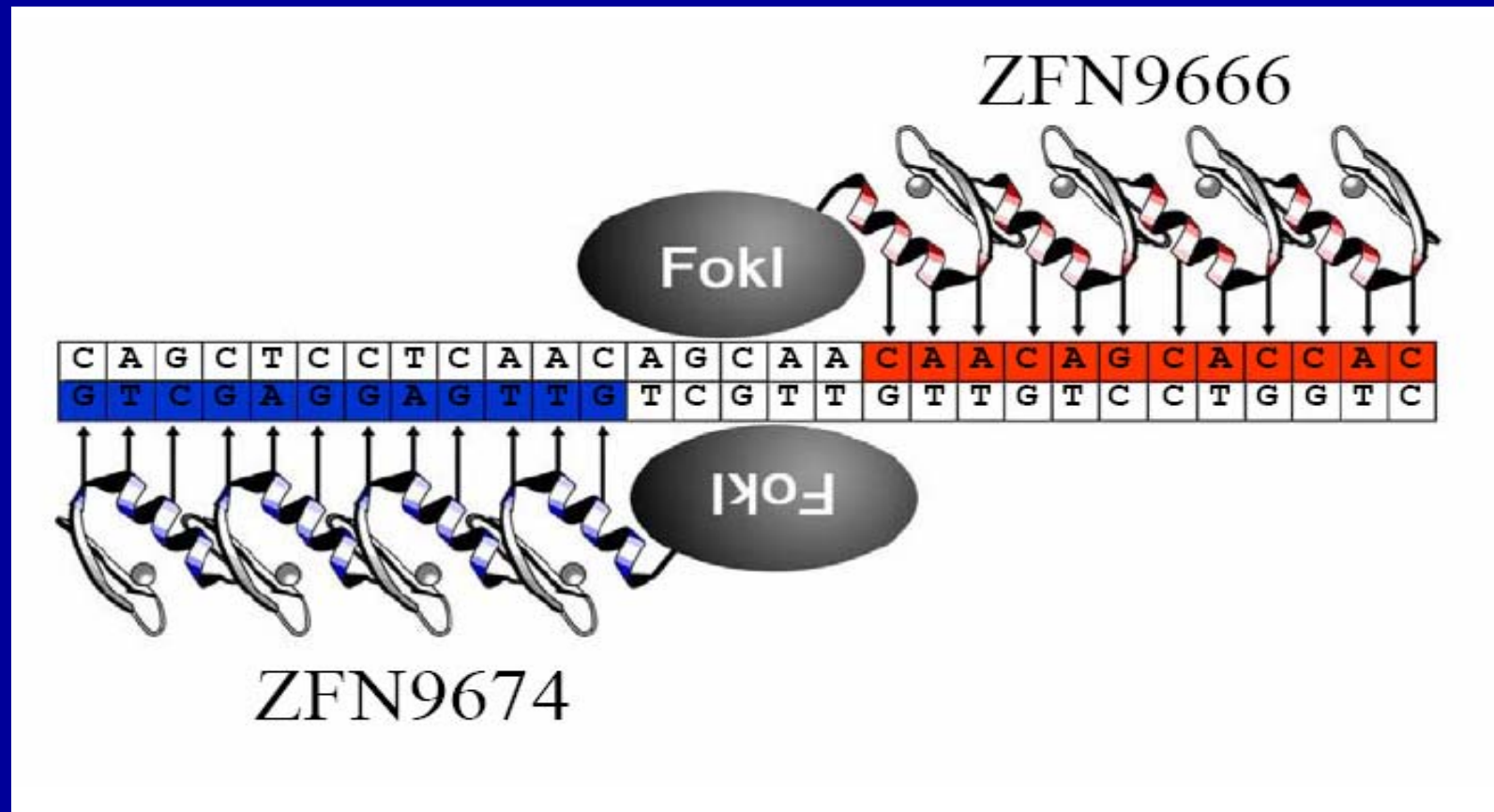
+

Grp.C 25,000 cells NSO IL2(2)\_ffluc-Hytk-pMG,GFP-DHFRdm-pEK i.c. &  $1 \times 10^6$  s.c. - Head only



BRAIN

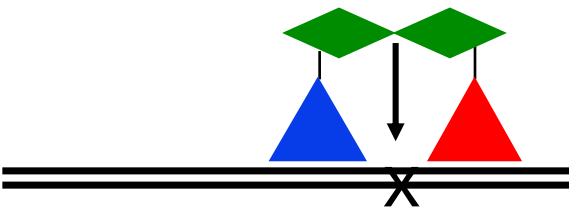
# ZFP-Fok I Fusion Proteins



# ZFN-Mediated Targeted Gene Disruption (TGD)

1. 

Endogenous gene targeted for disruption

2. 

ZFNs dimerize and introduce a double stranded DNA break in the gene

3. 

Break repaired by non-homologous end-joining (NHEJ) – resulting in loss of genetic information

4. 

Gene disrupted

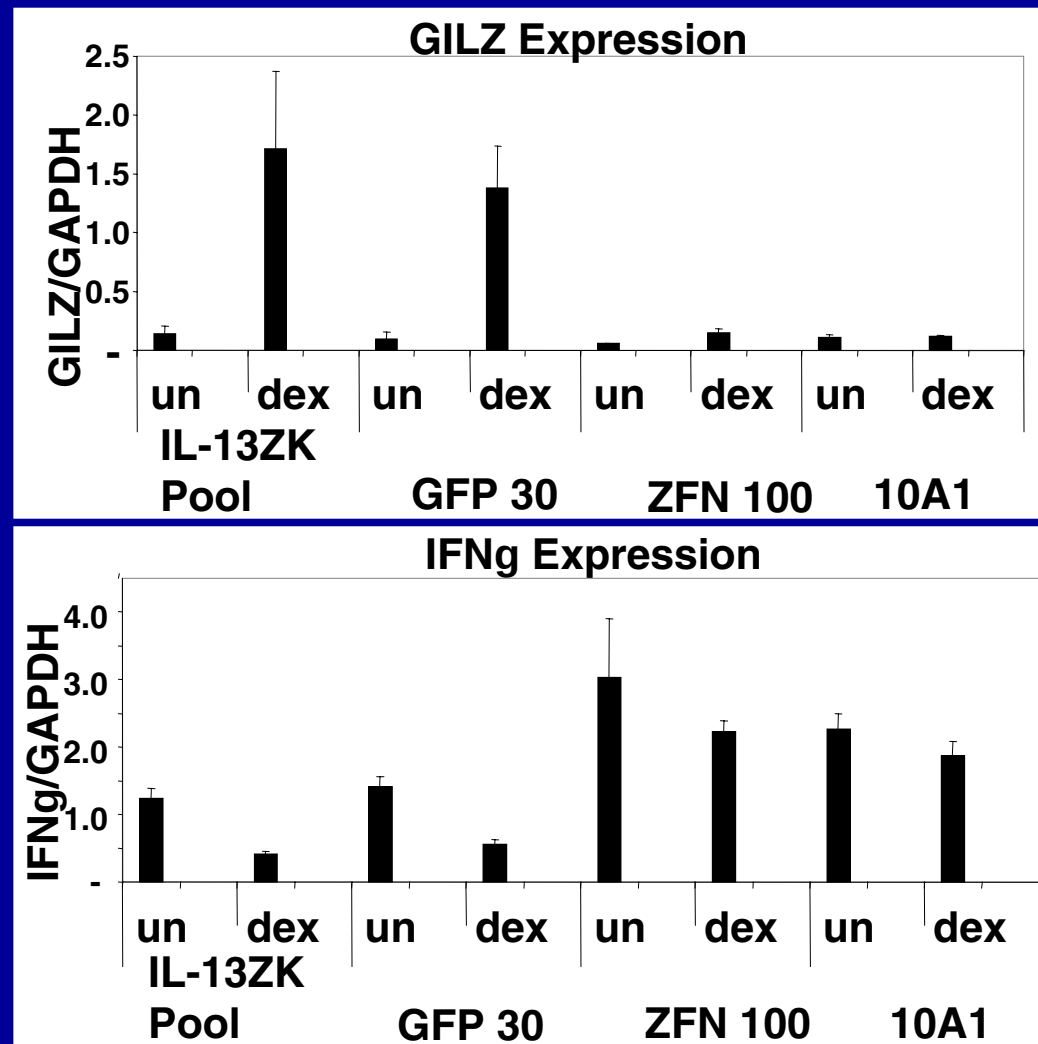


## GR (N-terminus)

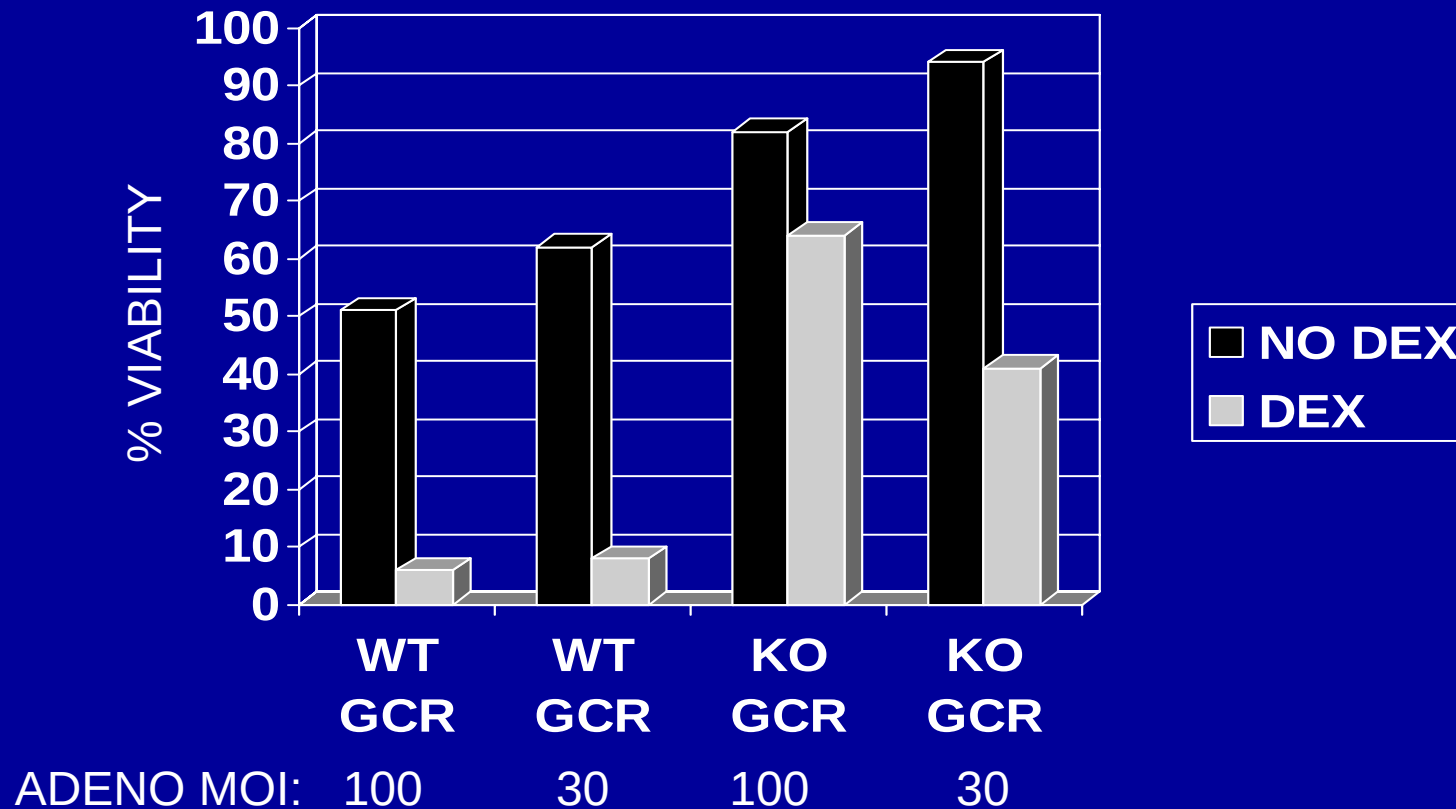
TFIIB



# RT-PCR Analysis Shows Loss of GR Target Gene Transcriptional Regulation in ZFN Modified T-Cells

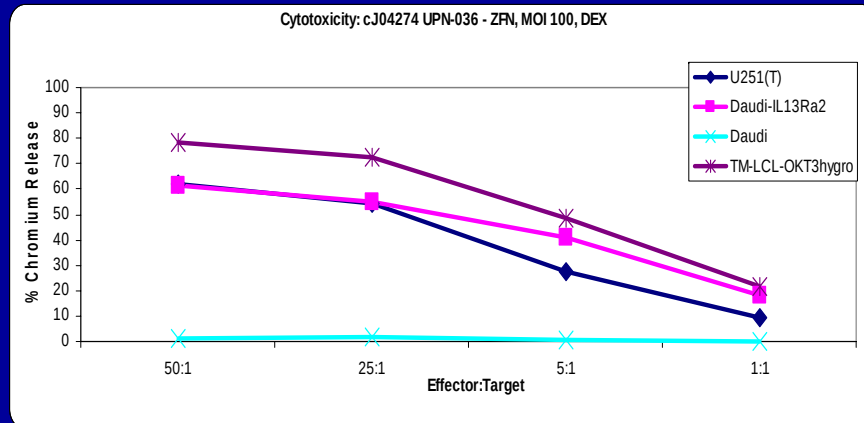


# ZFN-MEDIATED GLUCOCORTICOID DISRUPTION OF ZETAKINE<sup>+</sup> CTLs RESULTS IN RESISTANCE TO DEXAMETHASONE-TRIGGERED APOPTOSIS



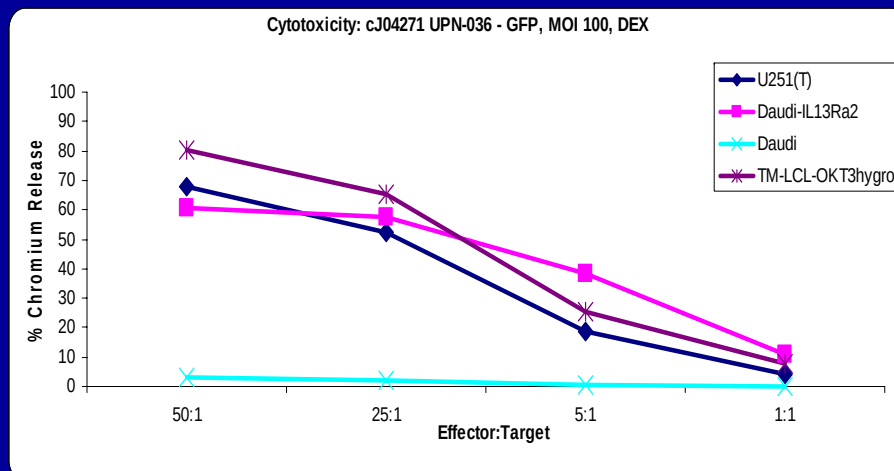
# GR-KO CTLs RETAIN ZETAKINE-REGULATED LYTIC ACTIVITY

**GR-ZFN Adeno Treated  
MOI 100**



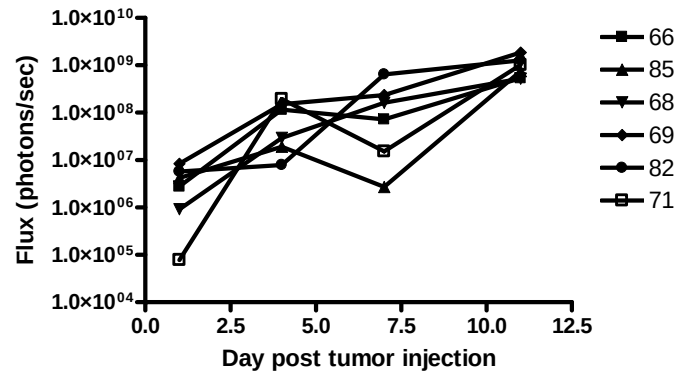
**GFP Adeno Control  
MOI 100**

**Dex 10e-4M**

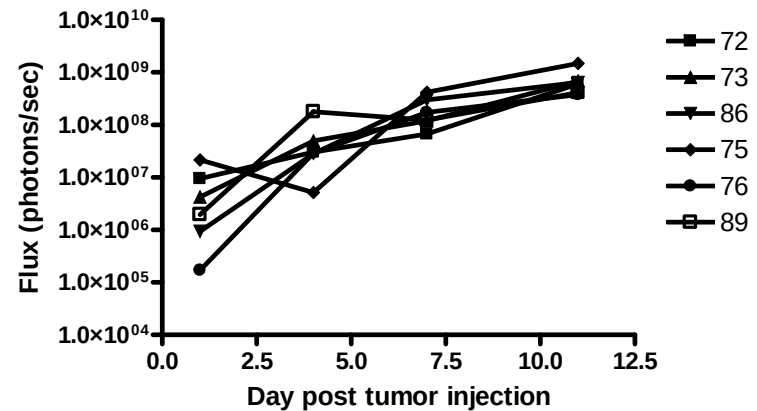


## GR- ZETAKINE+ CTL ARE DEX RESISTANT IN VIVO

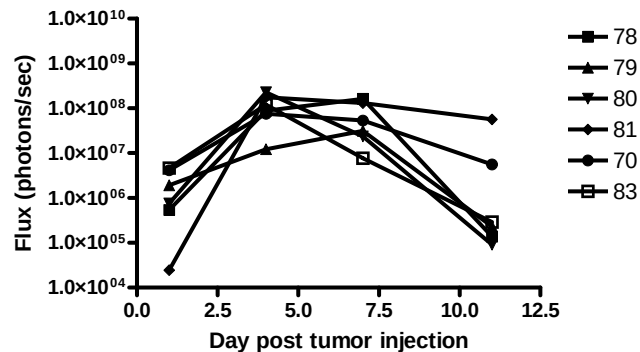
**0.2 x10<sup>6</sup> U87O-ffluczeo-IL2 i.c. Day 0**  
**PBS control i.c. Day 5 & 8**



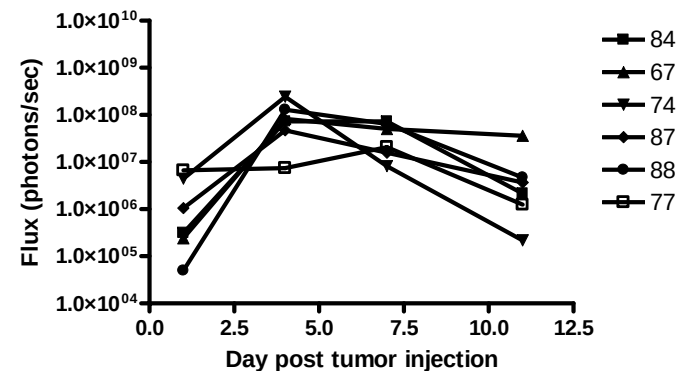
**0.2 x10<sup>6</sup> U87O-ffluczeo-IL2 i.c. Day 0**  
**PBS control i.c. Day 5 & 8**  
**4mg/kg Dex i.p. @ Day 5**



**0.2 x10<sup>6</sup> U87O-ffluczeo-IL2 i.c. Day 0**  
**2x10<sup>6</sup> UPN-036 - IL13-zeta\_ GR adeno**  
**@ MOI 100 i.c. Day 5 & 8**



0.2 x10<sup>6</sup> U87O-ffluczeo-IL2 i.c. Day 0  
2x10<sup>6</sup> UPN-036 - IL13-zeta\_ GR adeno  
@ MOI 100 i.c. Day 5 & 8  
4mg/kg Dex i.p. @ Day 5



# GR-KO IL13-ZETAKINE ALLOCLONE CLINICAL TRIAL

- UNRESECTABLE TUMORS
- DECADRON DEPENDENT PATIENTS
- INTRAPARENCHYMAL T-CELL  
INFUSIONS
- CONVECTION ENHANCED rHuIL-2  
DELIVERY

# Jensen Lab/Translational Research Team



**Reena  
Vishwanath**  
**COH Grad.  
Student**  
Tumor Tropism  
Project

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David DiGiusto, PhD  
Andrew Raubitschek, MD  
Stephen Forman, MD  
Michael Kalos, PhD

**Collaborators-**  
Stanley Riddell FHCRC  
Sam Gambhir Stanford  
Sangamo Biosciences