



AMERICAN SOCIETY OF GENE & CELL THERAPY  
13<sup>TH</sup> ANNUAL MEETING | Washington, DC USA May 19-22, 2010

***Dao Pan, Ph.D.***

***Division of Experimental Hematology  
Cincinnati Children's Hospital Medical Center***

**Cell and Gene Therapy for CNS in  
Lysosomal Storage Diseases**



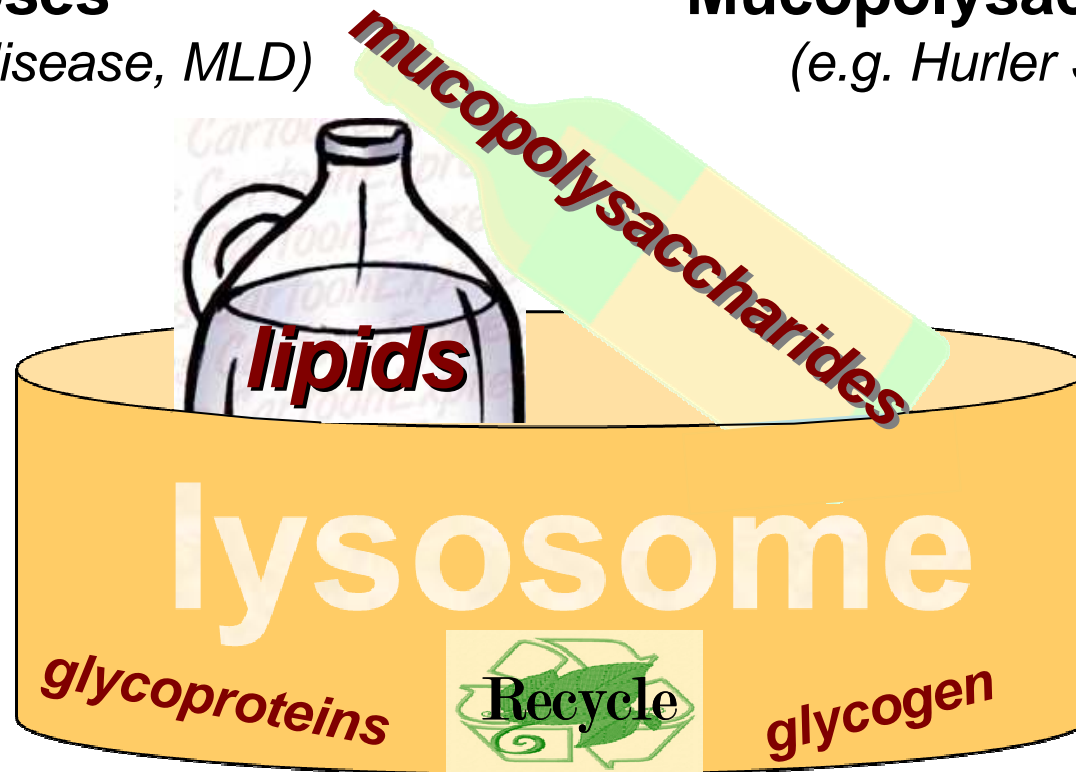
# Lysosomal Storage Diseases (LSD)

## Lipidoses

(e.g., Gaucher disease, MLD)

## Mucopolysaccharidoses

(e.g. Hurler Syndrome)



## Glycoproteinosis

(e.g., Mannosidosis)

## Pompe disease

# LSD Overview

- **Currently ~46 different genetic disorders, mostly autosomal recessive (except Hunter and Fabry which are X-linked)**
- **Occurring incidence of 1 in ~7,000**
- **Phenotype-genotype heterogeneity**
- **Shorten life-span with onset typically in infancy to early childhood**
- **Multi-organ symptoms:**
  - ✓ **hepatosplenomegaly,**
  - ✓ **Pulmonary and cardiac problems**
  - ✓ **Musculoskeletal disease**
  - ✓ **CNS dysfunctions**

# CNS Manifestations in LSD

- **LSD predominately involve CNS**
  - **Up to 70% of all LSD have CNS involvements**
  - **Symptoms:**
    - ✓ **Developmental regression**
    - ✓ **Seizures**
    - ✓ **Mental retardation**
- **Extra Challenges for CNS treatment**
  - **The progression of CNS manifestations --- “irreversible” deficits**
  - **Impermeability of BBB**

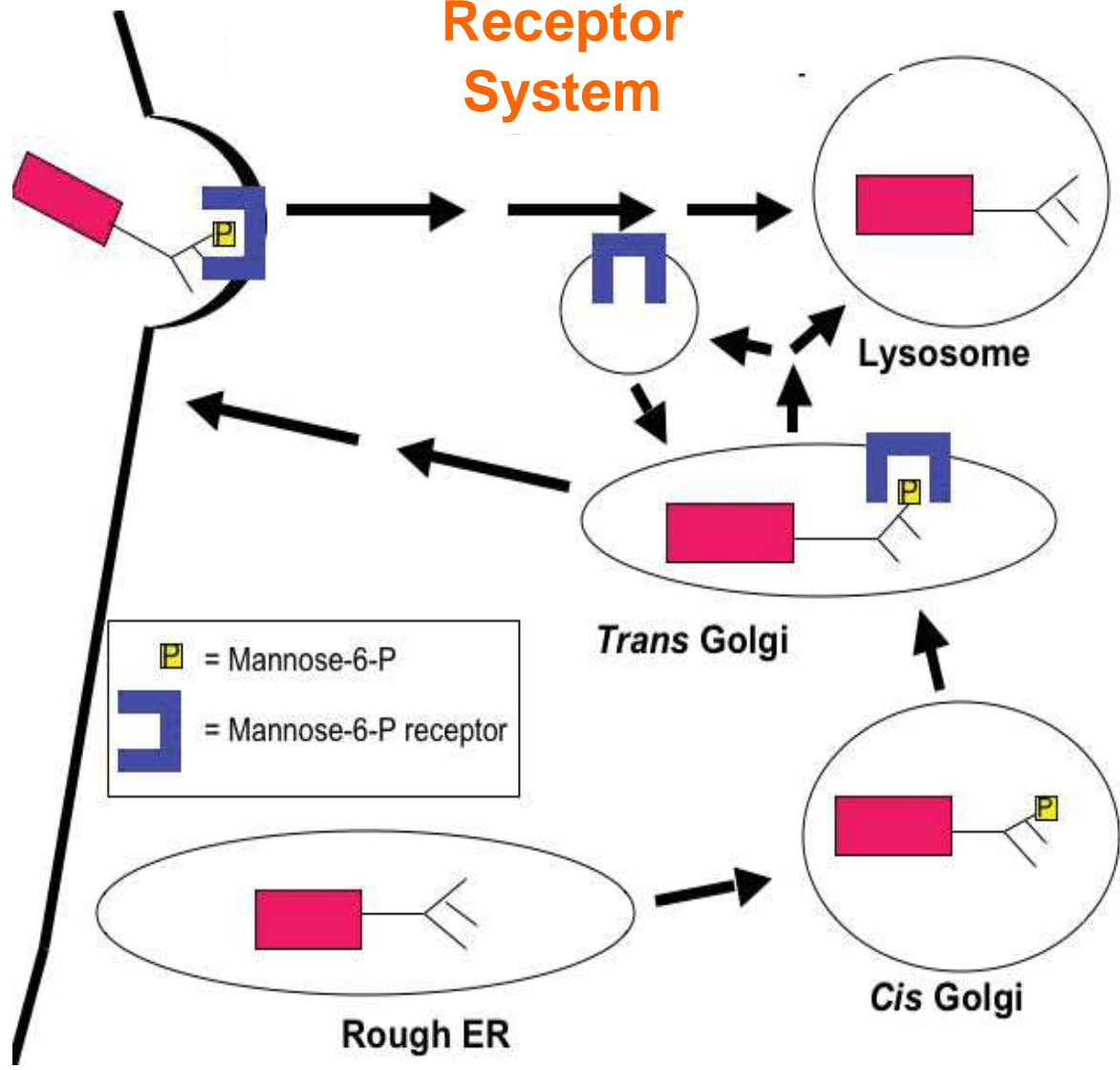
Intracellular & Intercellular Lysosomal Enzyme Transfer



**Cross-Correction**

\*Mannose Receptor

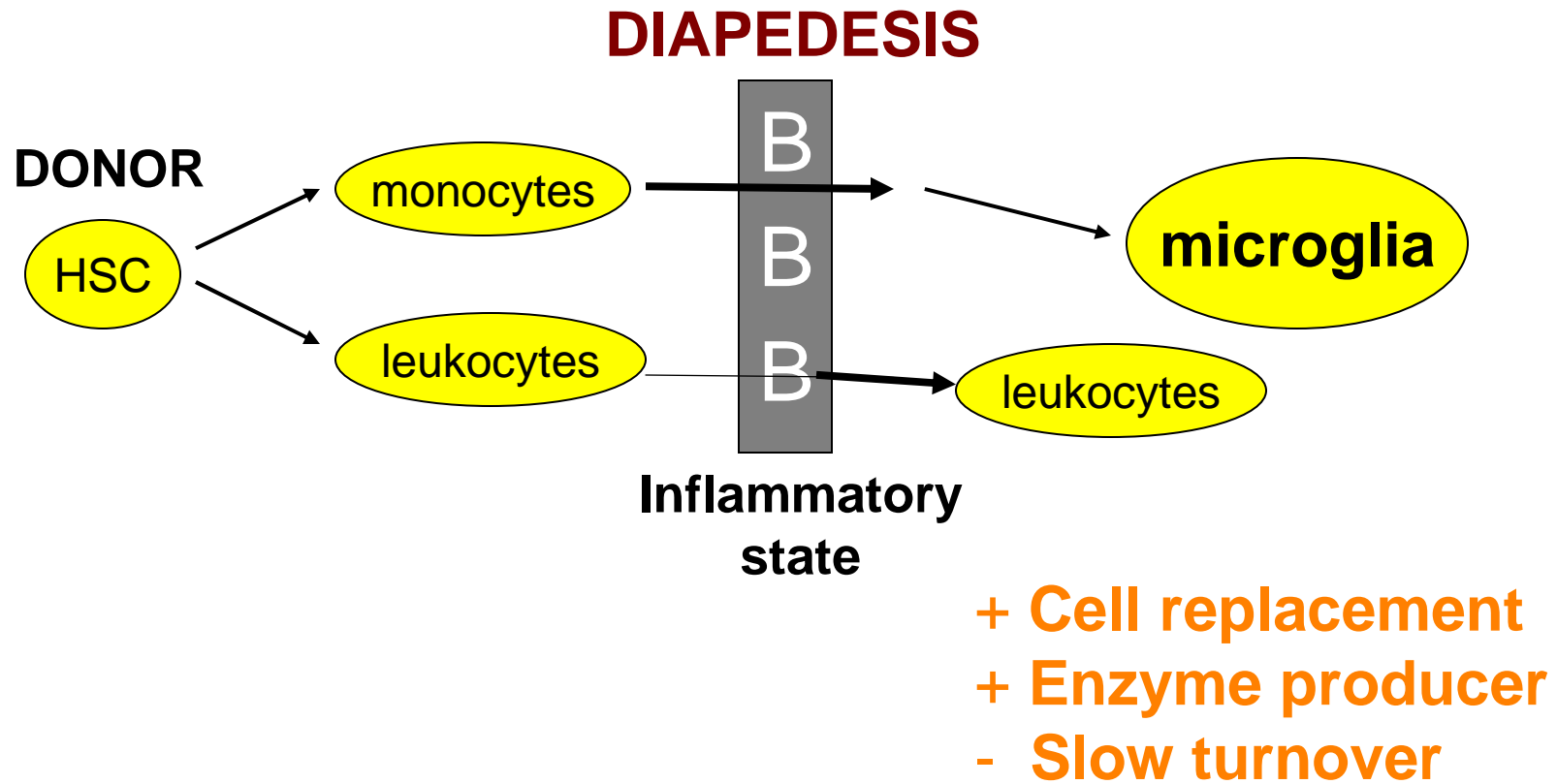
**Mannose-6-Phosphate Receptor System**



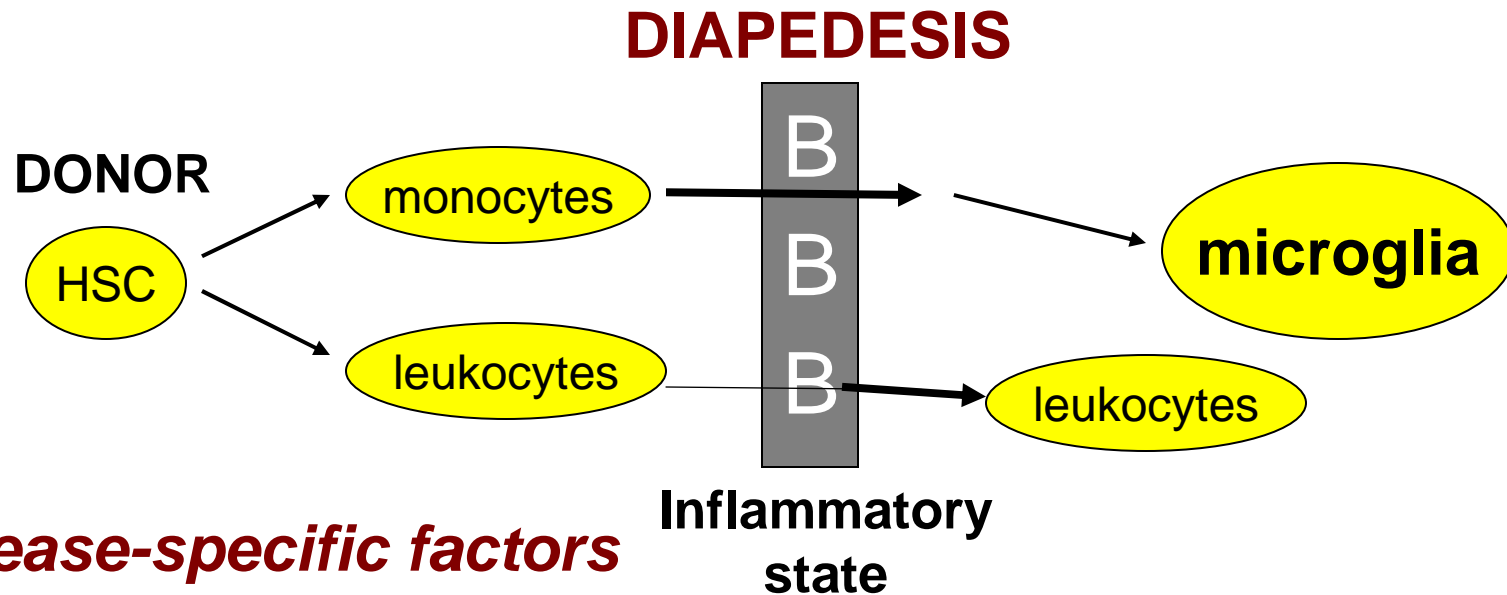
# Current Treatments

- **Enzyme replacement therapy**
  - + **Clinical improvement in non-neuronopathic LSD**
  - Require life-long repeated treatment (costly, immune concern)
  - Unlikely to treat CNS abnormalities in LSD
- **Allogeneic HSC transplantation**
  - + **Life-long therapeutic effects in somatic organs**
  - + **increase life-span**
  - Limited by the shortage of matched donor
  - Significant morbidity and a high risk of modality
  - ± Various CNS outcome

# CNS and HSC Transplantation in LSD



# CNS and HSC Transplantation in LSD



## *Disease-specific factors*

- Microglia involvement?
- Enzyme release rate?
- Demand for enzyme?

- + Cell replacement
- + Enzyme producer
- Slow turnover

- + Stabilization/prevention of CNS disease: e.g., MPS I
- No effect: e.g., MPS II, or neuropathic Gaucher

# New Strategies

- **Direct intracranial delivery**
  - **Stem cells: with or without gene transfer**
  - **Viral vectors**
- **Peripheral delivery via circulation**
  - **BBB-targeted gene transfer by IV injection**
  - **Hematopoietic stem cell gene transfer**
    - ✓ **ex vivo**
    - ✓ **in situ gene transfer**

# Direct Intracranial Delivery

- **Advantages**
  - + **Refined, on-target administration**
  - + **Controlled distribution**
- **Disadvantages**
  - **Invasive surgery**
  - **limited-distribution**

# **Direct Intracranial Delivery: Stem Cells**

# Sources of Stem Cells

## Stem Cell

- Self-renewal
- Multi-potential
- Highly proliferative

## Advantage

- Long-term benefits
- Repair damage brain by cell replacement
- Propagate- and transduce-able

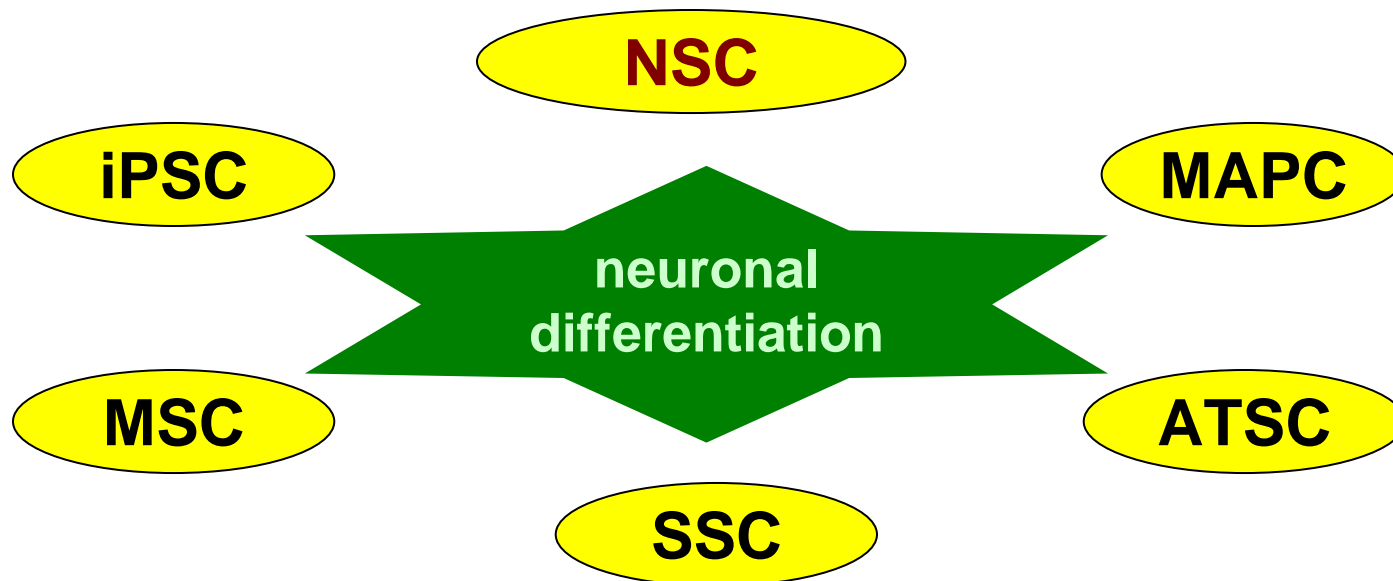
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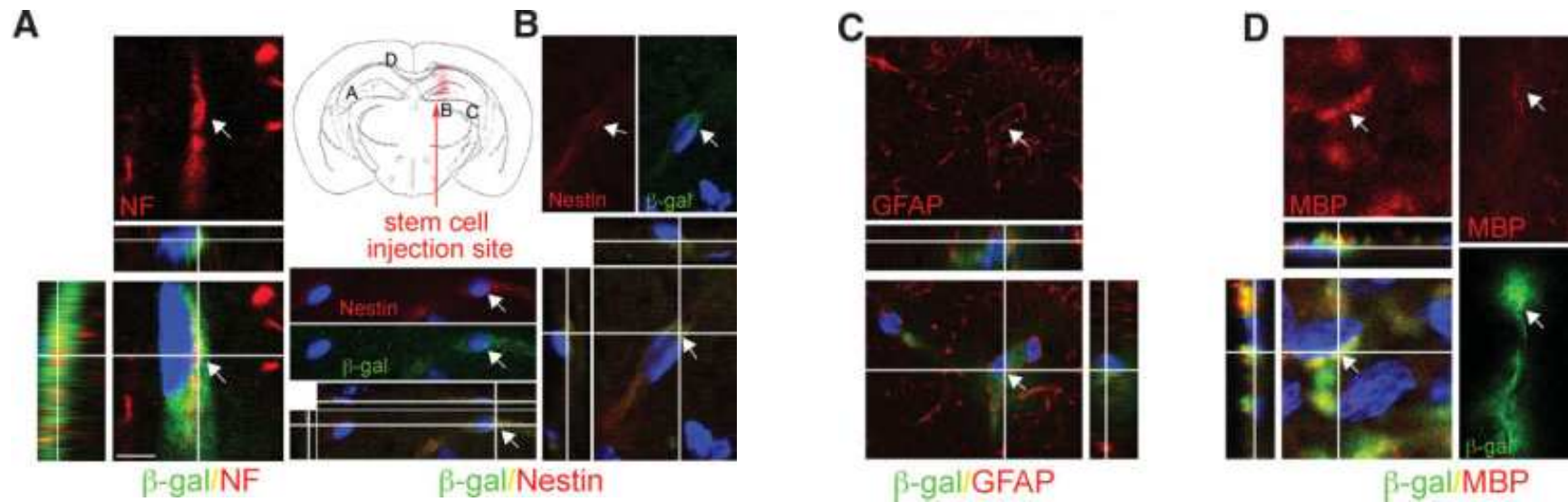
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## Advantage

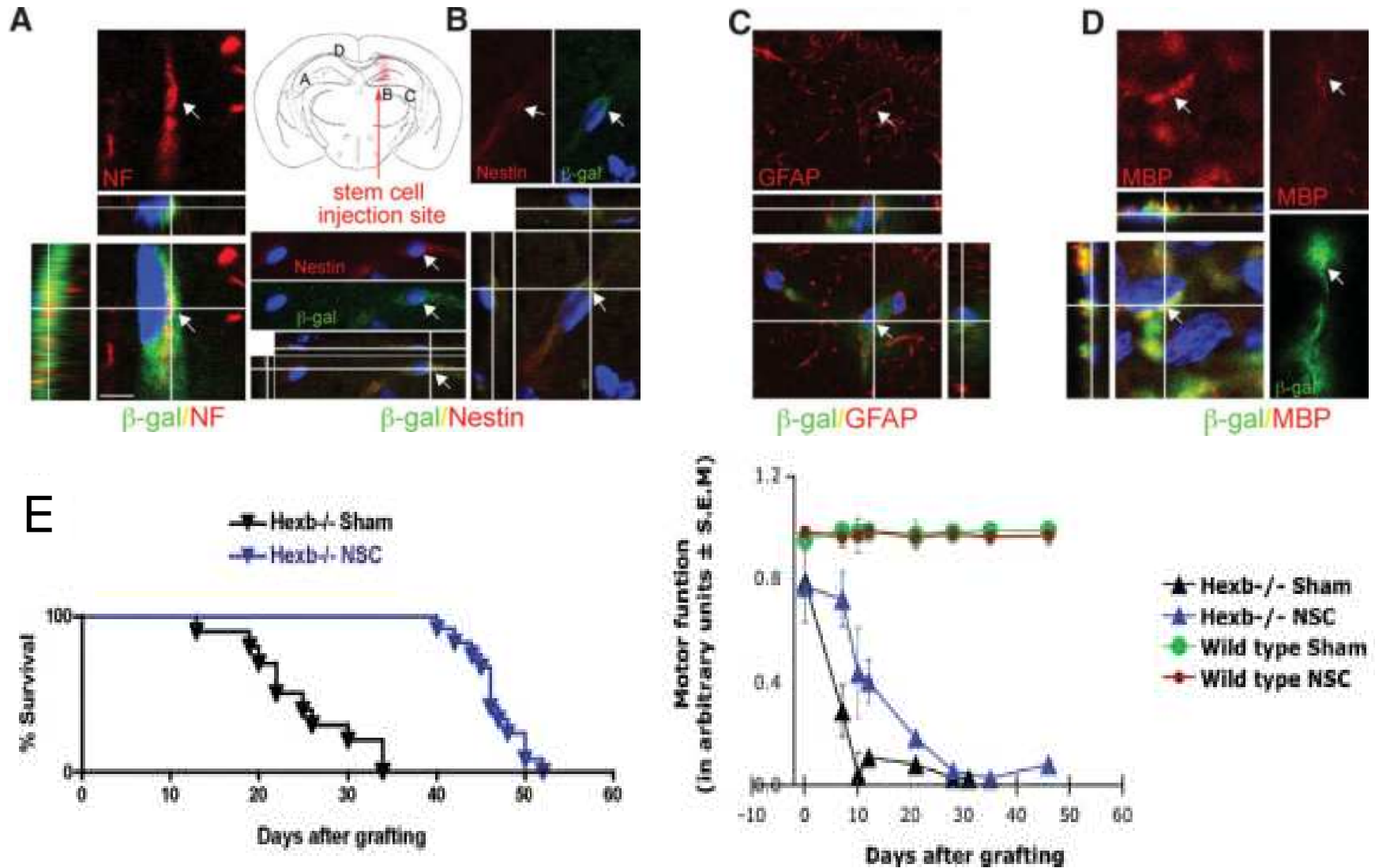
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# Intracranial Injection of NSC into Sandhoff Mice Resulted in Cell Migration and Differentiation with Therapeutic Benefit



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Jeyakumar et al., Stem Cell, 2009

# NSC/NPS for CNS in LSD

**mNPS in MPS VII mice**  
*Snyder, Nature 1995*



- ✓ Intraventricular injection in newborn
- ✓ Over-expression by RV
- ✓ Storage reduction in neurons and glial cells

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- ✓ Intracranial injection
- ✓ Delay onset and/or prolong life



## hNSC in Sandroff mice

*Newborn, Lee, Nat Med 2007*

*Adult, Jeyakumar, Stem Cells, 2009*

## hCNS-SCns in INCL mice

*Tamaki, Cell Stem Cells, 2009*

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## Phase I Clinical Trial in Batten

*NCT 00337636*

# **Direct Intracranial Delivery: Viral Vectors (AAV)**

# AAV Injection for CNS in LSD

- **Various rAAV serotypes have been evaluated in several animal models of LSD**
    - **AAV2, AAV5 and AAV9 can introduce robust but relatively local gene transfer**
    - **a variety of tropism among cell types**
    - **Different therapeutic response (MPS I vs. GLD)**
  - **Phase I clinical trial for Batten Disease was approved 2004 using AAV2 (*Worgall, Hum Gene Ther, 2008*).**
- ⇒ **Local delivery may be impractical to meet the demand of widespread CNS benefits in treating neuropathic LSD**

# **Peripheral Delivery: HSC Gene Therapy**

# Ex Vivo HSC Gene Therapy Using RV

- + **Overexpression leads to enzyme release and cross-correction in all tested LSD including Gaucher**
- + **Phase I clinical trial for Gaucher Disease was initiated early 90th (RV/LTR) resulting in low, transient gene-marking in non-myeloalated patients (*Dunbar, Hum Gene Ther, 1998*). --- no significant therapeutic benefits.**
- **Relatively low transduction efficiencies in successfully engrafted HSC by RV in most clinical trials.**
- ⇒ **HIV-based lentiviral vectors**
  - ✓ **Transduce non-dividing cells**
  - ✓ **Can be concentrated**

# Improve Efficiency: Lentiviral Vectors with LTR Promoters

## MLD in mice

*Biffi, J Clin Invest 2006*



- ✓ Over-expression by LV
- ✓ Reversal of CNS deficits in symptomatic MLD mice
- ✓ Microglia-mediated cross-correction in brain

- ✓ ~35% transduction efficiency
- ✓ CNS and systemic benefits superior to BMT with normal donor



## Pompe Disease in mice

*Van Til, Blood 2010*

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## Pompe Disease in mice

*Van Til, Blood 2010*

## ALD\* Clinical Trial

*Cartier, Science 2009*



- ✓ LV-mediated, GT in patient's CD34+
- ✓ ~15% transduction efficiency in HSC
- ✓ CNS benefit comparable to BMT with normal donor

# Insertional Mutagenesis

+ LSD are good candidates for stem cell-mediated therapy using integrated vectors

- ✓ Potential life-long therapeutic effects
- ✓ No need for precise transgene expression

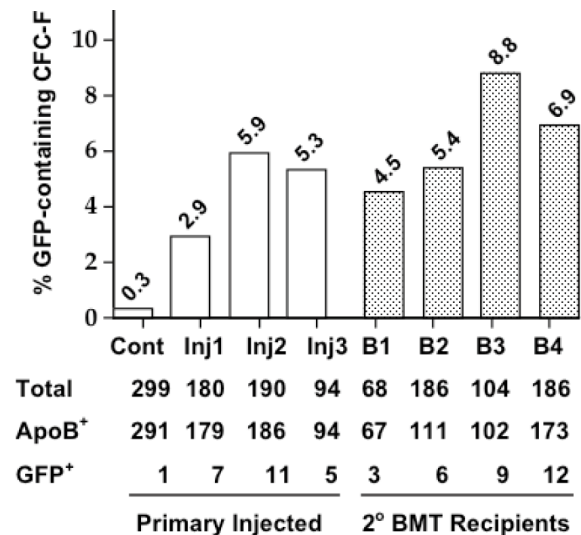
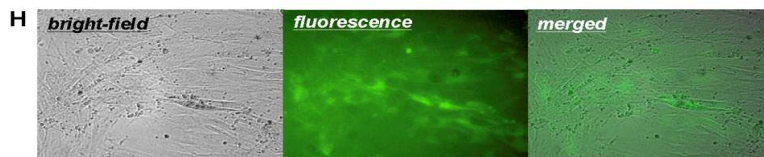
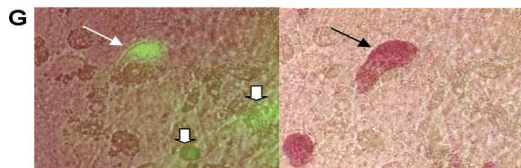
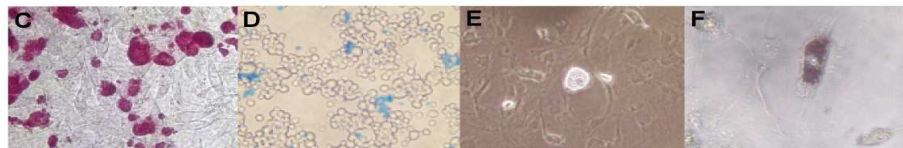
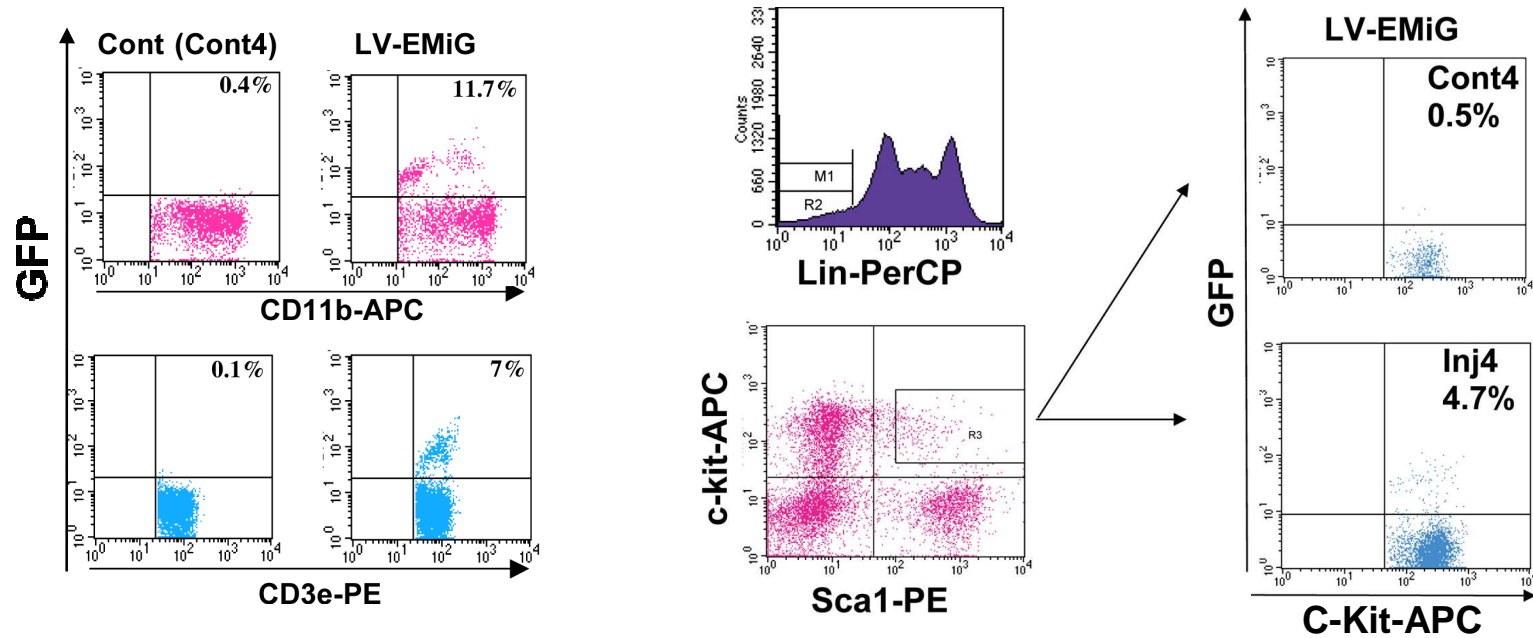
– Risk of insertional oncogenesis

- ✓ viral LTR promoters
- ✓ Integration-site bias of RV vs. LV

⇒ In situ bone marrow stem cell gene transfer

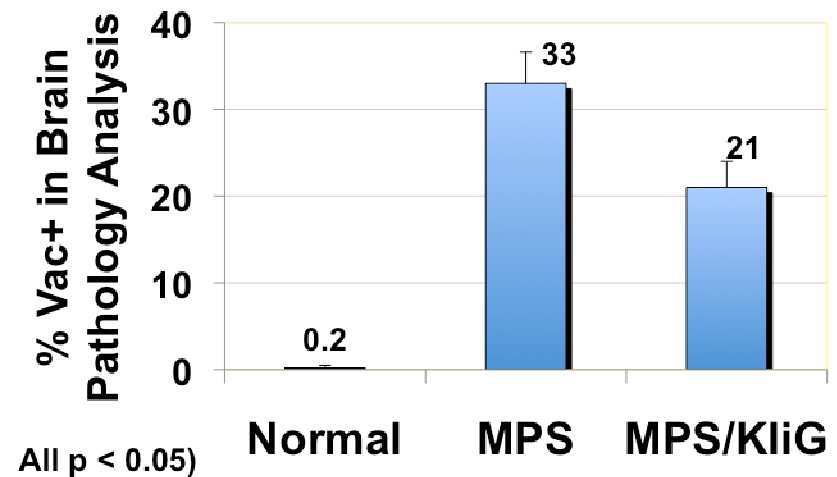
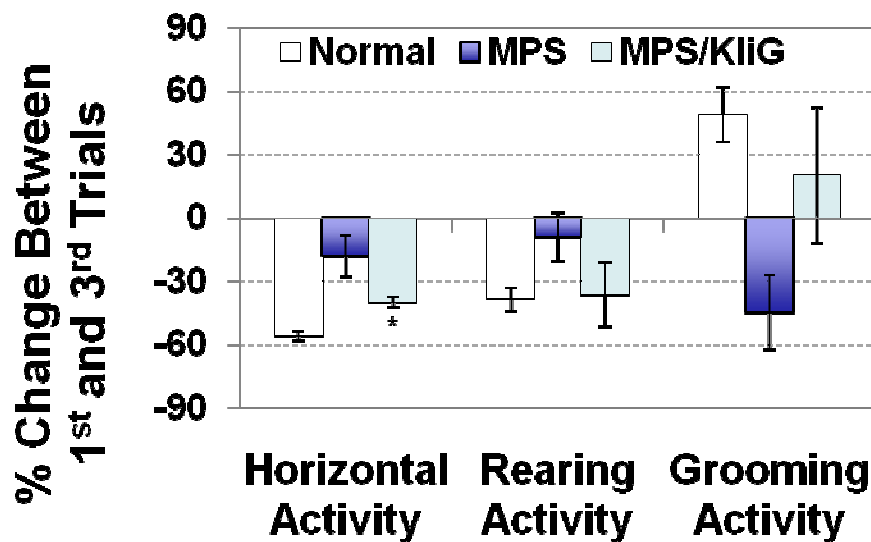
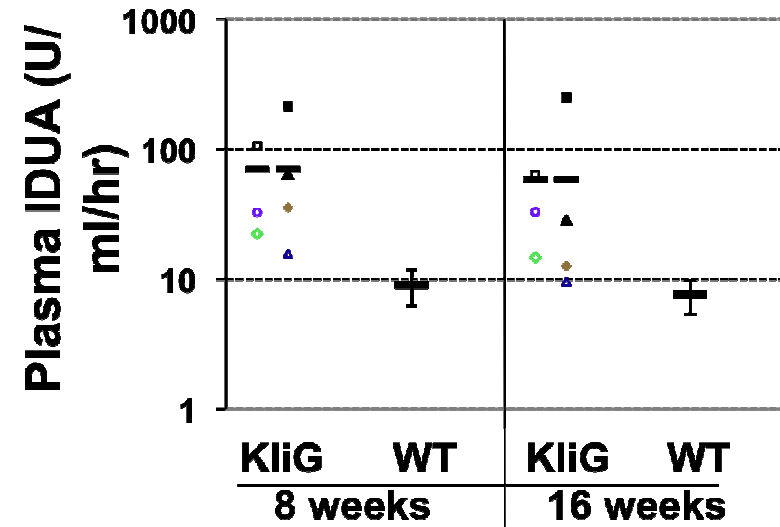
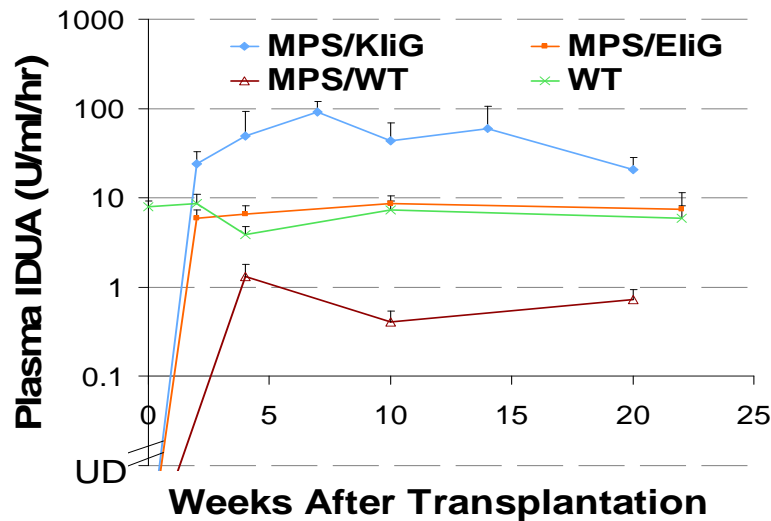
⇒ Lineage specific transgene expression

# In Situ Gene HSC Transduction by LV



Worsham et al., Mol Ther, 2006

# CNS Benefits in MPS I Mice by Lentiviral-mediated Erythroid-specific IDUA Production



Wang et al., PNAS, 2009

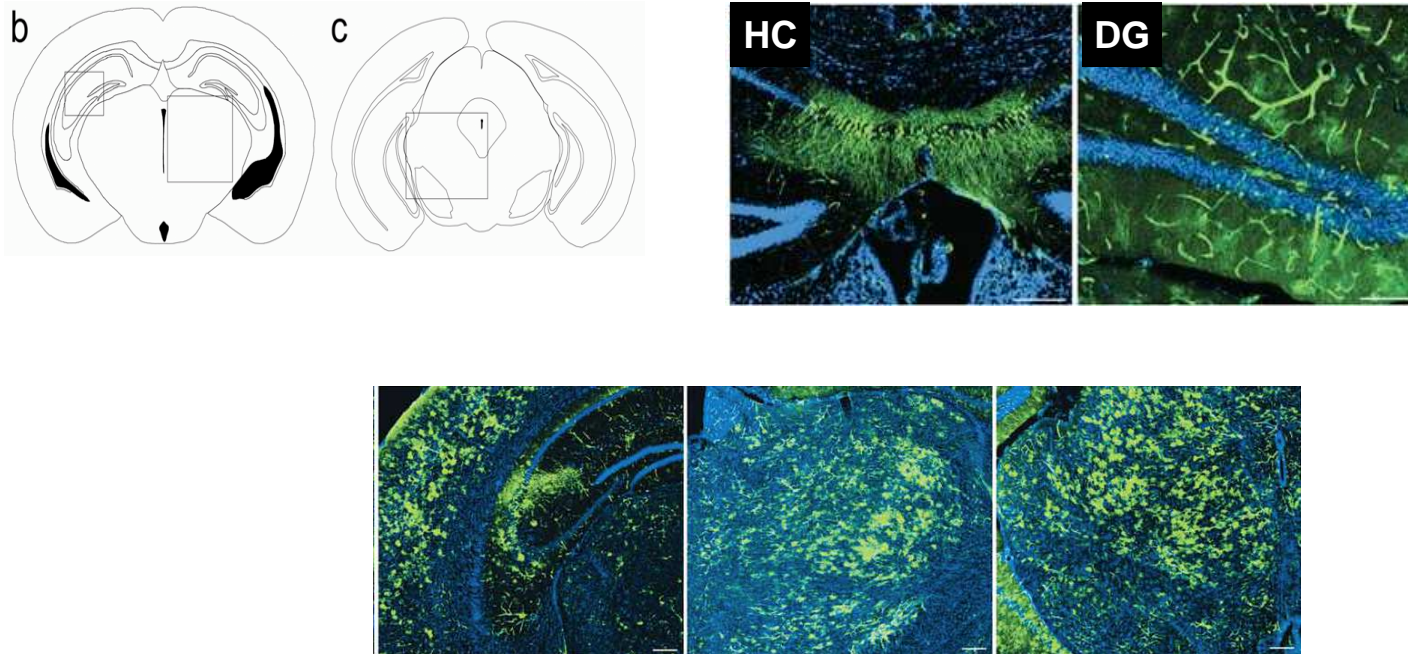
# **Peripheral Delivery: Intravenous Injection of Viral Vector**

# Intravenous Injection of Viral Vectors for CNS Benefits

- **IV injection of RV and LV in LSD mice**
  - ✓ **Therapeutic effects for visceral and CNS in mice targeting various cell types when treated at birth**
- **Hydrodynamic injection of plasmids/Sleeping Beauty transposon results in wide-spread therapeutic effects by long-term hepatic-derived protein production**
- **IV injection of rAAV**
  - ✓ **Different cell-type tropism in brain, depending on serotypes, purity, and the route of delivery, as well as developmental stages of the brain.**
  - ✓ **AAV 9 – new comer for CNS and PNS entry**
  - ✓ **Epitope-modified AAV for CNS targeting**

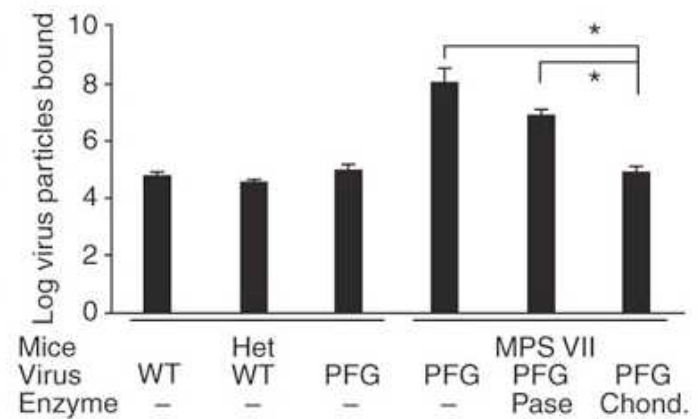
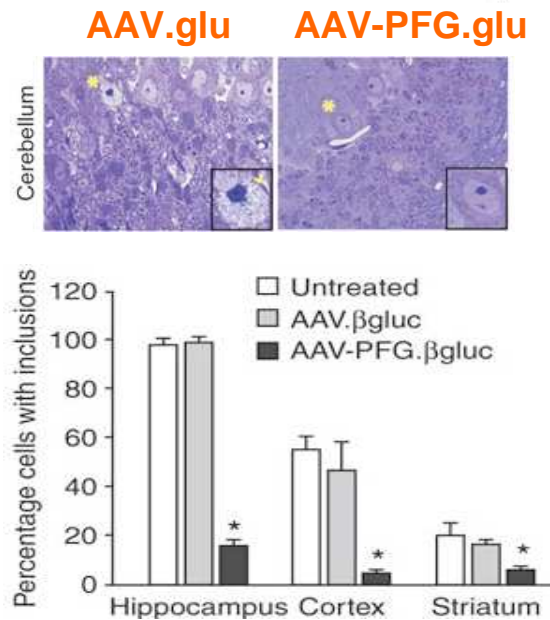
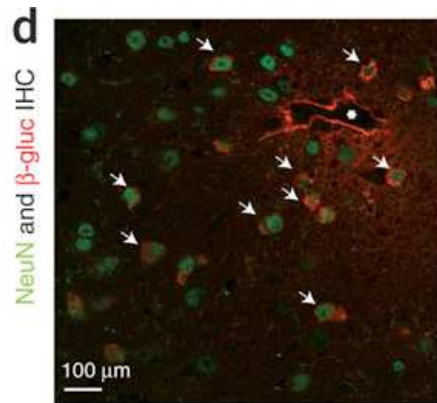
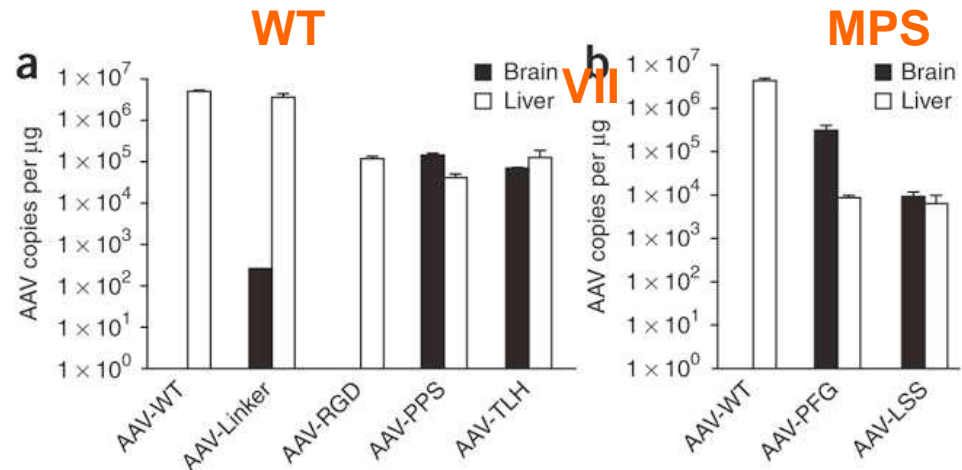
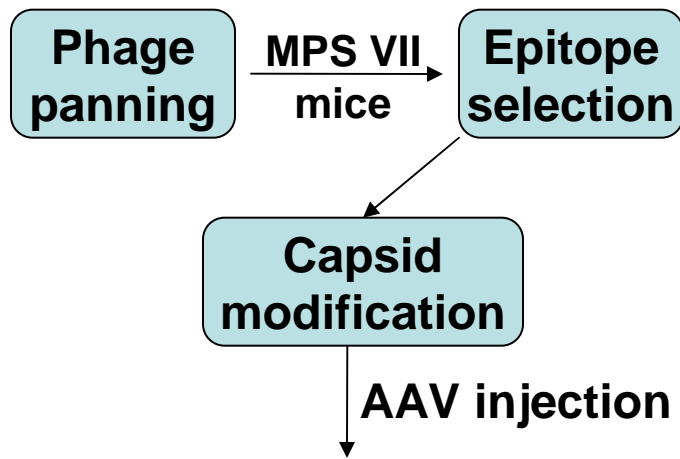
# CNS Transduction by IV Injection of scAAV 9

Wide-spread transduction in neonatal brain  
Restricted transduction in adult mice



*Foust... Kaspar, Nat Biotech, 2009*

# Epitope-modified AAV2 for CNS-direct Transfer by IV Injection



Chen et al., Nat Med, 2009

# Conclusion

## *Cell- and Gene-based Therapy for CNS in LSD*

- **Allogeneic HSC transplantation has different, disease-specific therapeutic response due to diverse pathogenesis and enzyme biology.**
- **Intracranial cell- or vector-injection may present a complementary approach to patients with visceral correction by HSCT.**
- **Autologous HSC gene therapy has showed better therapeutic effects than HSCT in some LSDs, with relatively low efficiency and risk concerns of oncogenesis.**
- **Systemic delivery of vectors (AAV, LV, and non-viral plasmids) could result in long-term protein production in circulation with therapeutic benefits on CNS and peripheral organs.**
- **A comprehensive approach would be needed in treating neuropathic LSDs which have high phenotype-genotype heterogeneity.**

# **Thank You for Your Attention 😊**

## **References:**

- Enns and Huhn, 2008, Neurosurg. Focus, 24: E11**
- Jeyakumar et al., 2009, Stem Cells, 27: 2362-3270**
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- Foust KD et al, 2009, Nat Biotech, 27: 59-65**
- Chen et al., 2009, Nat Med, 15: 1215-1218**