Anti-tumor Effects of Activated Human Natural Killer Cells in Orthotopic Human Brain Tumor Models

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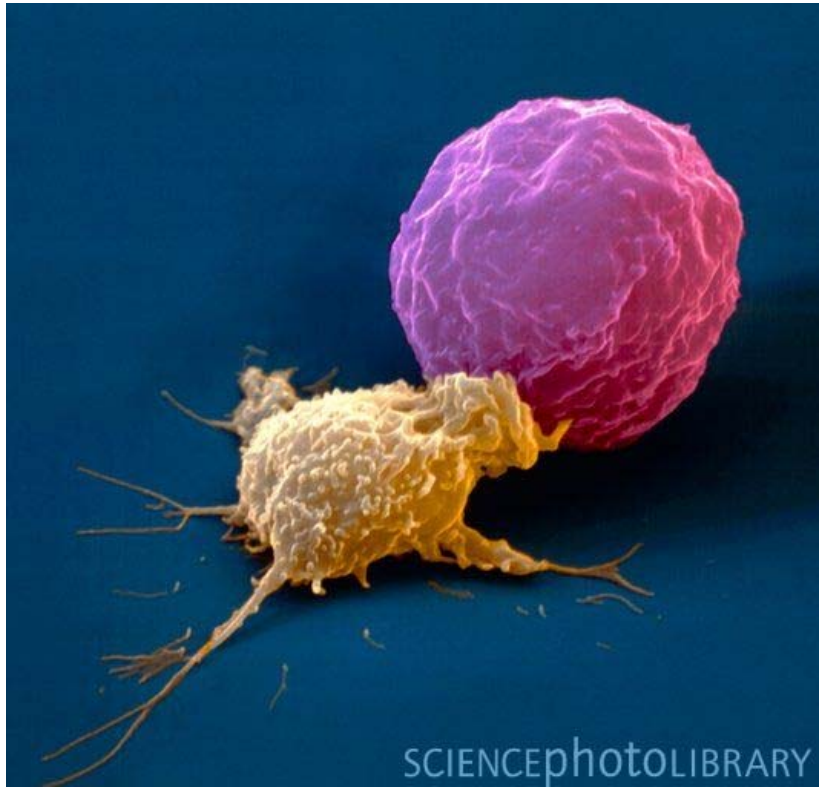

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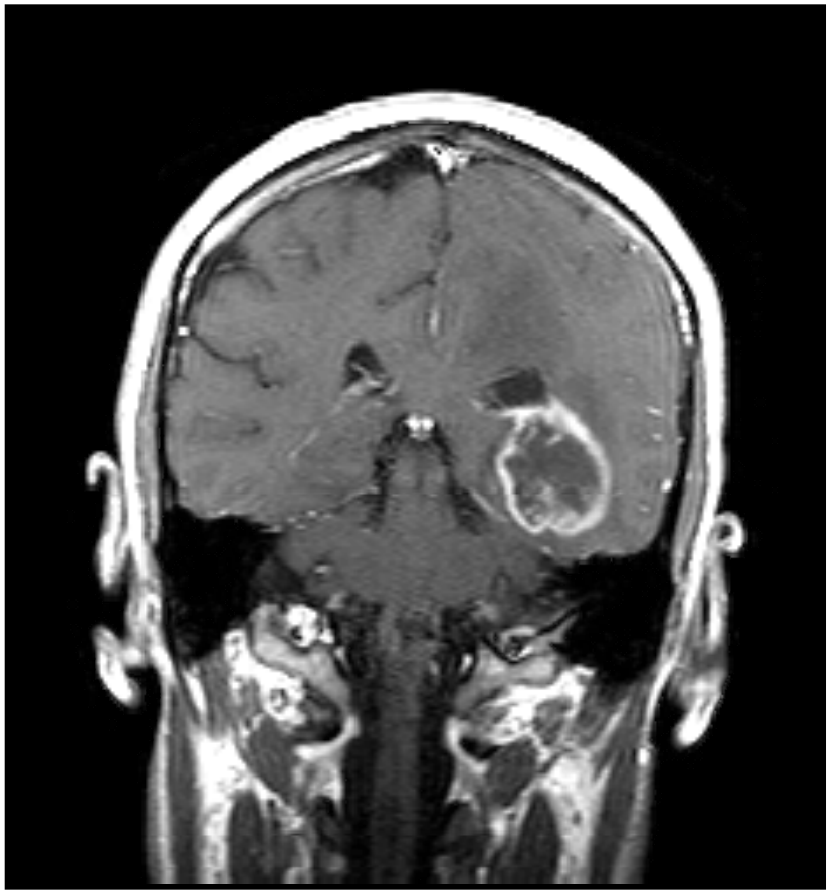
HEALTH SYSTEM

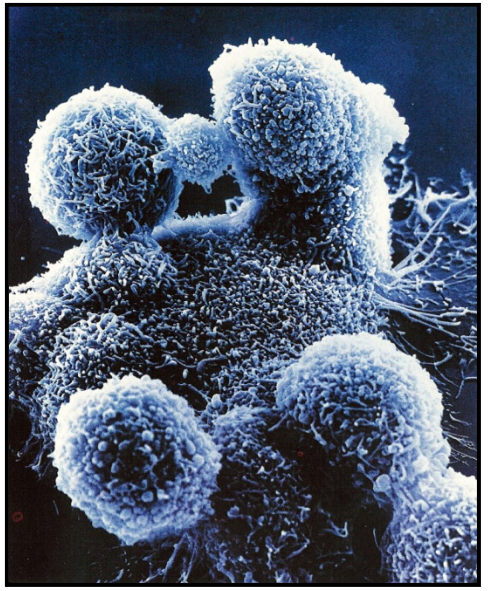
Glioblastoma Multiforme (GBM)

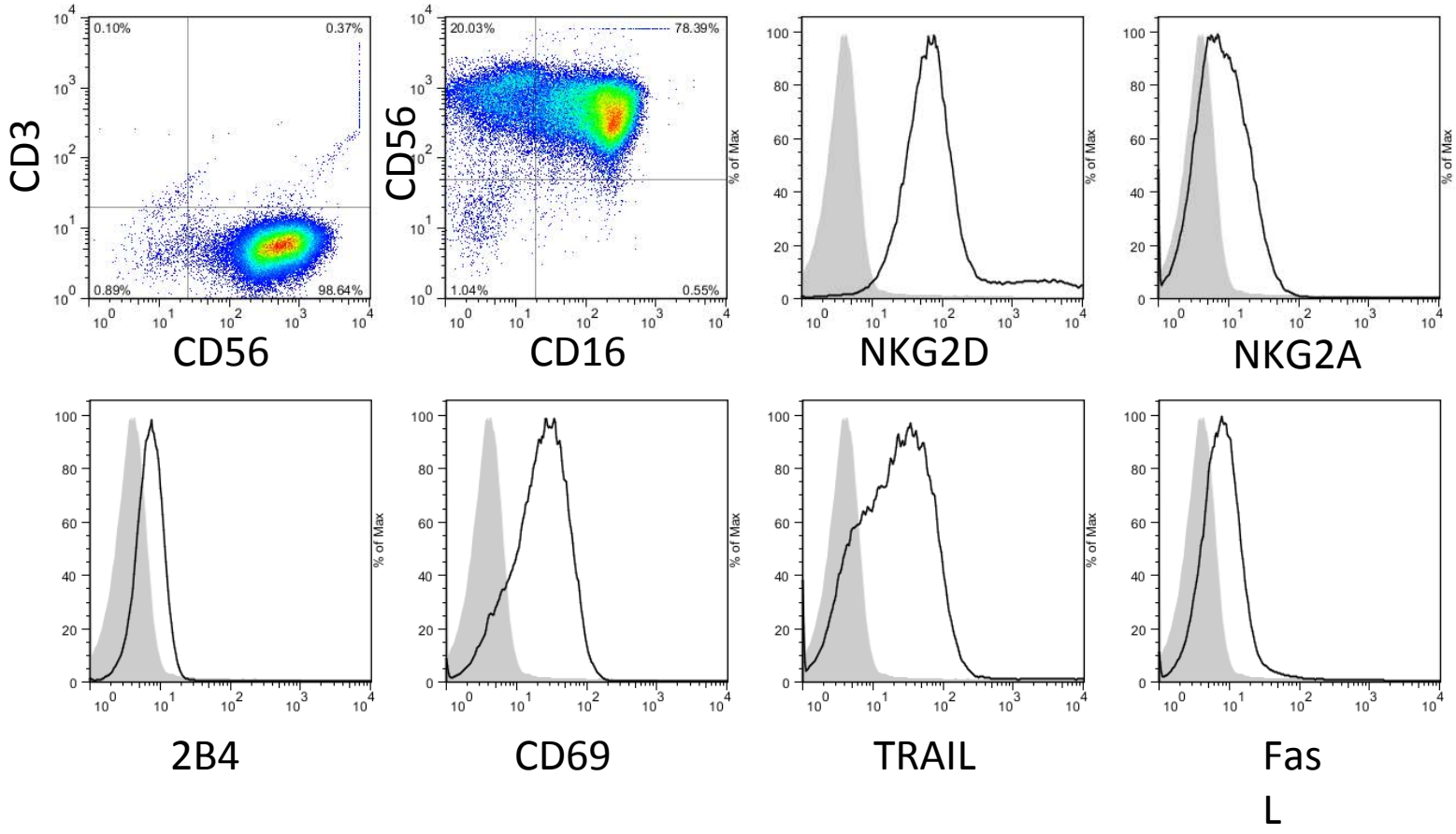
- In US 2.96 *new* cases per 100,000 people per year.
- Most frequent malignant brain tumor
- Multicentric in about 5%
- Carries an extremely poor prognosis and is rapidly fatal.
- Recurrence extremely common.
- Two varieties: primary GBM (~95%) and secondary GBM (~5%).



Natural Killer (NK) Cells

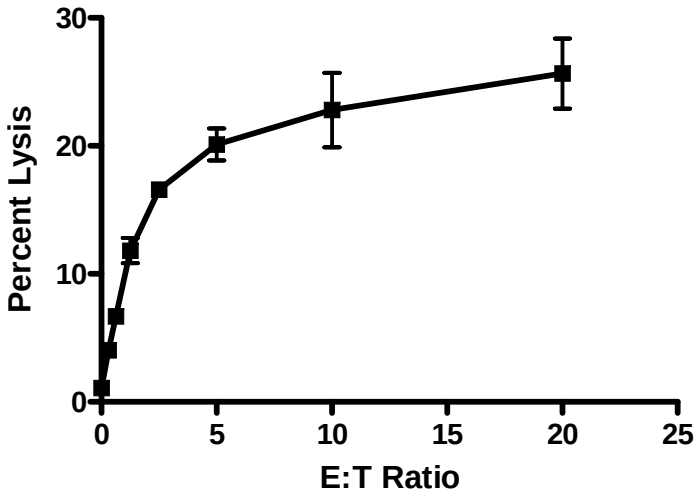
- Innate immune effector cells
- Kill virally-infected & tumor cells
  - perforin/granzyme-dependent pathway
  - Death Receptors (FasL, TRAIL)
- Possess various unique activation and inhibitory receptors
- Activated and expand with cytokines (IL2 and IL15)
- Local Administration in GBM may result in significant anti-tumor effects



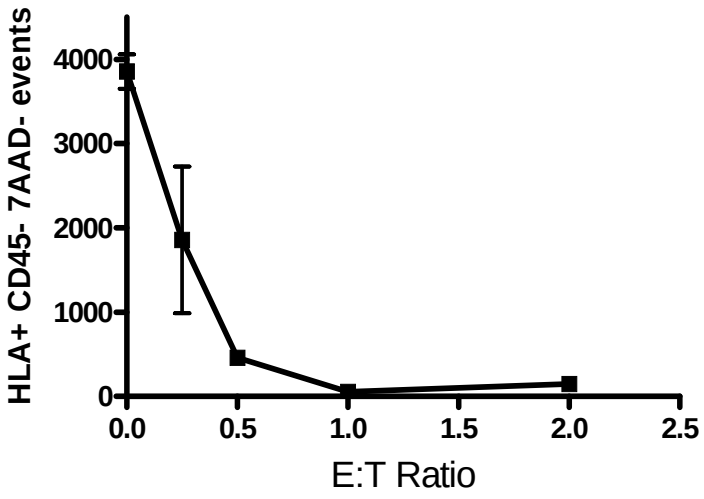


- NK cells cultured with feeder EBV-transformed lymphoma cell line and IL-2 for ~14 days.
- Roughly 1000-fold expansion of NK cells observed; NK cells display an activated phenotype by day 14.

Activated NK Cytotoxicity Toward GBM Cell Line U87MG

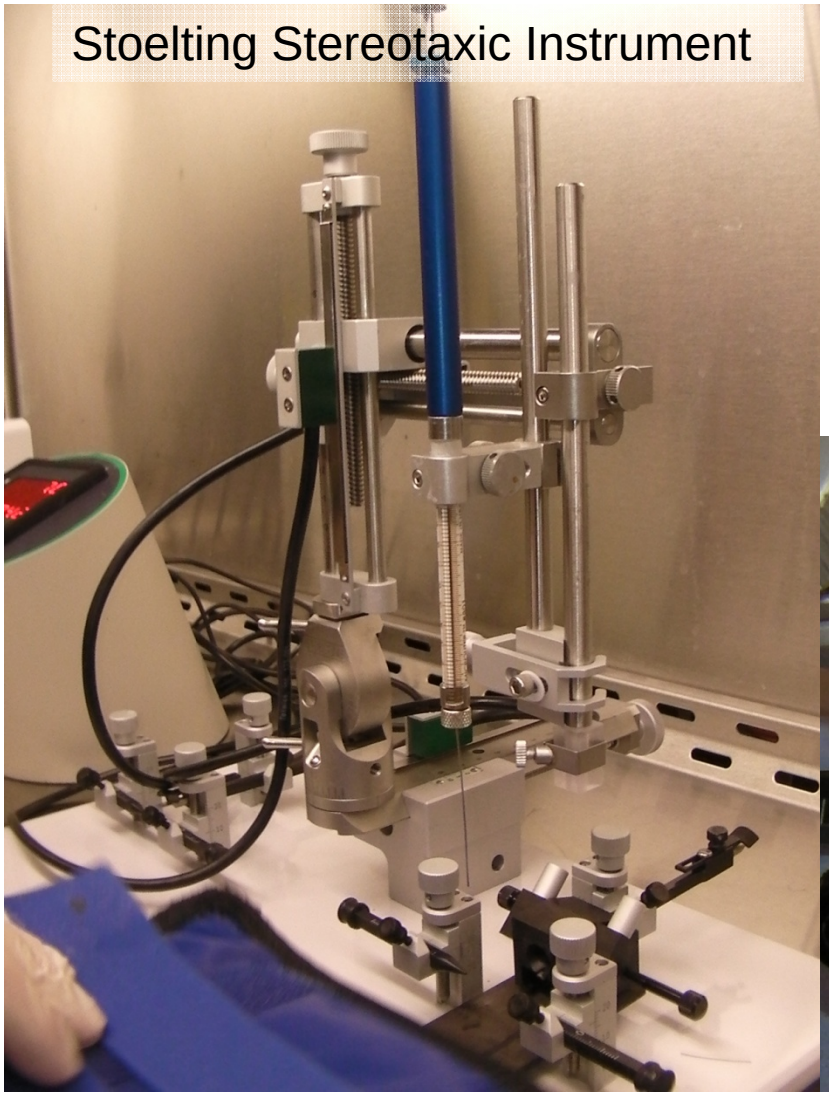


Activated NK cell killing against U87MG in a 4 hour ^{51} Chromium-release assay



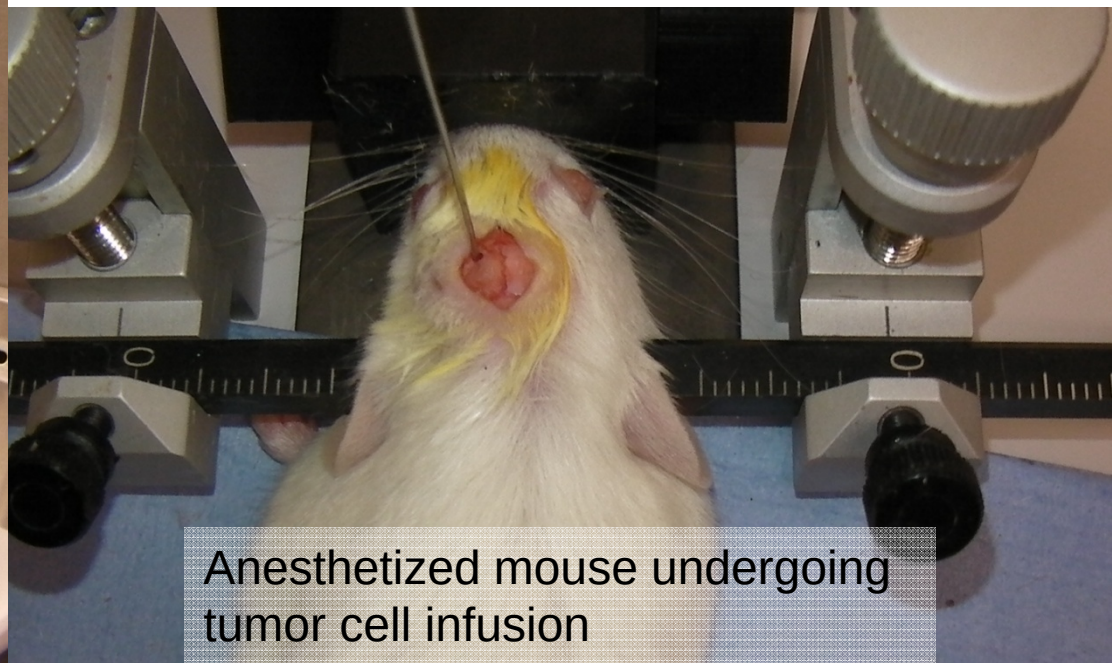
Activated NK cell killing against U87MG in a 12 hour cytotoxicity assay

Stereotactic Brain Engraftment



Stoelting Stereotaxic Instrument

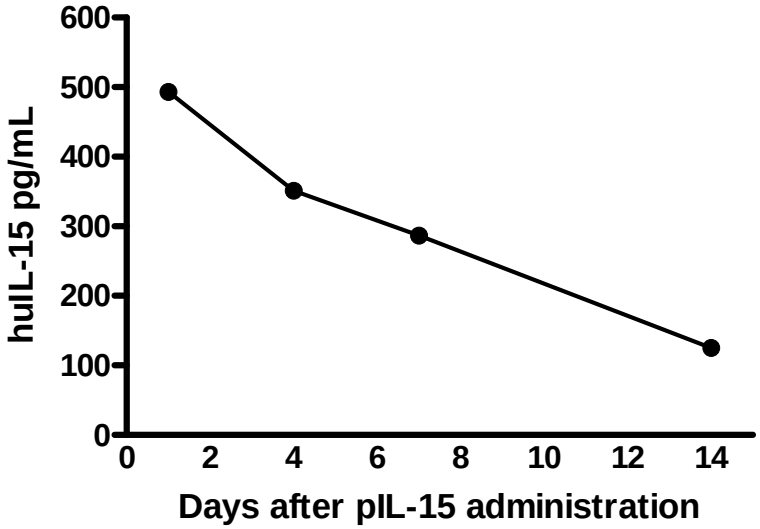
- Immobilized, anesthetized NOD-scid-IL2Rgamma KO (NSG) mice receive 5×10^4 U87-luc tumor cells injected in 2 μ L over 5 minutes.
- Cells injected 3.5mm beneath skull into brain parenchyma



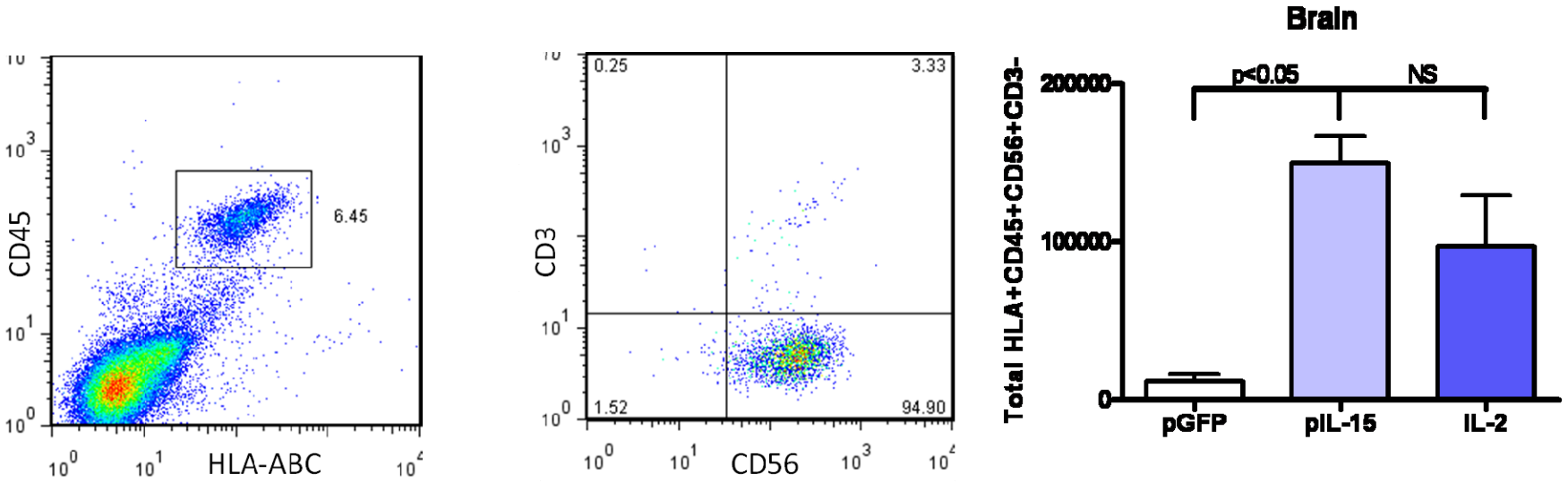
Anesthetized mouse undergoing tumor cell infusion

Hydrodynamic Gene Delivery

- Rapid injection of high volumes of plasmid DNA
- Force permeabilizes capillary endothelium and generate "pores" in membranes of the surrounding parenchyma cells in liver
- High amounts of IL15 produced for several days



Engraftment of Human Natural Killer Cells in Brains of NSG Mice



NSG mice were given 10ug hydrodynamic human IL-15 plasmid the day before NK cell infusion or 1000IU IL-2 on the day of, and two days after 10^6 NK cells injected intracranially. Mice were sacrificed 4 days after NK cell infusion and assessed for human cell engraftment.

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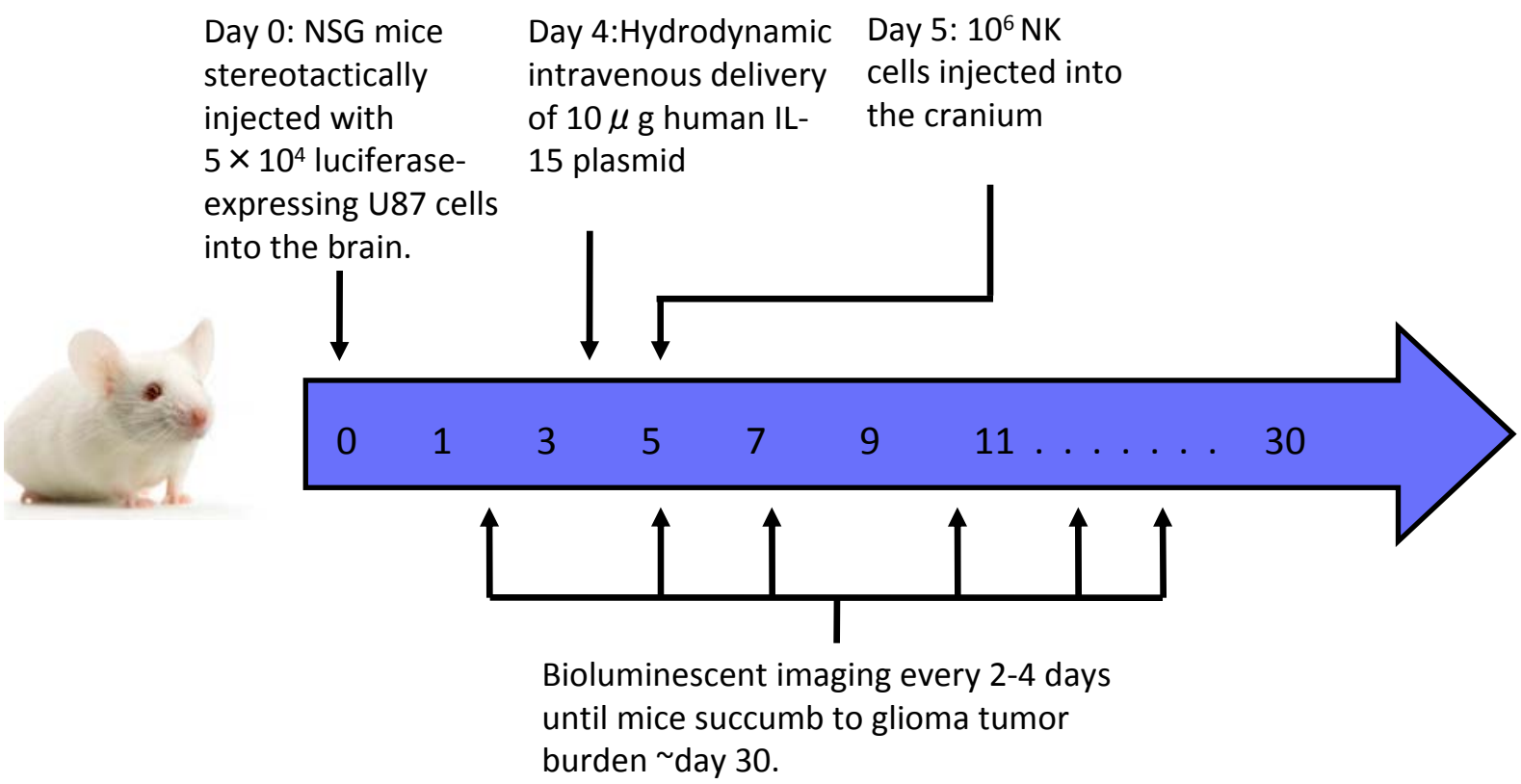
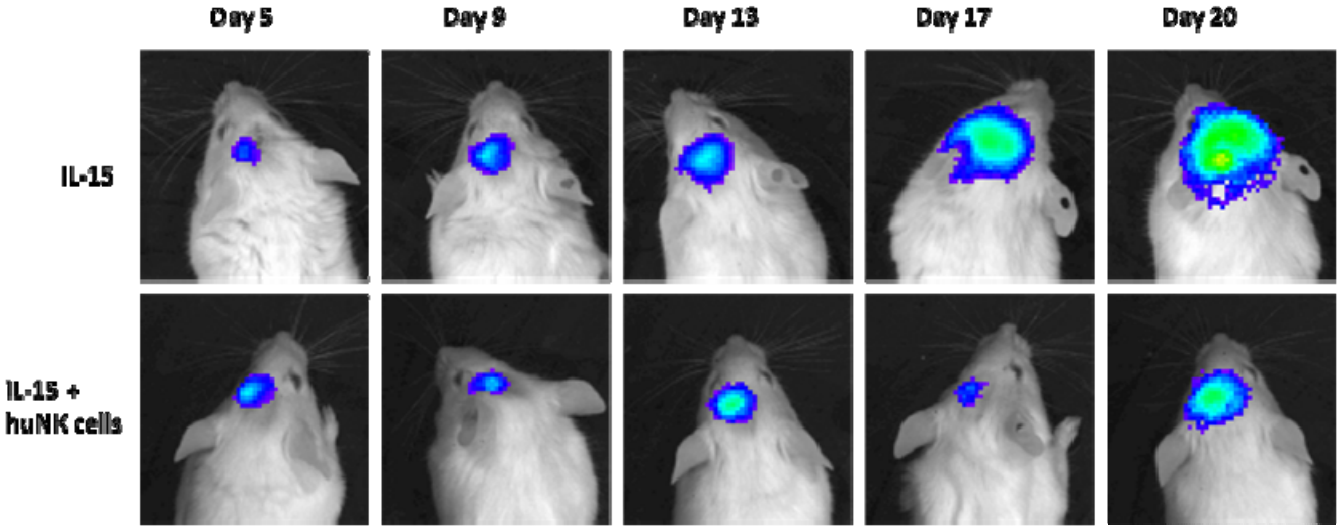
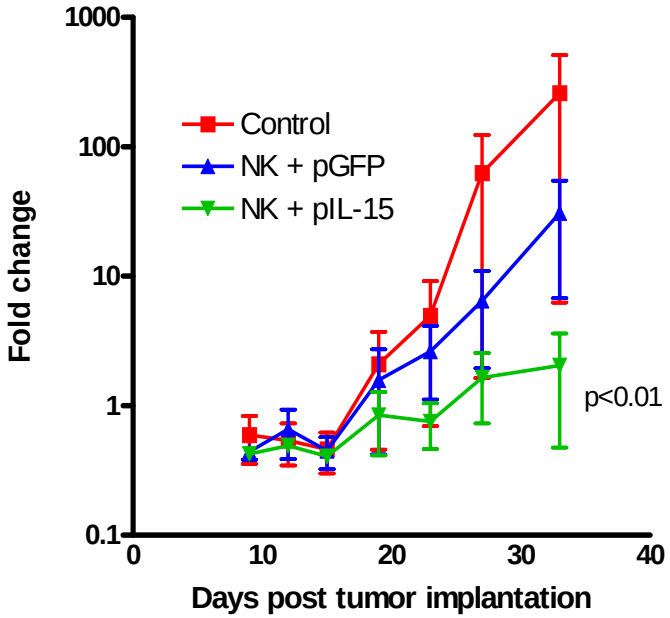
Schema for Orthotopic Tumor Model



Progression of GBM Growth After NK cell Immunotherapy in vivo





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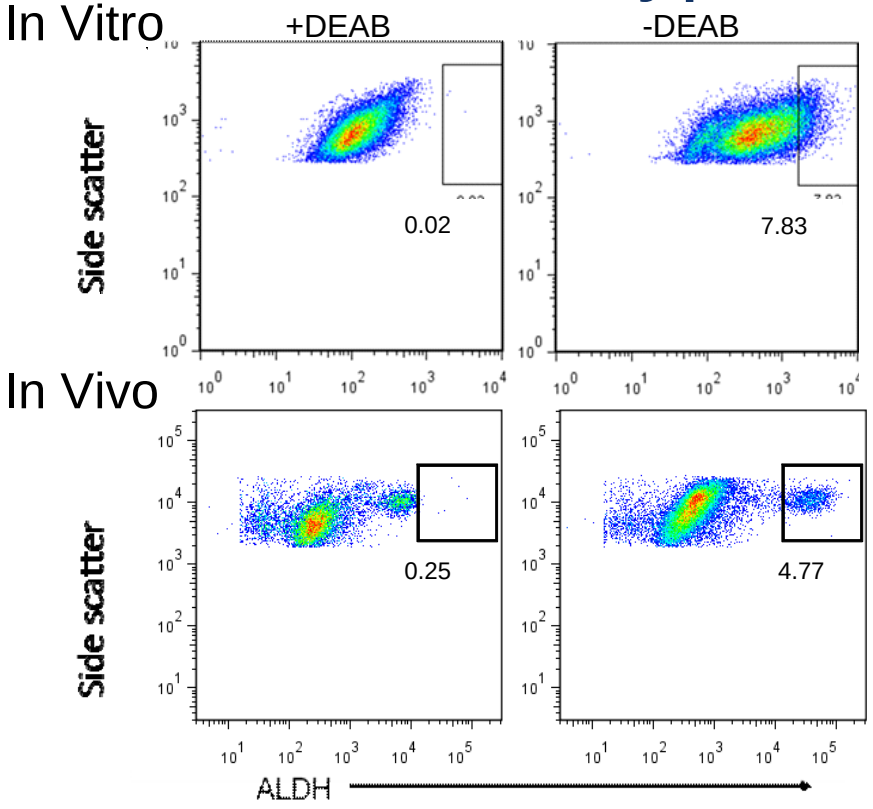
Glioblastoma Cell Lines Display Subpopulations of cells with Cancer Stem Cell Phenotype in vitro and in vivo



- Left: U87 glioblastoma cells display distinct populations which express high levels of aldehyde dehydrogenase (ALDH).

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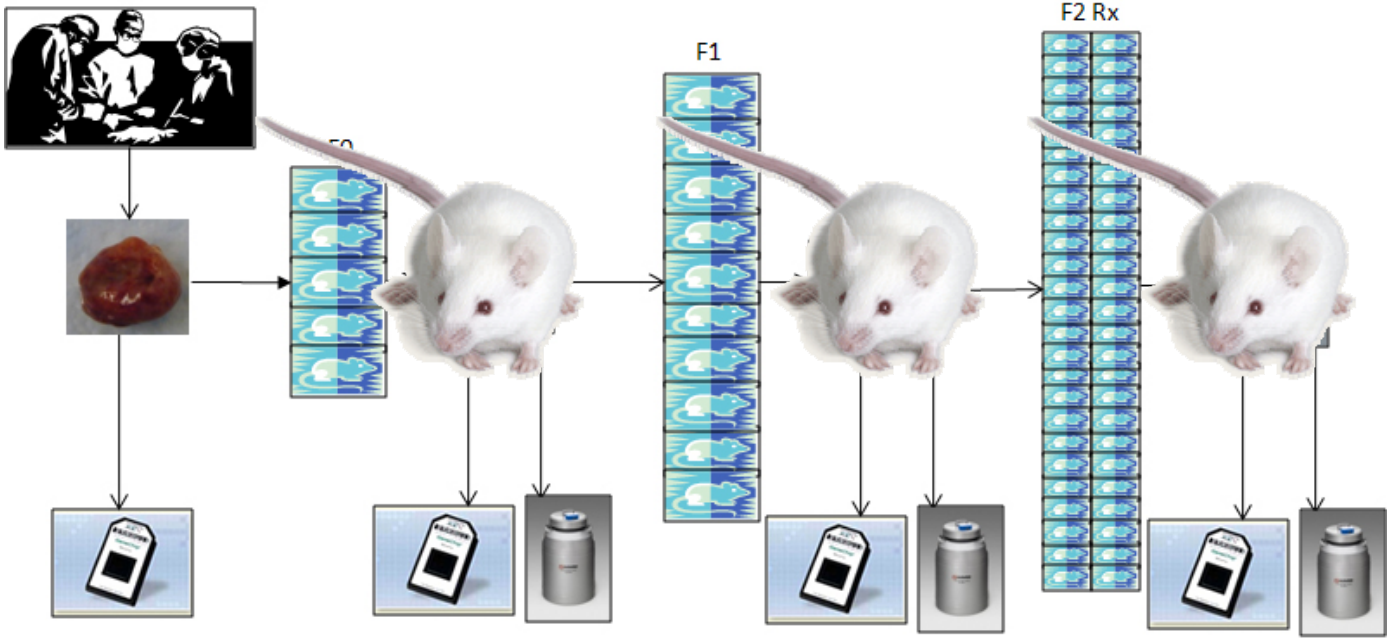
JAX-UCD Cancer Consortium PDX (Patient-Derived Xenograft) Program

The Jackson Laboratory

Leading the search for tomorrow's cures



JAX-UCD Cancer Consortium PDX Models

Tumor ID	Date Implanted	Tissue	Diagnosis	Tumor ID	Date Implanted	Tissue	Diagnosis
BL-0262f	3/29/2010	Bladder	Urothelial CA	KD-0459f	10/9/2009	Kidney	Medullary Carcinoma
BL-0269f	4/12/2010	Bladder	Urothelial CA Invasive	KD-0459PE	4/9/2010	Kidney	Pleural effusion
BL-0284f	5/19/2010	Bladder TURBT	Carcinoma	KD-0531f	8/20/2010	Kidney	Kidney TCC
BL-0293f	7/14/2010	Bladder	Pending	LG-0476f	1/5/2010	Lung	Squamous Cell CA
BL-0304f	8/27/2010	Bladder TURBT	Pending	LG-0481f	1/21/2010	Lung	Adenocarcinoma
BL-0307f	9/17/2010	Bladder	Urothelial CA	LG-0487f	2/11/2010	Lung	Adenocarcinoma
BL-0308f	9/29/2010	Bladder	Urothelial CA	LG-0494f	3/15/2010	Lung	Adenocarcinoma
BN-0261f	3/1/2010	Brain	Oligodendroglioma	LG-0502f	4/1/2010	Lung	Adenocarcinoma
BN-SN194f	7/21/2010	Brain	GBM	LG-0505f	4/8/2010	Lung	Metastatic Sarcoma
BN-SN195f	8/11/2010	Brain	GBM	LG-0506f	4/8/2010	Lung	Metastatic Melanoma
BN-SN199f	8/18/2010	Brain	Asytrocytoma	LG-0517f	4/19/2010	Lung	Neuroendocrine CA
BN-SN207f	10/14/2010	Brain	GBM	LG-0520f	4/27/2010	Lung	Squamous Cell CA
BN-SN210f	10/21/2010	Brain	Astrocytoma	LG-0521f	5/3/2010	Lung	Adenocarcinoma
BN-SN211f	10/27/2010	Brain	GBM	LG-0525f	5/10/2010	Lung	Adenocarcinoma
BN-SN213f	11/3/2010	Brain	GBM	LG-0531f	6/3/2010	Mediastinum	Mucoepidermoid
BN-SN215f	11/11/2010	Brain	GBM	LG-0536f	6/23/2010	Lung	Squamous Cell Carcinoma
BN-SN217f	12/20/2010	Brain	GBM	LG-0464f	12/10/2009	Lung	Adenocarcinoma
BN-SN218f	12/22/2010	Brain	GBM	LG-0541f	7/1/2010	Lung	Adenocarcinoma
BR-0555f	2/22/2010	Breast ($ER^+/PR^+/Her2^-$)	Carcinoma	LG-0542f	7/1/2010	Lung	Adenocarcinoma
BR-0558f	3/30/2010	Breast ($ER^-/PR^-/Her2^-$)	Carcinoma	LG-0551f	7/12/2010	Lung	Squamous Cell Carcinoma
BR-0564f	4/7/2010	Ductal ($ER^+/PR^+/Her2^-$)	Carcinoma	LG-0552f	8/9/2010	Lung	Stage IV NSCLC
BR-0579f	4/13/2010	Breast ($ER^-/PR^-/Her2^+$)	Carcinoma	LG-0556f	8/2/2010	Lung	Adenocarcinoma
BR-0588f	6/22/2010	Breast ($ER^-/PR^-/Her2^-$)	IDC	LG-0559f	8/30/2010	Lung	Squamous Cell CA
BR-0594f	6/25/2010	Breast ($ER^+/PR^+/Her2^-$)	Carcinoma	LG-0567f	9/20/2010	Lung	Squamous Cell CA
BR-0605f	8/24/2010	Breast ($ER^+/PR^-/Her2^-$)	IDC	LG-0570f	11/1/2010	Lung	Pending
BR-0620f	9/21/2010	Breast ($ER^-/PR^-/Her2^-$)	Carcinoma	LG-0571f	9/28/2010	Lung	Adenocarcinoma
CD-SF8517f	10/13/2010	Bone	Chordoma	LG-0574f	9/30/2010	Lung	Pending
CN-0319f	11/3/2009	Colon	Villous Adenoma	LG-0577f	10/28/2010	Lung	Pending
CN-0330f	1/8/2010	Colon	Adenocarcinoma	LG-0578f	10/5/2010	Lung	Pending
CN-0375f	11/3/2010	Liver	Colon CA Metastatic	LG-0580f	10/7/2010	Lung	Pending

Green = Developed to P1 (Histology, Microarray and CNV data collected)
Gray = Developed to P0 log phase growth

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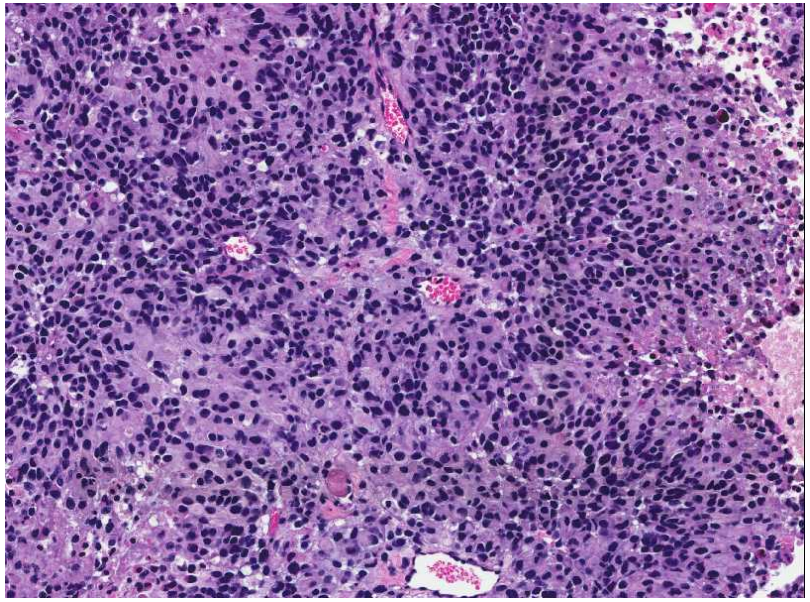
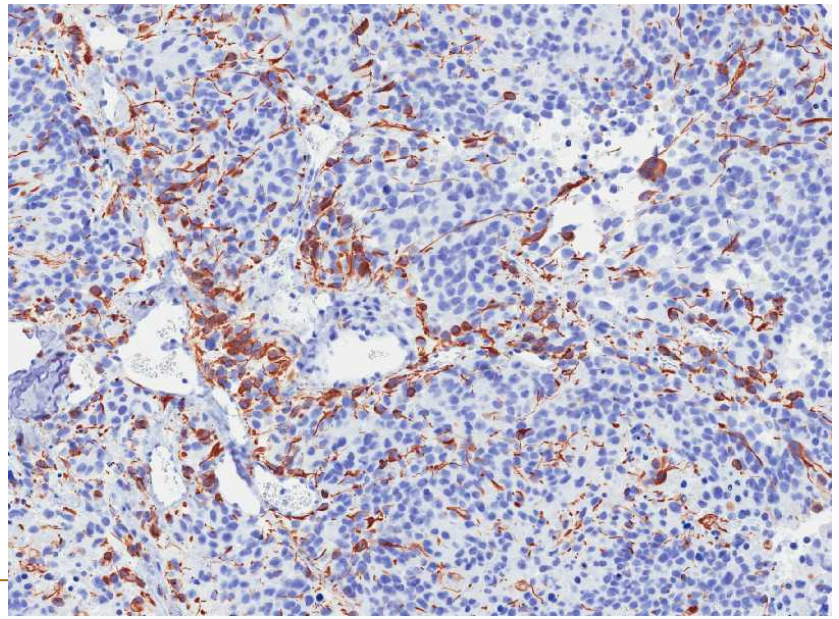
Example of PDX Tissue Specimen: BN-SN194F001P0 Glioblastoma



“Classic” Glioblastoma with angiogenesis, pseudopalisade pattern and necrosis

GFAP IHC

Characteristically, there is less expression in Glioblastoma compared to low or high grade Astrocytoma



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Conclusions

- Human NK cells engraft in the brains of immunodeficient NSG mice and this engraftment is augmented with IL2 or IL15.
- Human NK cells exert significant anti-tumor effects towards orthotopically implanted human GBM with no overt toxicity.
- CSC phenotype cells from GBM cell lines as well as primary GBM engraft in NSG mice.
- This xenogeneic model may allow assessment of NK cell efficacy in cancer.