NCL Nanotechnology Characterization Laboratory

NCI Alliance for Nanotechnology in Cancer

"Autophagy and lysosomal dysfunction as emerging mechanisms of nanomaterial toxicity"

11th Annual GRIDS2025
Innovations in Lysosomal Disorders—New Frontiers in Diagnosis and Treatment

Tysons, Virginia
November 16-17, 2025.

Stephan Stern, Ph.D., DABT
Director of Research and Development
Nanotechnology Characterization Lab (NCL)
Frederick National Laboratory for Cancer Research

NATIONAL® CANCER INSTITUTE

Frederick National Laboratory for Cancer Research

Disclosures

Stephan Stern has no relevant financial relationships with ineligible companies to disclose.

Disclosure will be made when a product is discussed for an unapproved use.

This continuing education activity is provided by AffinityCE, The Lysosomal and Rare Disorders Research and Treatment Center (LDRTC), and CheckRare CE. AffinityCE, CheckRare CE and LDRTC staff, planners, and reviewers, have no relevant financial relationships with ineligible companies to disclose. AffinityCE adheres to the ACCME's Standards for Integrity and Independence in Accredited Continuing Education. Any individuals in a position to control the content of a CME activity, including faculty, planners, reviewers, or others, are required to disclose all relevant financial relationships with ineligible companies. All relevant financial relationships when present, have been mitigated by the peer review of content by non-conflicted reviewers prior to the commencement of the activity.

This activity has been supported by educational grants from commercial supporters. Please see the final program for a list of all supporters.

Learning OBJECTIVES

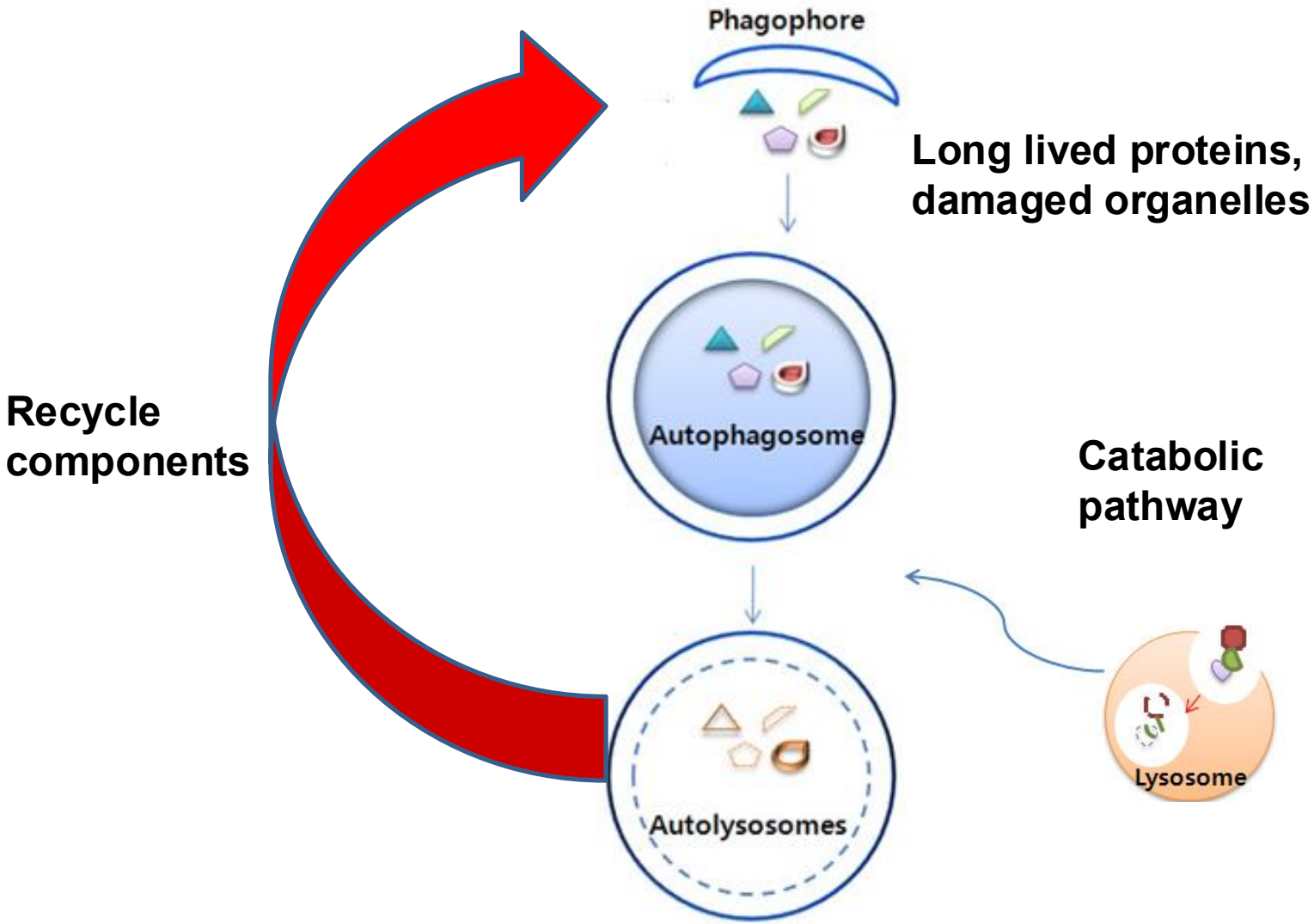
At the conclusion of this activity, participants will be able to:

1. Understand methods to measure autophagy and lyosomal perturbations by nanomaterials.

2. Describe mechanisms of nanomaterial-induced autophagy and lysosomal dysfunction and potential toxic effects.

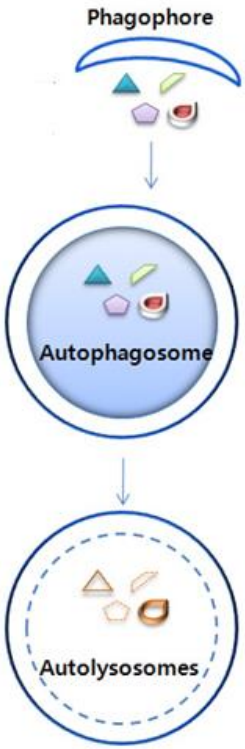
3. Describe characteristics of nanomaterials the cause autophagy and lysosomal dysfunction.

The Autophagy Pathway- Novel Target of Nanomaterial Toxicity?



Lee JA. 2009 BMB Rep. 42:324-30.

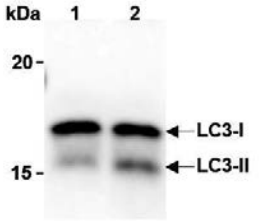
Monitoring Autophagy

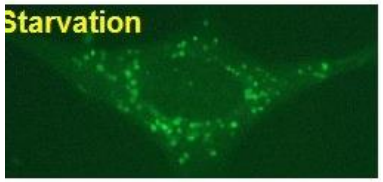


1) Degradation of autophagy substrates- p62 western (marker of autophagy flux)

↑ Induction/↓ blockade

2) LC3- I → II cleavage (biomarker)- Western, GFP-LC3 punctate, luciferase - LC3



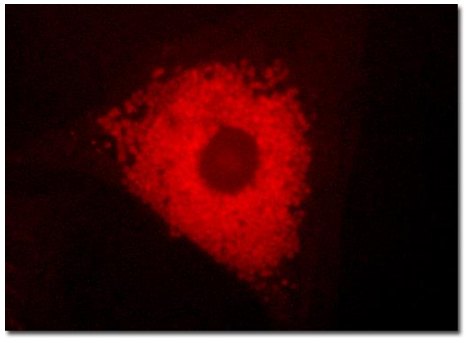


GFP-LC3 punctate

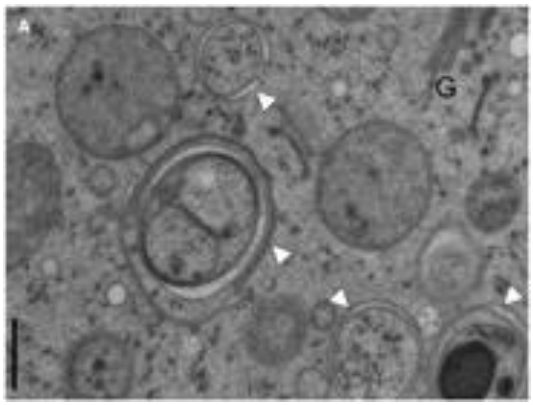
3) Autophagic vacuoles- TEM

4) Increased autolysosomal structures- monodansylcadaverine, cationic dyes

↑ Induction/↑ blockade



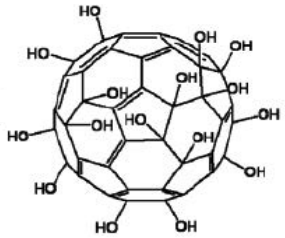
Lysotracker Red



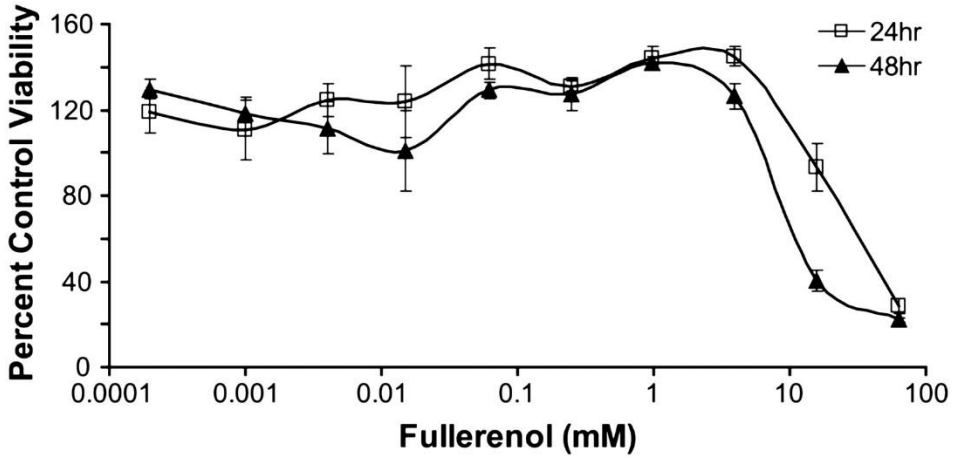
Autophagic vacuoles- TEM

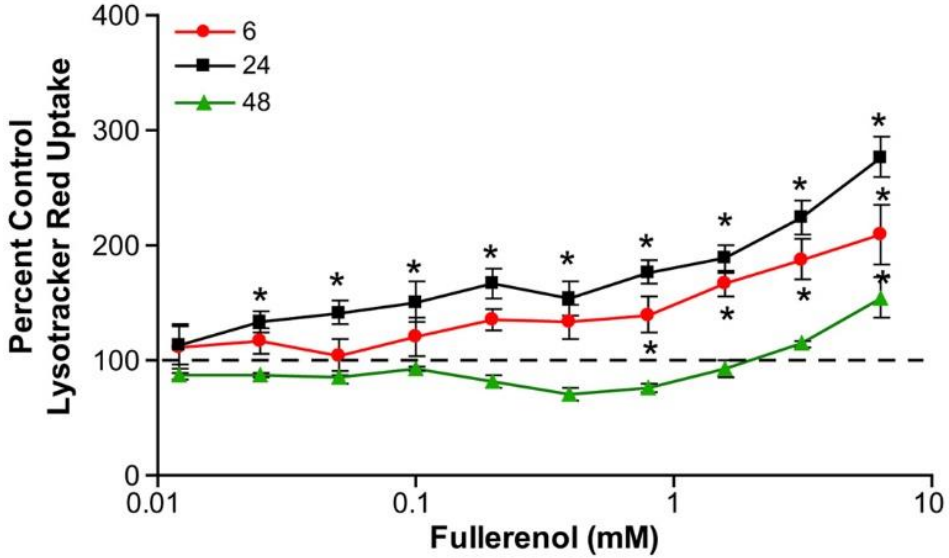
Neun BW, Stern ST. Methods Mol Biol. 2011;697:207-12.

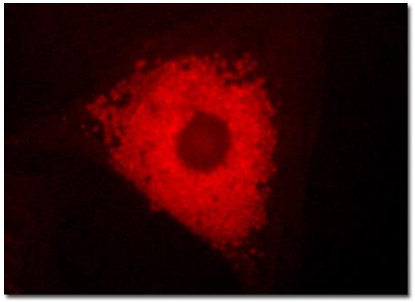
Case Study: Fullerenol – Cytotoxicity in Kidney Cells Associated with Lysosome Accumulation



fullerenol



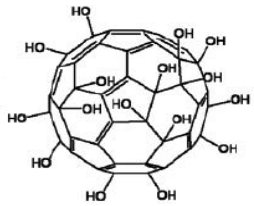




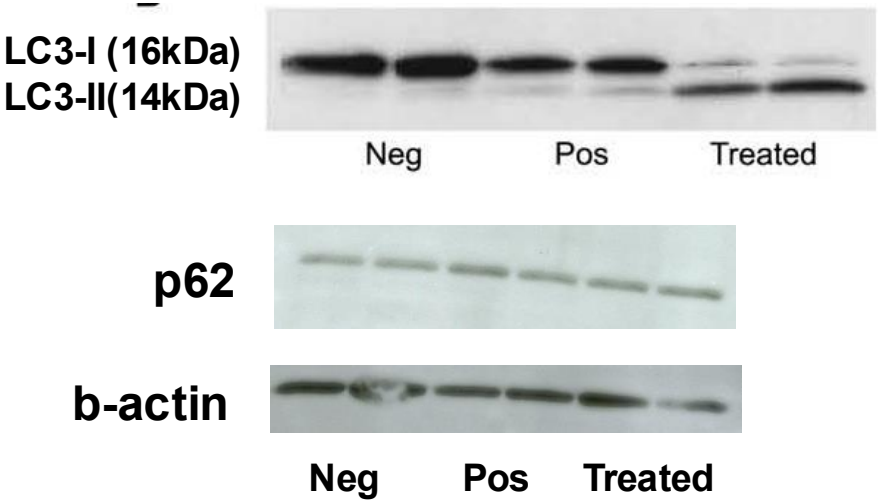
Fullerenol cytotoxity in porcine kidney cells associated with lyosome accumulation.

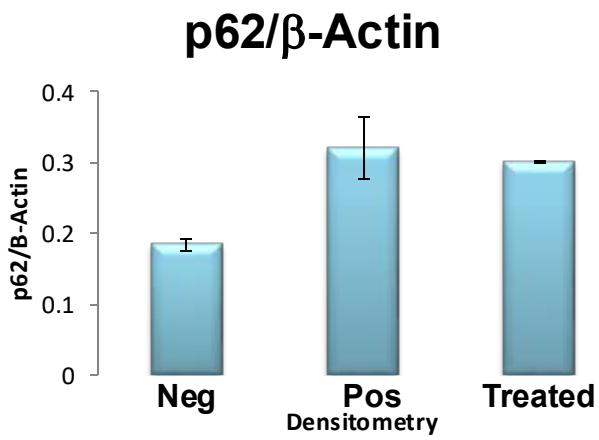
Johnson-Lyles DN, et al, Toxicol Appl Pharmacol. 2010 Nov 1;248(3):249-58.

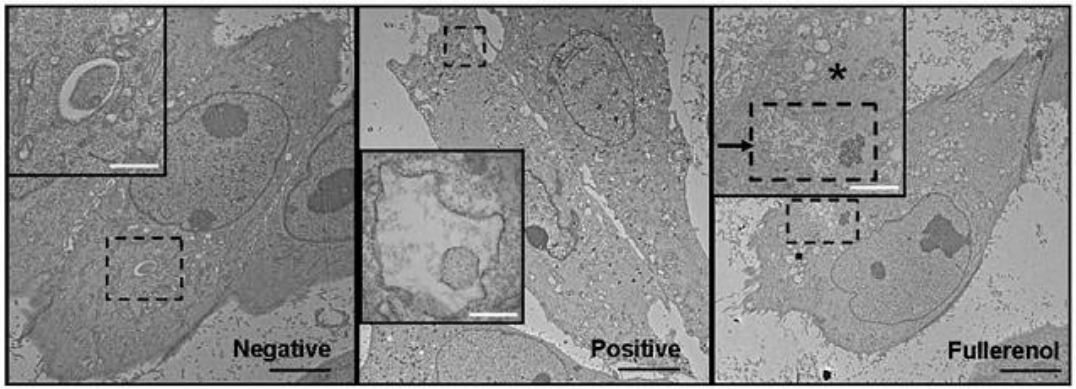
Fullerenol – Cytotoxicity in Kidney Cells Associated with Autophagy Blockade



fullerenol



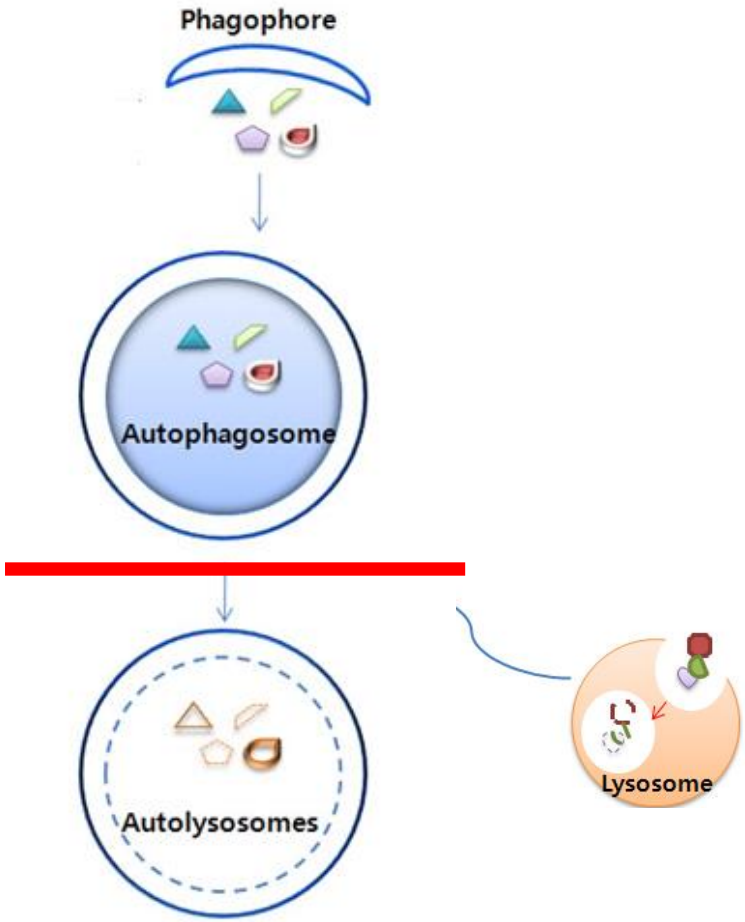




Fullerenol cytotoxity in porcine kidney cells associated with autophagy blockade, as evidenced increased LC3-II, autophagosomes (TEM) and increased p62.

Johnson-Lyles DN, et al, Toxicol Appl Pharmacol. 2010 Nov 1;248(3):249-58.

Fullerenol Autophagy Blockade

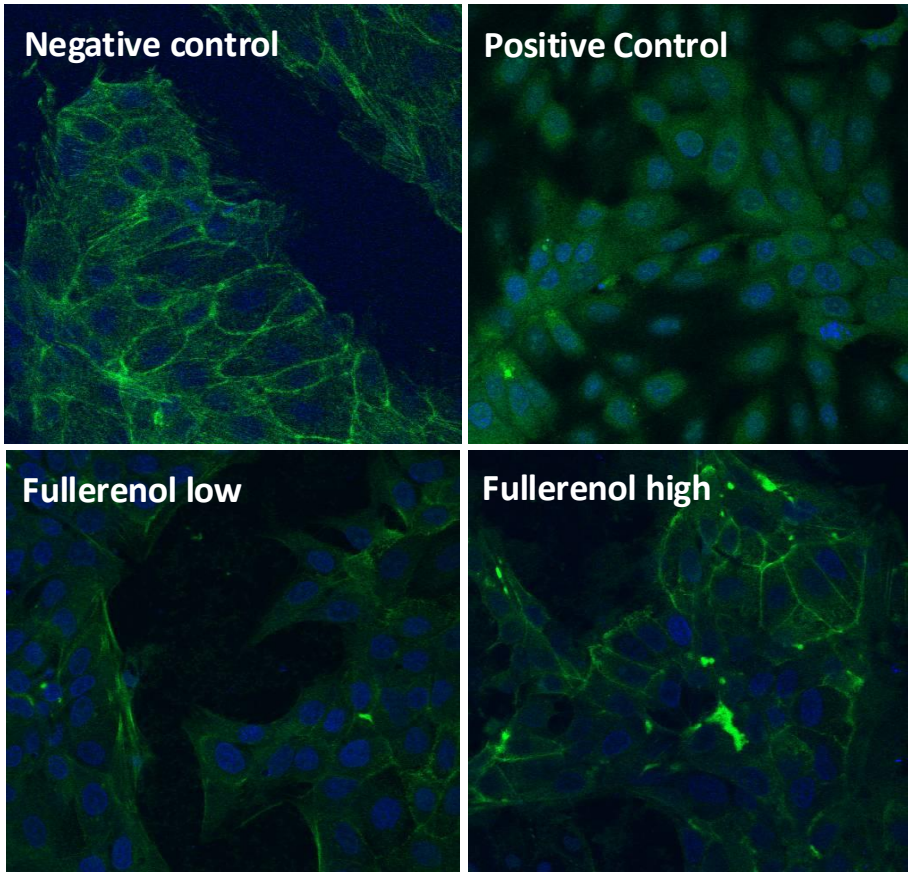


Apparent blockade of autophagy flux by fullerenol

Fullerenol – Actin and Mitochondrial Perturbation

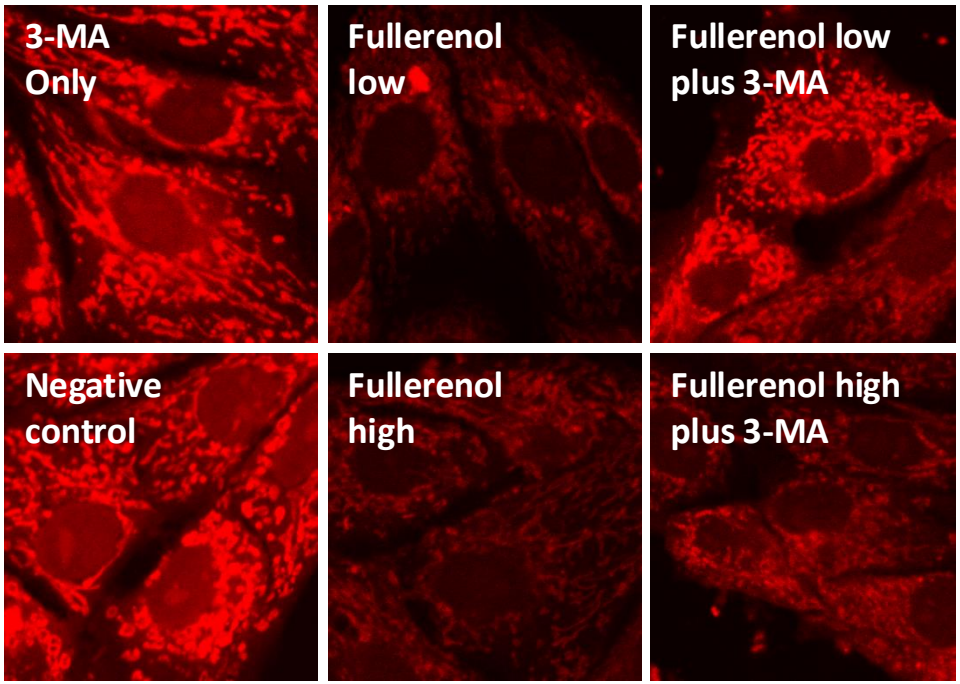
NCL Nanotechnology Characterization Laboratory | NCI Alliance for Nanotechnology in Cancer

Actin (Green, Phalloidin) and Nuclei (Blue, Hoechst)



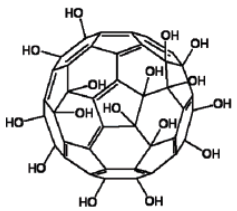
40x magnification

Mitochondria (Mitotracker Red)



148x magnification

24hr exposure; negative – media; actin positive-nocodazole (50 µM); fullerenol low – (0.6 mM); fullerenol high (3 mM); 3-MA (2 mM)

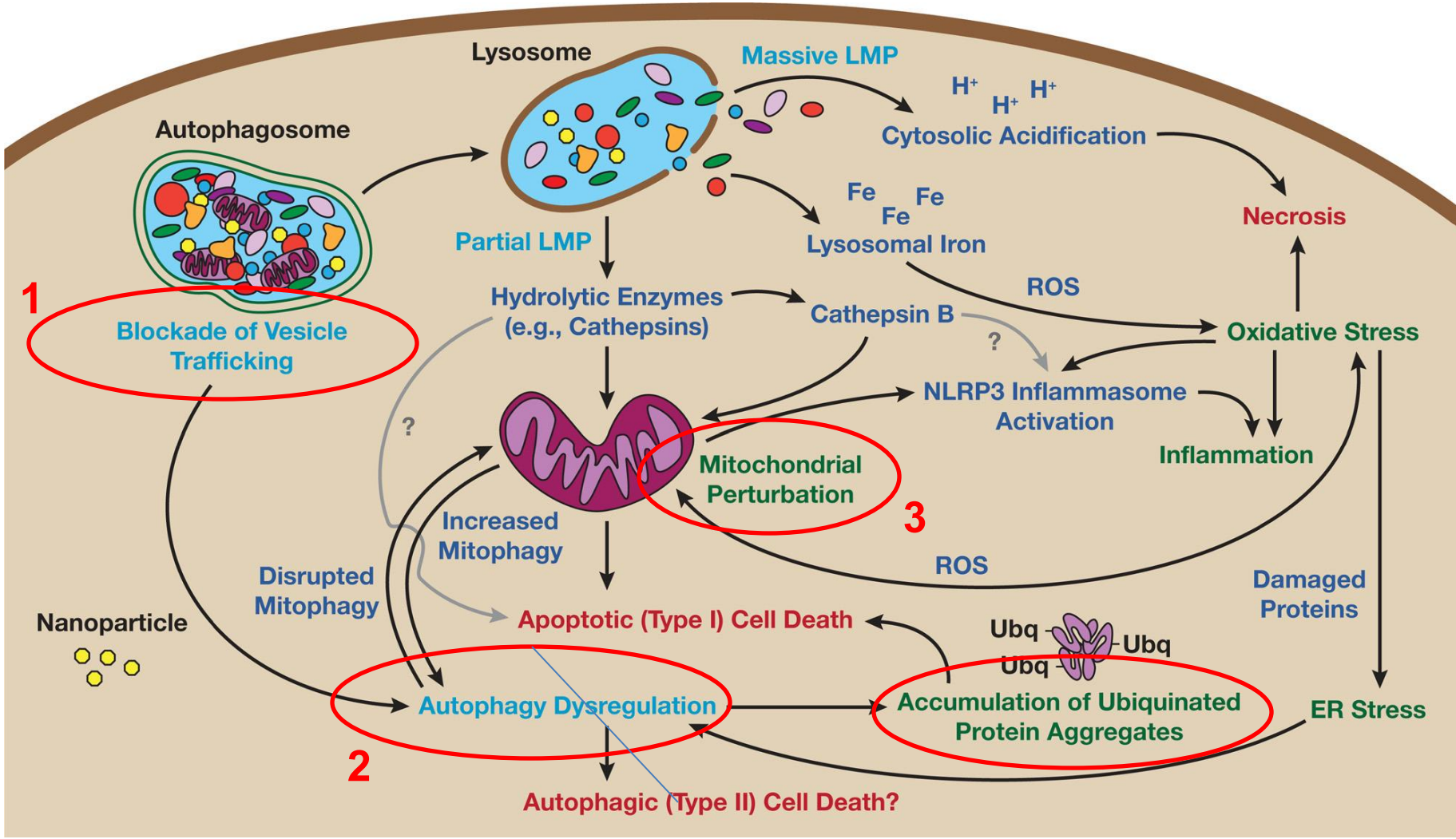


Fullerenol

- Fullerenol disrupts actin cytoskeleton structure and mitochondrial electron transport in LLC-PK1 cells.
- 3-MA, inhibitor of autophagy, prevents loss of mitochondrial function.

Johnson-Lyles DN, et al, Toxicol Appl Pharmacol. 2010 Nov 1;248(3):249-58.

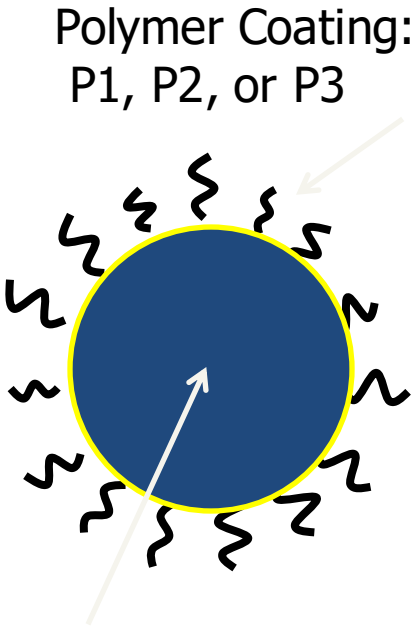
Mechanisms of Autophagy and Lysosomal Dysfunction Toxicity



Autophagy dysfunction has been shown for over 11 types of nanomaterials, from metallic to carbon based (**biopersitance appears to be a significant factor)**.

Stern et al. Particle and Fibre Toxicology 2012, 9:20

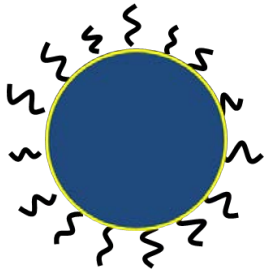
Case Study:
Metal Oxide Nanoparticle CT Imaging Agent



Metal Oxide Nanoparticle CT Contrast agent

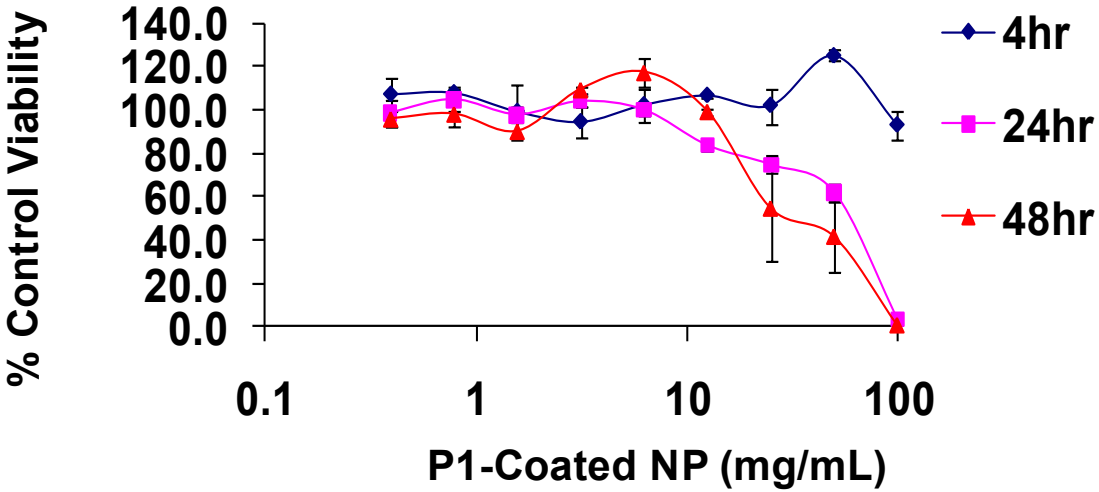
- NCL tested metal oxide nanoparticles with three different polymer coatings, designated here as P1, P2, P3.
- NCL's in vitro tests correlated with in vivo results and showed that the biocompatibility depended on the coating.

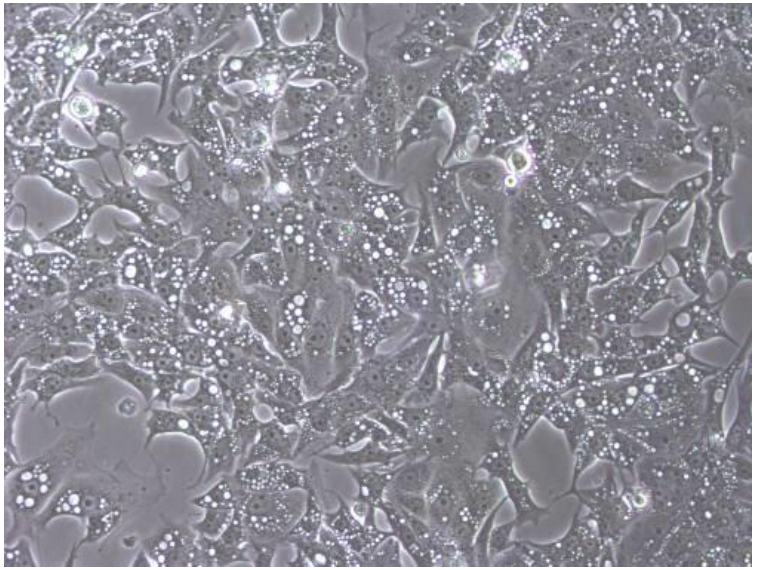
P1 Metal Oxide Nanoparticle Imaging Agent - Proximal Tubule Cytotoxicity



In Vitro

P1-Coated Metal Oxide Nanoparticle





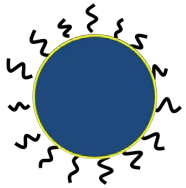
P1-Coated Metal Nanoparticle (25mg/ml), 24hr

20x magnification

Extensive Vacuolization

P1 metal oxide NP cytotoxity in porcine proximal tubule cells associated with vacuolization.

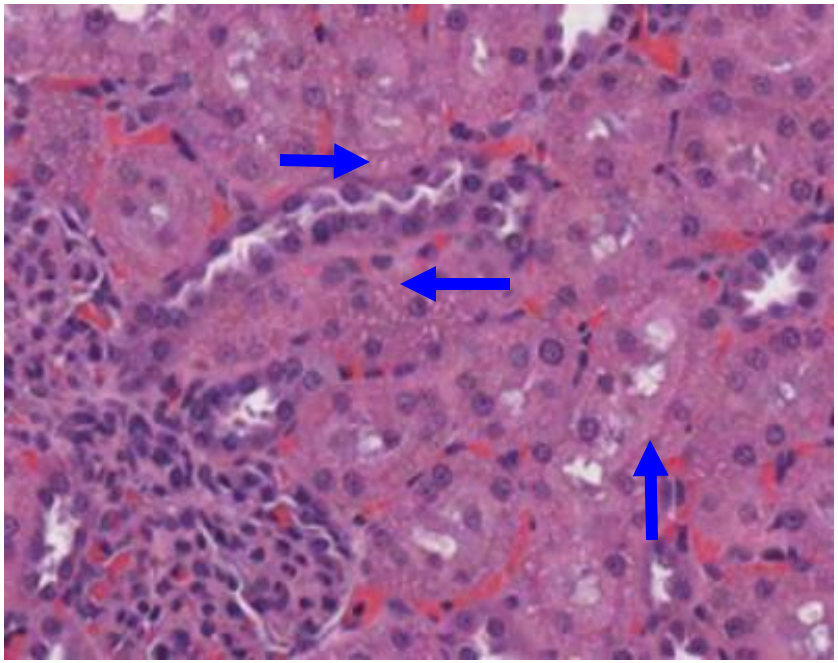
P1 Metal Oxide Nanoparticle Imaging Agent - Rat Renal Toxicity



In Vivo

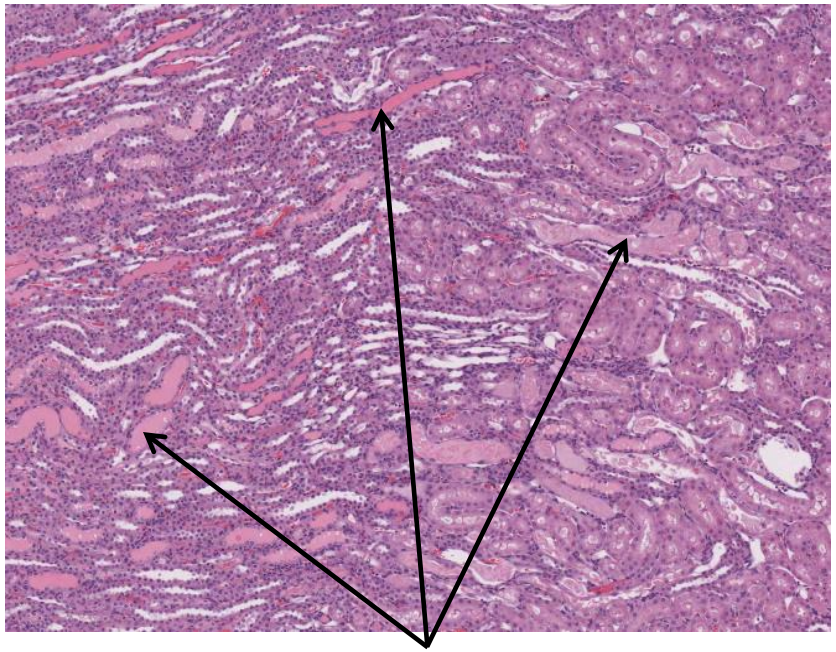
P1-Coated Metal Oxide Nanoparticle

6h TOT Rats Tx w/ 1.5 g/kg



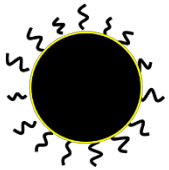
Proximal tubular vacuolization

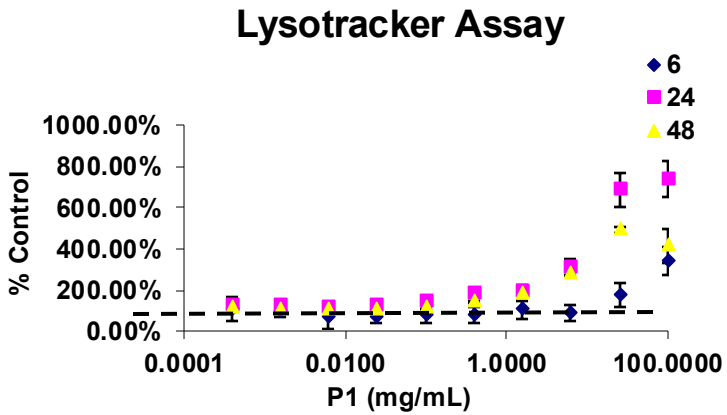
3-Day TOT Rats Tx w/ 1.5 g/kg

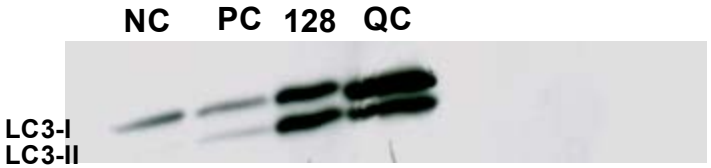


Dilated, re/degenerative tubules, protein casts, and mineralization; Correlated with increased BUN/KIM-1

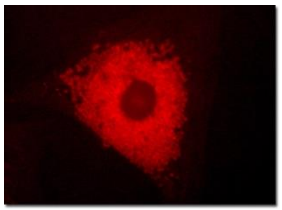
P1 Metal Oxide Nanoparticle Imaging Agent - Autophagy dysfunction



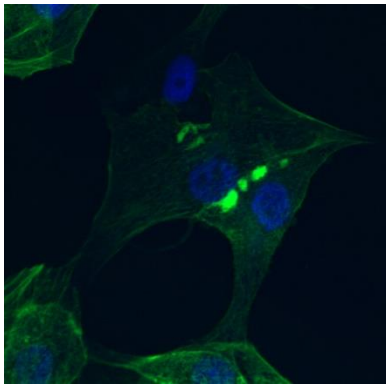




P1 (25mg/ml), 24hr



Lysotracker Red

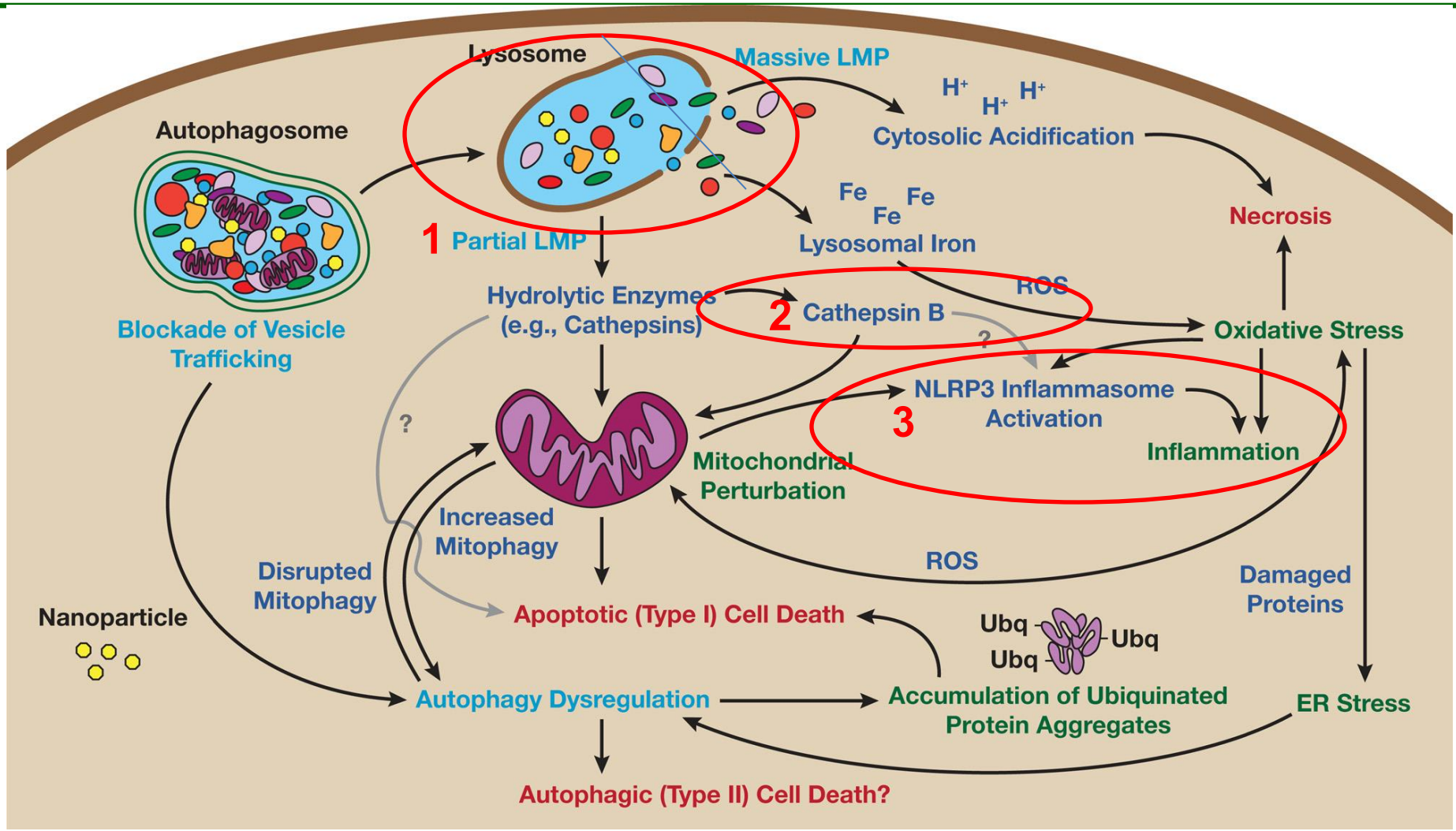


P1 (25mg/ml), 24hr

Actin clumping

- P1 Metal NP induced-cytotoxity in porcine kidney cells associated with:
  - Autophago/lysosome accumulation
  - Actin cytoskeleton perterbation
- P2- and P3-coated particles did not have this response and were free of nephrotoxicity in vivo.

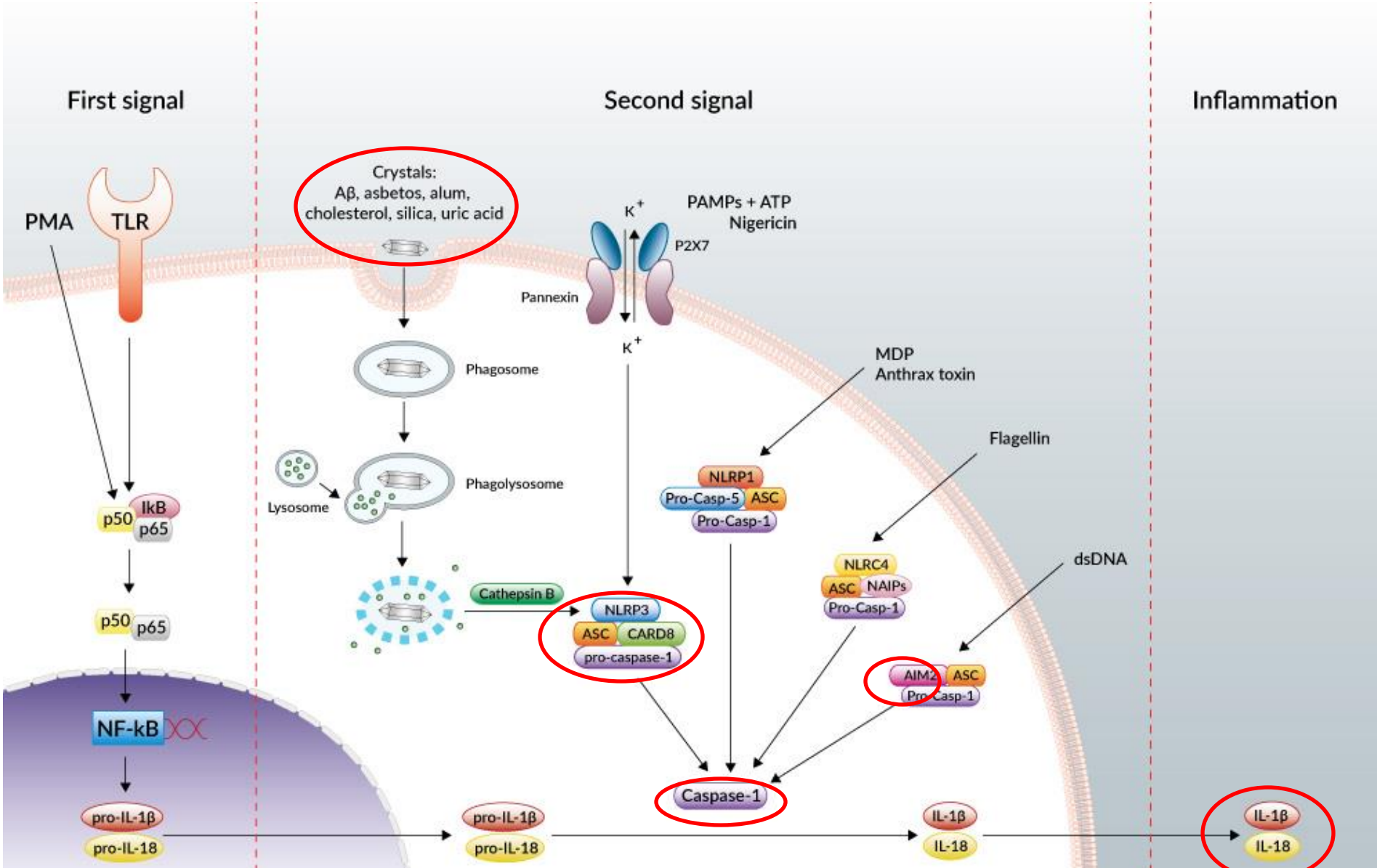
Lysosomal membrane permeability and NLRP3 Inflammasome activation



Lysosomal membrane permeability and NLRP Inflammasome activation can be a toxic inflammatory mechanism (silica) or a therapeutic mechanism for vaccines (Alum)

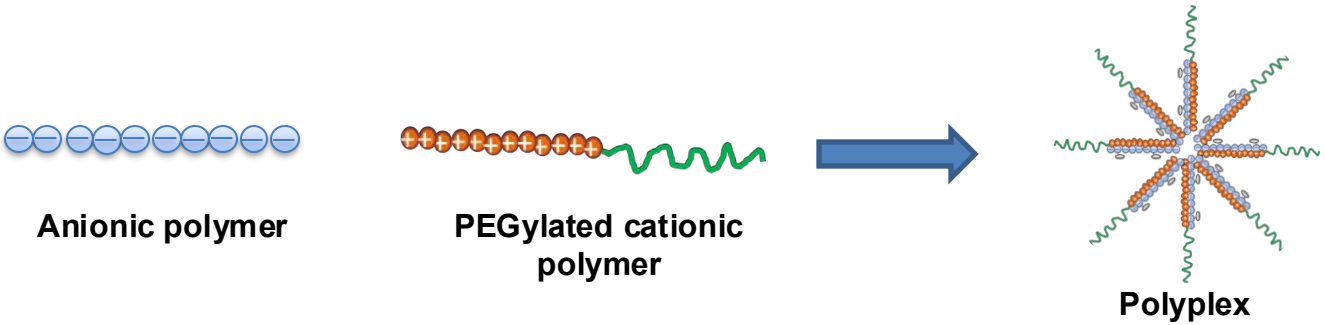
Stern et al. Particle and Fibre Toxicology 2012, 9:20

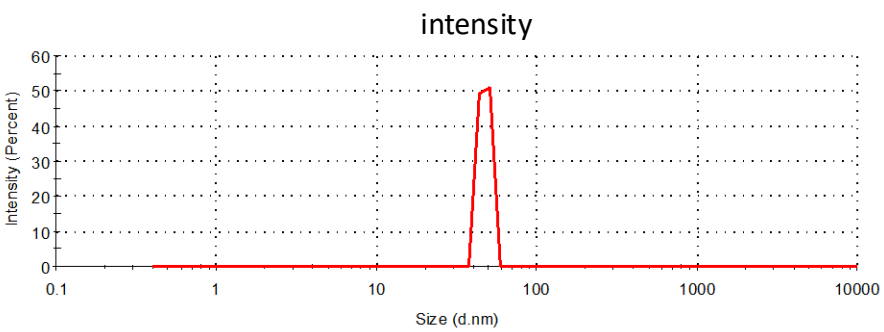
NLRP3 Inflammasome components

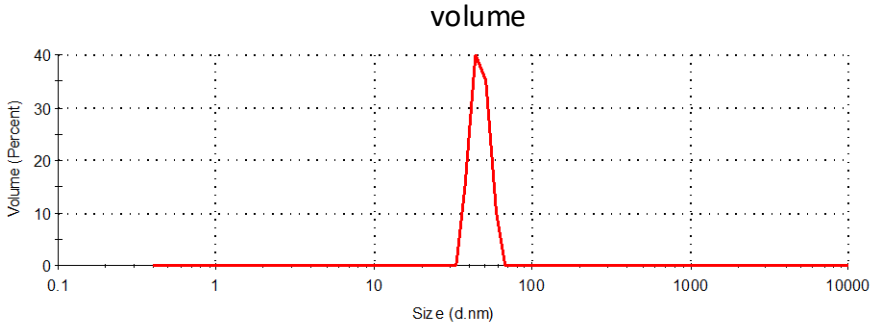


https://www.invivogen.com/review-inflammasome

Case Study: Polyplex Adjuvant





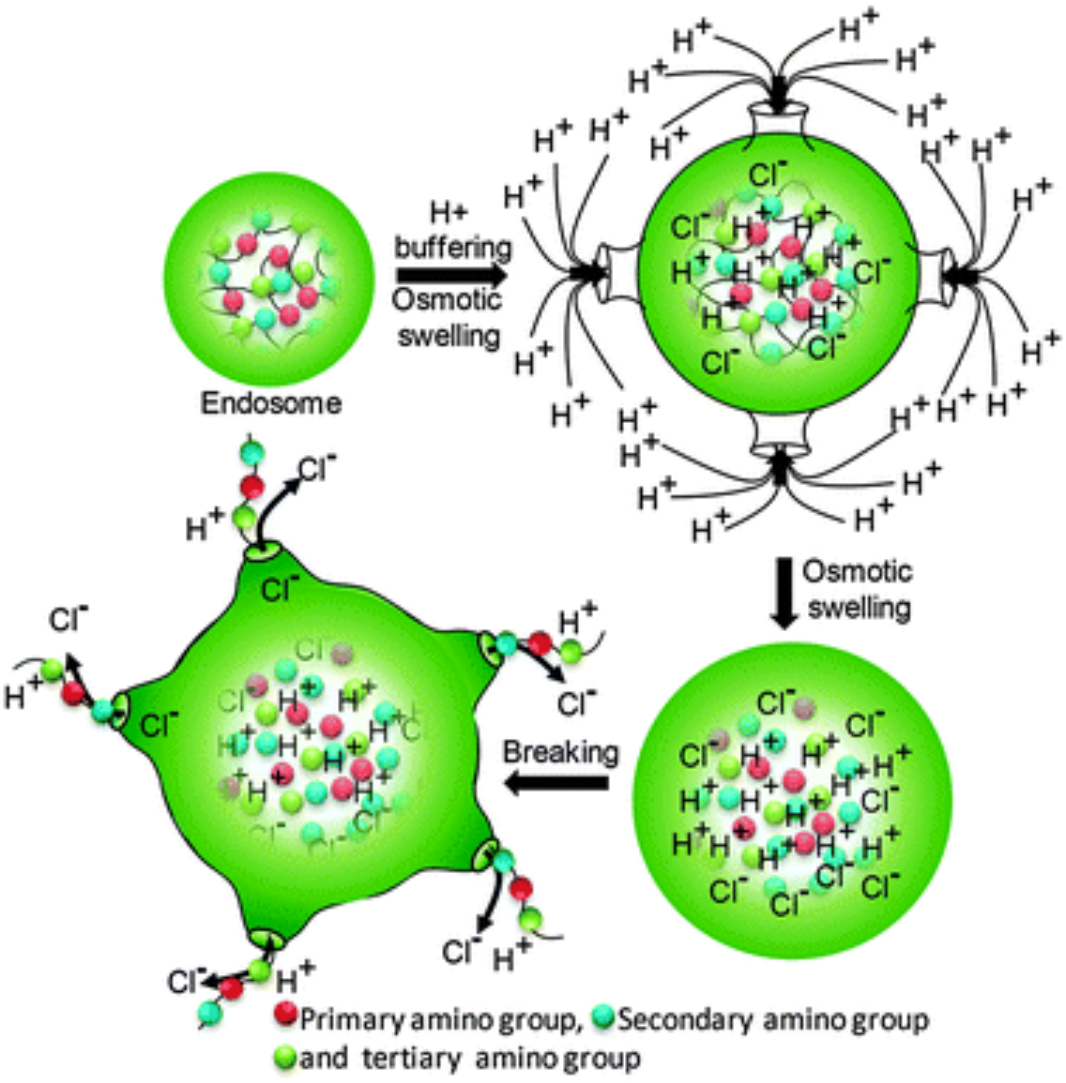


Low volume quartz cuvette, b = 10 mm, 25 °C, 633 nm laser λ, 90° scattering angle
Samples run at 10-fold dilution in PBS, data presented as mean ± SD (n=10)

Sample	Z avg (nm)	PDI	Int-Peak (nm)	% Int	Vol-Peak (nm)	% Vol
Fresh preparation	45.9 ± 0.3	0.033 ± 0.018	47.1 ± 0.6	100 ± 0.1	43.9 ± 0.8	100 ± 0.1
6 months (4 °C)	48.8 ± 0.7	0.011 ± 0.009	49.3 ± 0.7	100 ± 0.1	48.1 ± 0.6	100 ± 0.1
Freeze/thaw at -20 °C	47.5 ± 0.4	0.037 ± 0.021	47.4 ± 0.3	100 ± 0.1	46.8 ± 0.4	100 ± 0.1

- Polyplex forms monodisperse nanoparticle with ~50 nm diameter
- Greater than 6-month storage stability at 4 ° C

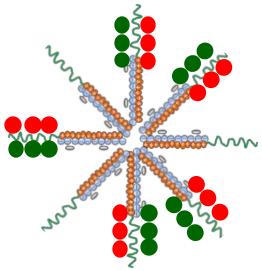
Proton sponge theory for cationic nanoparticle LMP



Chem.Soc.Rev., 2010, 39, 3031–3056

Polyplex stability in vitro

THP-1 cells
8-well chamber slide

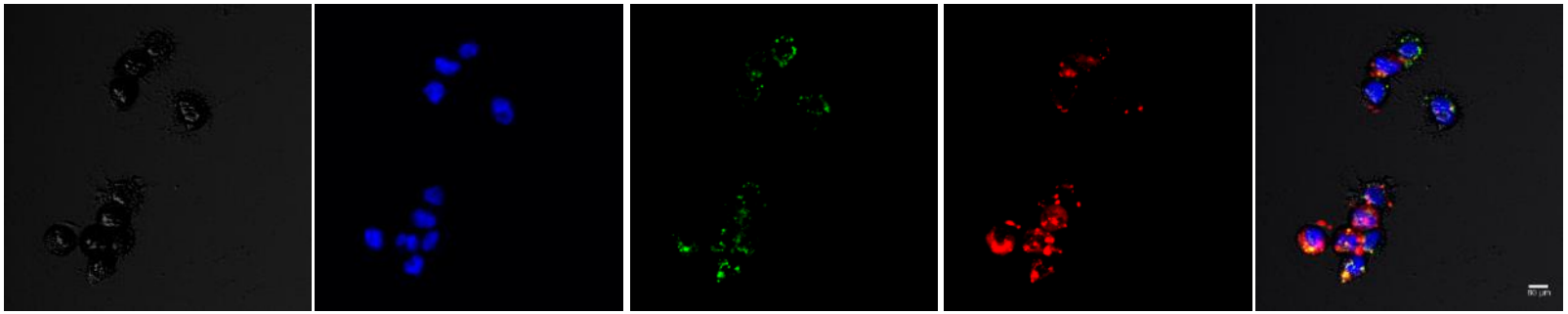


Dual Fluorochrome tagged Polyplex

Yellow merge if polymers stay together as polyplex

Green or **red** if polyplex falls apart

Confocal microscopy
200X

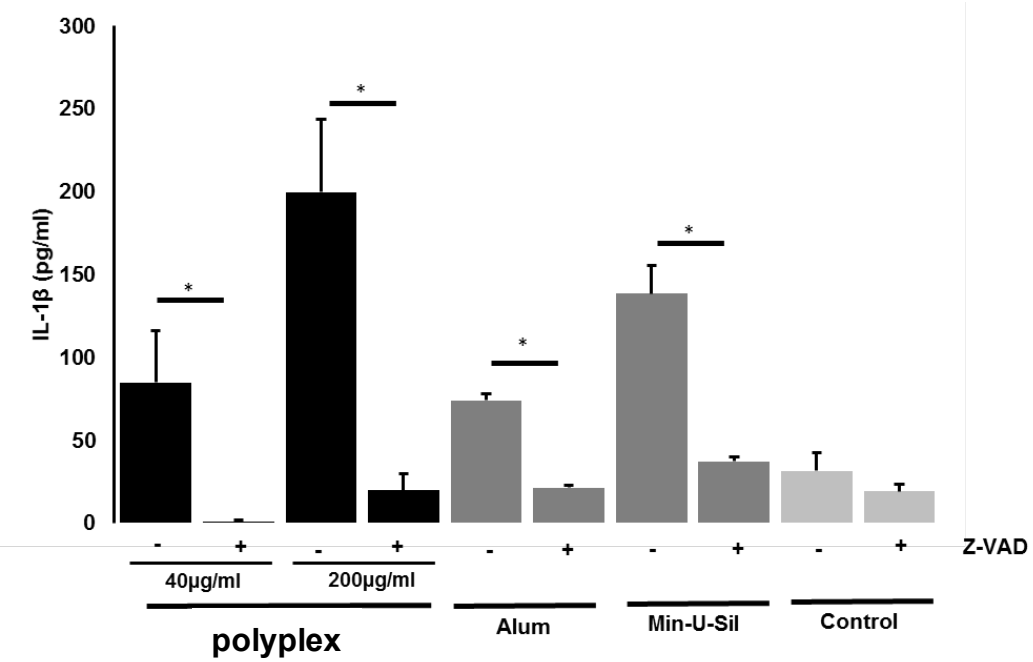


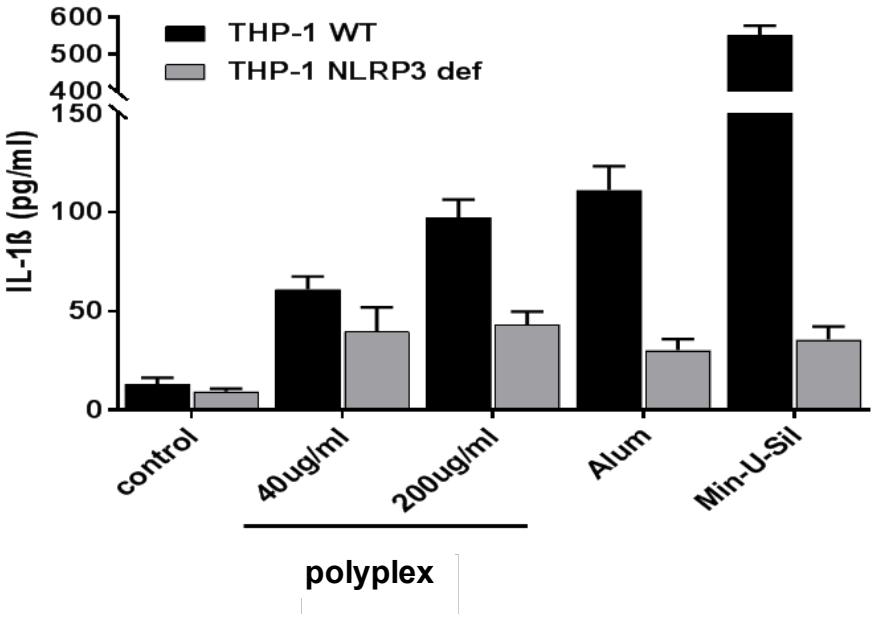
Phase-Contrast | **Hoechst** | **Alexa 488** | **Alexa 594** | **Merge**

Polyplex remains intact during cell internalization in differentiated THP-1 cells, macrophage like

THP-1 cells plated with 50nM PMA and treated with 10uM of dual tagged polyplex for 4h. Cells were fixed with 4%PFA and counterstained with Hoechst for 5mins; Anionic polymer tagged to AlexaFluor 488 and Cationic polymer to AlexaFluor 594

Polyplex mediated NLRP3 inflammasome activation dependent on caspase-1

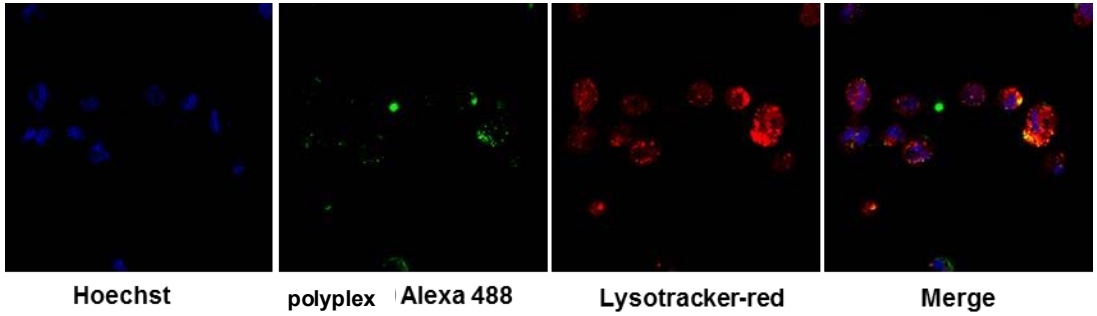




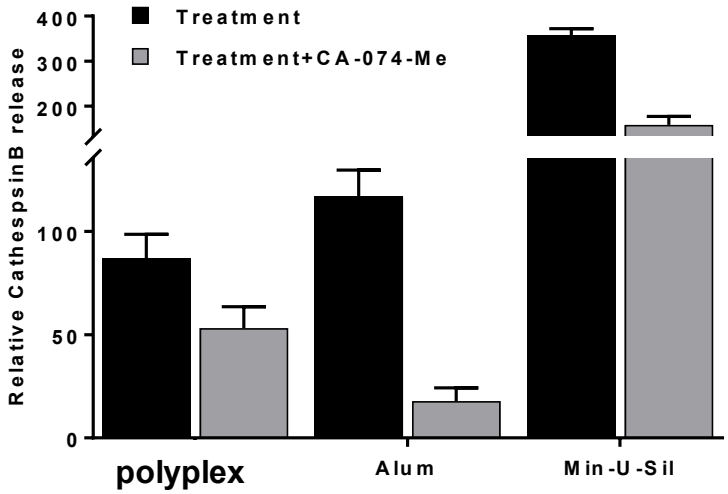
Pan caspase inhibitor (Z-VAD) decreased IL-1B release in polyplex treated THP-1 cells

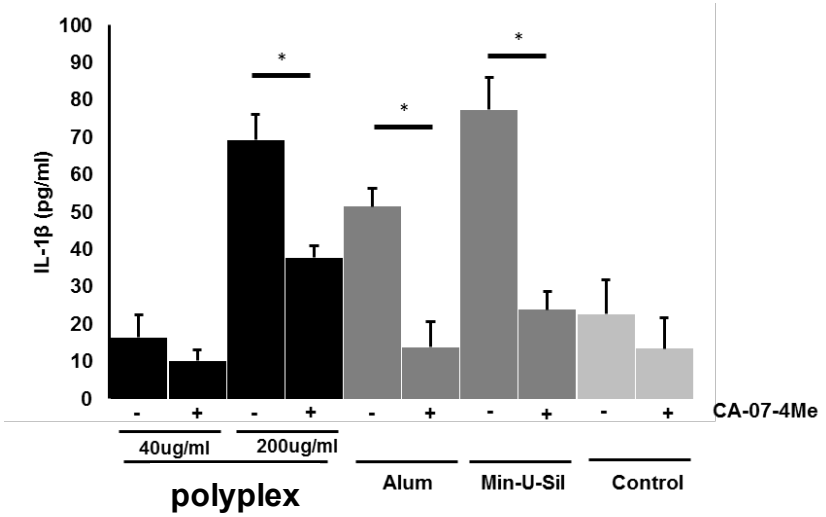
No IL-1B release in polyplex treated caspase-1 deficient THP-1 cells

Polyplex causes lysosomal membrane permeabilization (LMP) following cellular uptake



PMA-primed THP-1 cells were treated with 200µg/ml of fluorescently labeled Polyplex (AlexaFluor 488) for 24 hr. After incubation, cells were stained with a lysosomal-specific dye, lysotracker red and Hoechst to stain the nuclei.





- Polyplex accumulates in lysosome following cellular uptake.
- Pre-treatment with cathepsin B inhibitor decreased cathepsin activity and IL-1B release supporting a LMP mechanism.

Summary/Conclusions

Nanoparticle-Autophagy Dysfunction Hypothesis

- **Many biopersistant/cationic nanoscale particles induce autophagy and lysosomal dysfunction, with mechanisms including cytoskeleton disruption, lysosomal overload and lysosomal membrane permeabilization, resulting in**

  - Loss of phagocytotic function, lysosomal trafficking
  - Accumulation of damaged organelles (mitochondria)
  - Accumulation of protein aggregates
  - Inflammasome activation and inflammation (adjuvants)
  - Autophagic cell death?

- **Possible pathological consequences of autophagy and lysosomal dysfunction:**

  - Cardiopulmonary toxicity (COPD, endothelial dysfunction)
  - Immunotoxicity (Immunosuppression/Immunostimuation)
  - Neurodegeneration (Parkinson's, Alzheimer's……)
  - Other target organ toxicities? (lysosomal overload nephropathy-fullerene, lung toxicity-cationic dendrimer, renal tubular injury-metal NP)

Stern ST, Johnson DN. Autophagy. 2008 Nov 16;4(8):1097-100.

The NCL Team


Edward Cedrone


Jeffrey Clogston


Rachael Crist


Marina Dobrovolskaia


Matthew Hansen


Krishna Kattel


Ruvanthi Kularatne


Barry Neun


Timothy Potter


Sarah Skoczen


Jie Xu

Program Evaluation and CE Credit

We need your assistance. Your opinion is important.

Please go to the link below and help us evaluate the program. You can also claim continuing education credit at this link.

ldrtc.cds.affinityced.com

Blood-Brain Barrier Delivery for Lysosomal Storage Disorders with IgG-Lysosomal Enzyme Fusion Proteins

Ruben Boado, Ph.D.

University of California at Los Angeles, CA, USA

GRIDS2025

DISCLOSURES: Ruben Boado has no relevant financial relationships with ineligible companies to disclose.

Blood-Brain Barrier Challenge: Bottleneck of CNS drug development

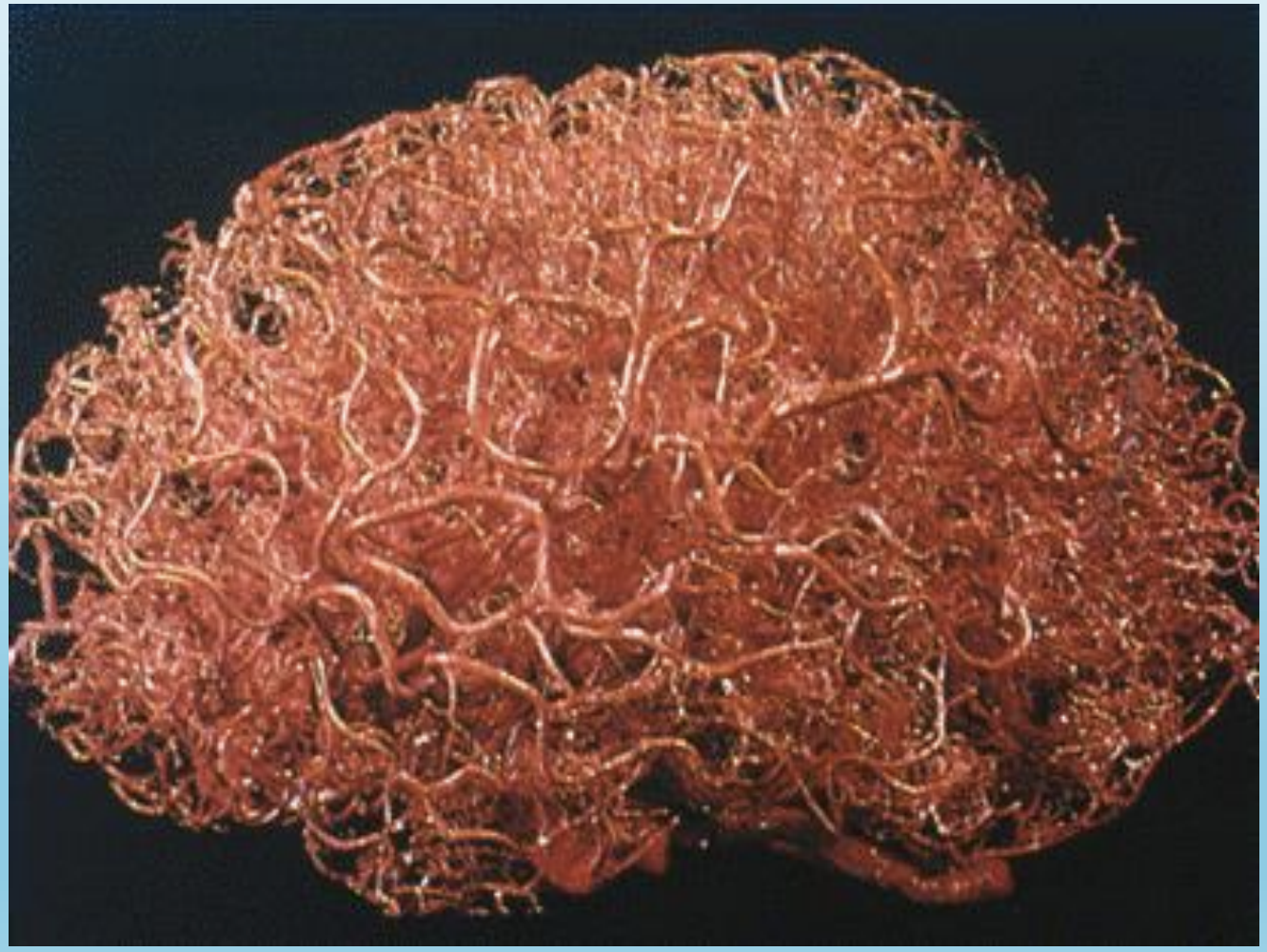
- **BBB protects brain from microorganisms, peripheral cytokines and neurotramsmitters**
  - 100% of large-molecule therapies do not cross BBB
  - 98% of small-molecule therapies do not cross BBB
  - Therefore, most available treatments do not address neurological complications

Microvasculature of the Brain: Anatomical Site of the Blood-Brain Barrier

There are >100 billion capillaries in the human brain

The total length of brain capillaries is 400 miles

The surface area of the brain endothelium is 20 M^2

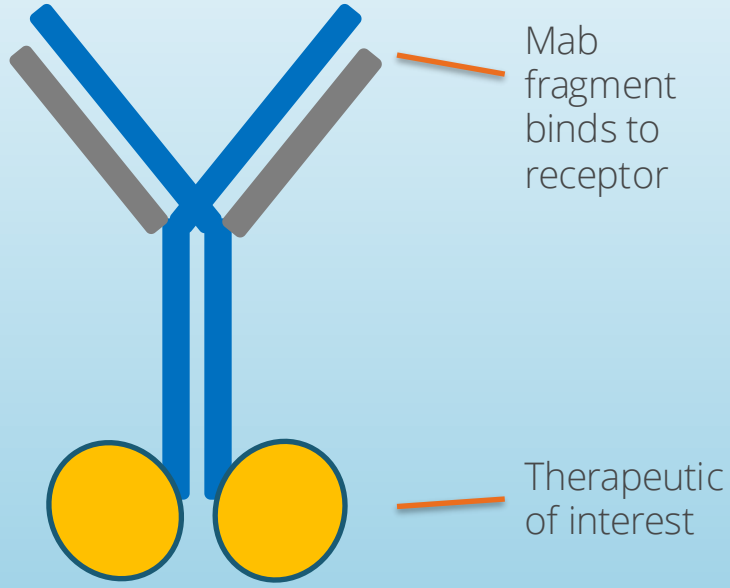


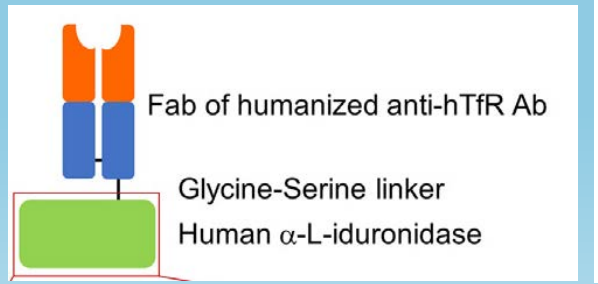
The trans-vascular route for drug delivery to the brain

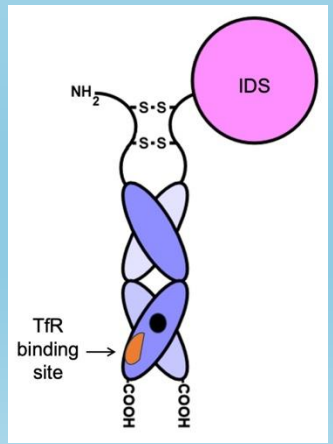
Every neuron is perfused by its own capillary

Brain penetrating IgG-Lysosomal Enzyme Fusion Proteins

- Target body's natural active transport systems present on BBB
  - Insulin receptor
  - Transferrin receptor
- Form by a transport and a therapeutic domain, respectively
  - Transport domain binds to a BBB receptor (i.e.: insulin receptor)
  - Therapeutic domain (enzymes, antibodies, recombinant proteins, even siRNA)

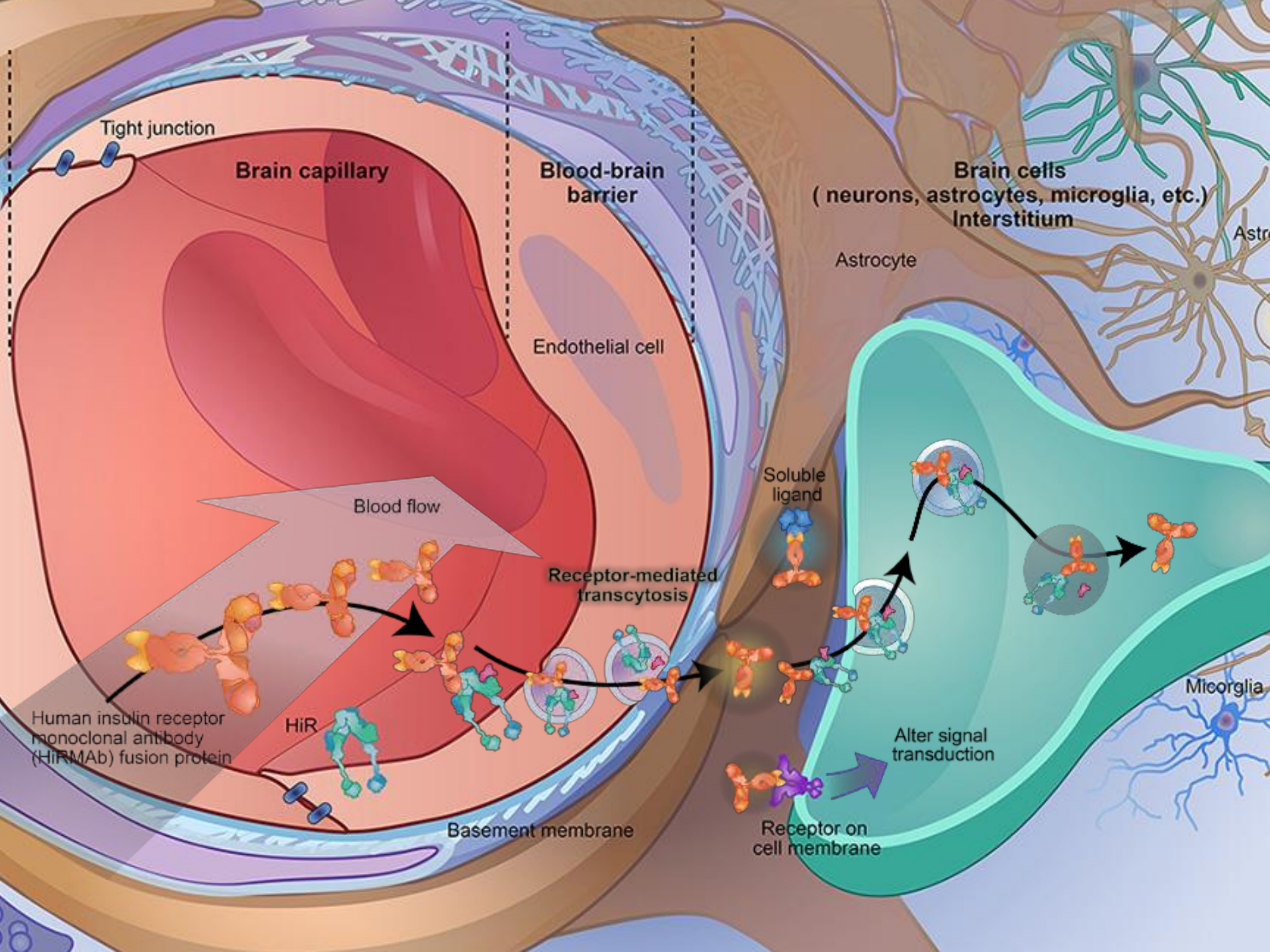






Advantages of IgG-Lysosomal Enzyme Fusion Proteins

- Non-Invasive, Brain Global Distribution of Biotherapeutics
- Highly specific for BBB-receptor and binds with high affinity
  - Minimal off-target effects
  - Low therapeutic dose levels
- Straightforward engineering and validated manufacturing process
- Reduced immunogenicity
- Extensive library of peer reviewed publications
  - > 50 publications on engineering of IgG-therapeutic fusion proteins


Tight junction
Brain capillary
Blood-brain barrier
Brain cells (neurons, astrocytes, microglia, etc.) Interstitium
Astrocyte
Endothelial cell
Blood flow
Soluble ligand
Receptor-mediated transcytosis
Human insulin receptor monoclonal antibody (HiRMAb) fusion protein
HiR
Alter signal transduction
Receptor on cell membrane
Basement membrane
Micorglia

Lysosomal Storage Disorders: Urgent Unmet Need

Potential to revolutionize treatments

- Enzyme replacement therapies (ERTs) have been developed for some select LSD's
- Available ERTs do not reach the brain in clinically relevant amounts and thus do not address the severe, progressive neurological complications of most LSDs:
  - Intellectual disability
  - Severe behavioral issues
  - Premature mortality
- Orphan drug status provides regulatory benefits
- Established LSD communities with large identified patient populations

Brain penetrating IgG-fusion proteins targeting the BBB human insulin receptor

Indication	Therapeutic Domain	IgG-Fusion Protein[1]
Hurler syndrome (MPS I)	Iduronidase (IDUA)	HIRMAb-IDUA (valanafusp alpha, AGT-181)
Hunter syndrome (MPS II)	Iduronate-2-sulfatase (IDS)	HIRMAb-IDS (AGT-182)
Hunter syndrome (MPS II)	Iduronate-2-sulfatase (IDS)	HIR-Fab-IDS (GNR-055)
Metachromatic leukodystrophy*	Arylsulfatase A (ASA)	HIRMAb-ASA (AGT-183)
Sanfilippo A (MPSIIIA)*	Sulfamidase (SGSH)	HIRMAb-SGSH (AGT-184)
Sanfilippo B (MPSIIIB)*	N-acetyl-alpha-D-glucosaminidase (NAGLU)	HIRMAb-NAGLU (AGT-187)
Niemann-Pick A/B*	Acid shingomyelinase (ASM)	HIRMAb-ASM (AGT-189)
Tay-Sachs*	Hexoaminidase A (HEXA)	HIRMAb-HEXA (AGT-192)
Batten Type 1*	Palmitoyl-protein thioesterase (PPT1)	HIRMAb-PPT1 (AGT-194)
GM1-gangliosidosis*	b-galactosidase (GLB1)	HIRMAb-GLB1 (AGT-195)

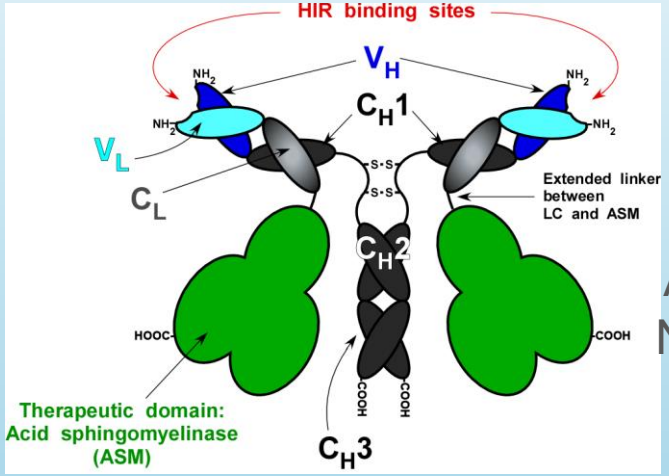
[1] The transport domain of these human fusion proteins is a monoclonal antibody (HIRMAb), or a Fab fraction (HIR-Fab-IDS), directed to the human BBB insulin receptor. *Represents an indication that has a primary CNS disease burden. From Boado RSC Pharmaceutics 2025 (DOI: 10.1039/d5pm00204d).

Brain penetrating IgG-fusion proteins targeting the mouse or human BBB transferrin receptor

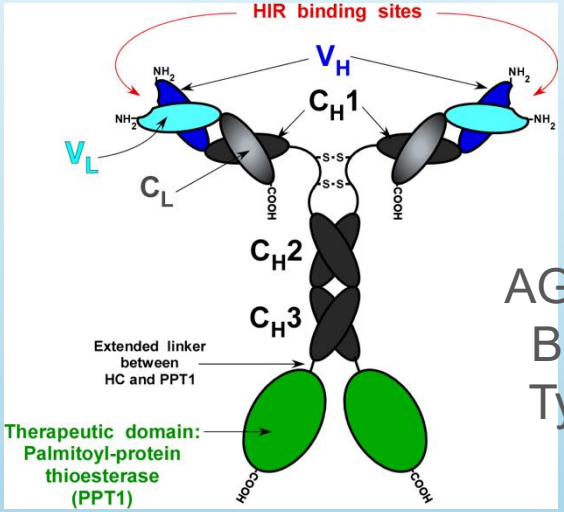
Indication	Therapeutic Domain	IgG-Fusion Protein[1]
Hurler syndrome (MPS I)	Iduronidase (IDUA)	mTfRMAb-IDUA
Hunter syndrome (MPS I)	Iduronidase (IDUA)	hTfR-Fab-IDUA (lepunafusp alfa, JR-171)
Hunter syndrome (MPS II)	Iduronate-2-sulfatase (IDS)	hTfRMAb-IDS (pabinafusp alfa, JR-141)
Hunter syndrome (MPS II)	Iduronate-2-sulfatase (IDS)	mTfRMAb-IDS
Hunter syndrome (MPS II)	Iduronate-2-sulfatase (IDS)	TfR transport vehicle, ETV:IDS (tividenofusp alpha, DNL310)
Sanfilippo A (MPSIIIA)*	Sulfamidase (SGSH)	hTfR-Fab-SGSH (postnafusp alfa, JR-441),
Sanfilippo A (MPSIIIA)*	Sulfamidase (SGSH)	TfR transport vehicle, EVT:SGSH (DNL126)
Sanfilippo A (MPSIIIB)*	N-acetyl-alpha-D-glucosaminidase (NAGLU)	hTfR-Fab-NAGLU (JR-446)

1 The transport domain of these fusion proteins is a monoclonal antibody directed to the mouse BBB-transferrin receptor (mTfRMAb) or the human BBB transferrin receptor (hTfRMAb). *Represents an indication that has a primary CNS disease burden. From Boado RSC Pharmaceutics 2025 (DOI: 10.1039/d5pm00204d).

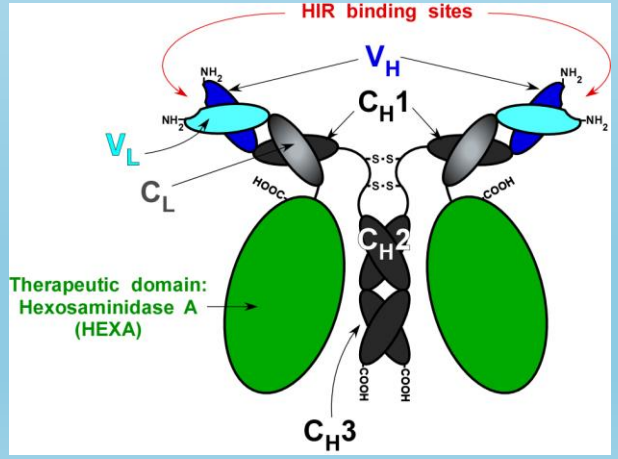
Bi-functional IgG-lysosomal enzyme fusion proteins for brain drug delivery (Sci Rep 2019)



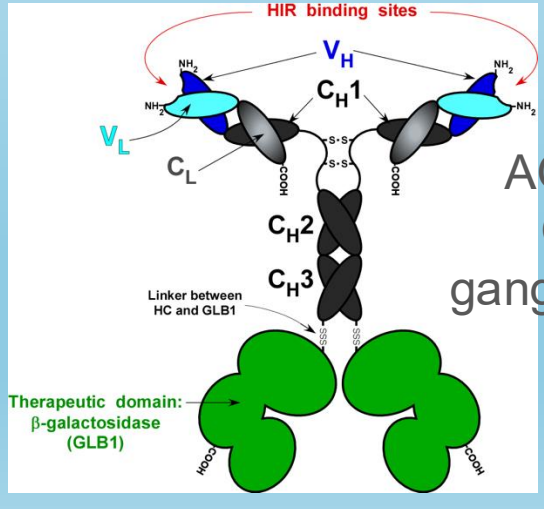
AGT-189
Niemann-Pick A/B



AGT-194
Batten Type 1

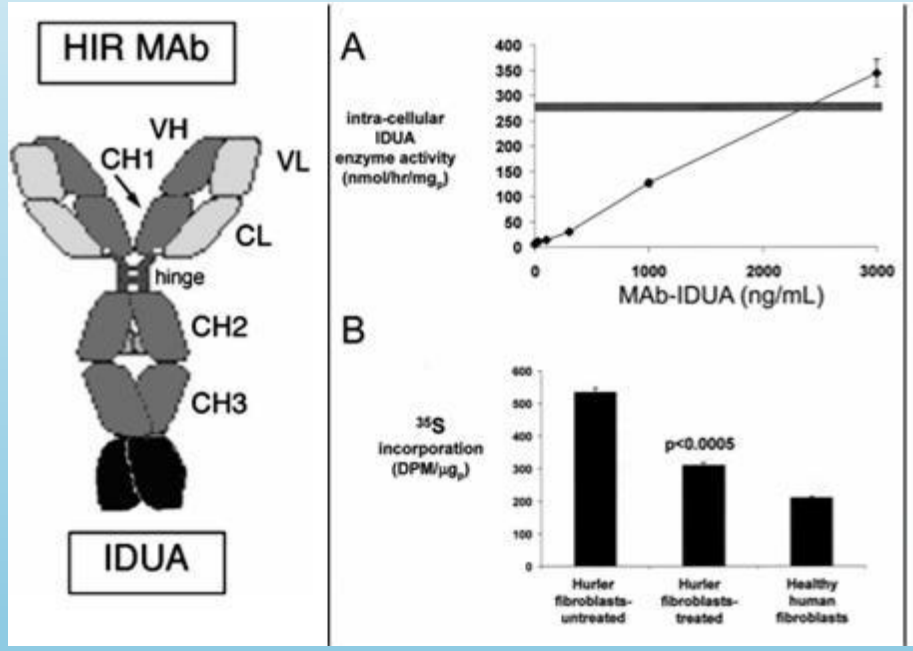


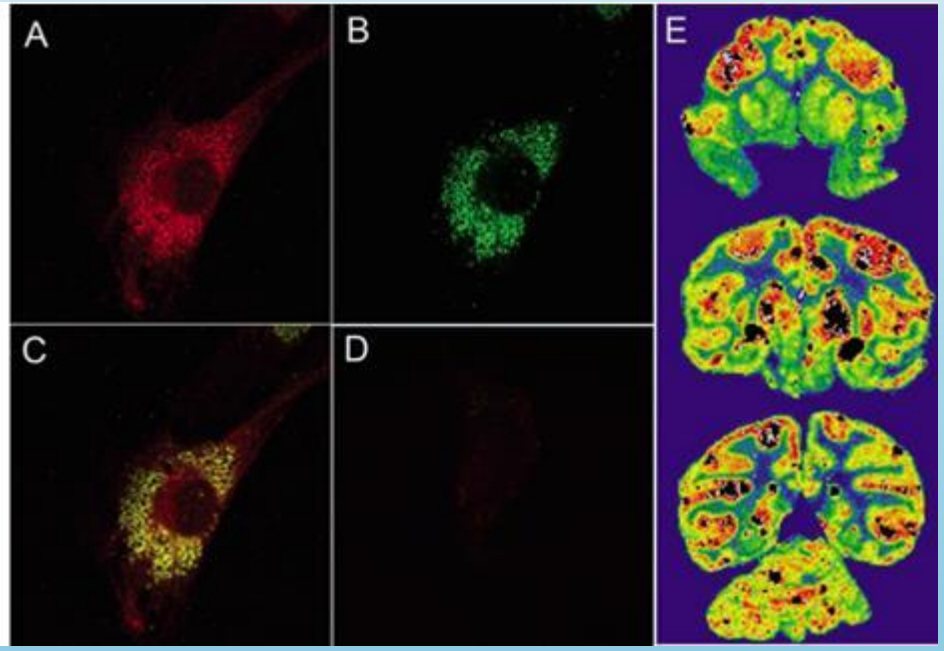
AGT-192
Tay-Sachs



AGT-195
GM1-gangliosidosis

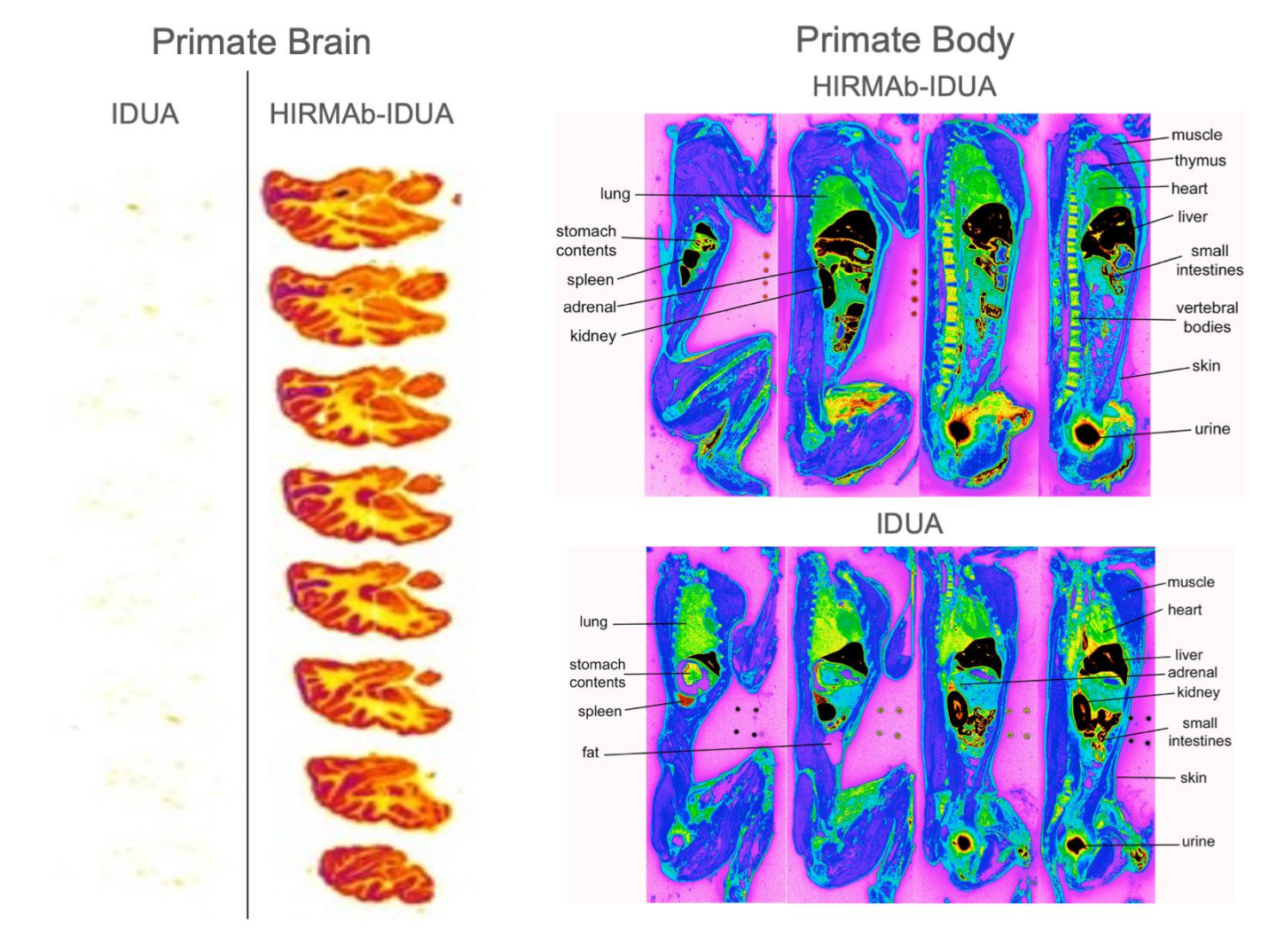
Genetic engineering and validation of a HIRMAb-iduronidase (IDUA) (valanafusp alpha, AGT-181) fusion protein for Hurler's syndrome





(*Biotech Bioeng 2008*)

Biodistribution of AHIRMAb-IDUA and Laronidase (IDUA) in the Rhesus monkey



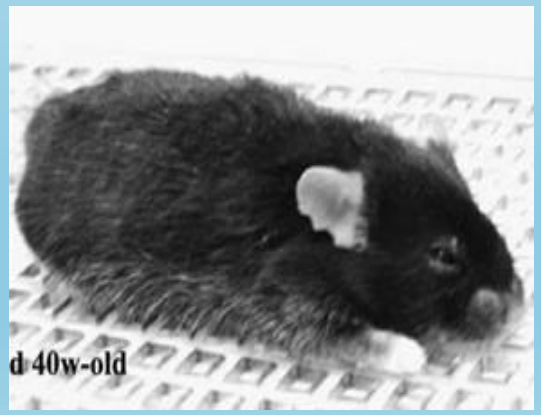
- Lysosomal enzyme (IDUA) alone does not cross the BBB, owing to absence of M6PR expression at BBB.
- IDUA fused to HIRMAb Trojan horse distributes to all parts of brain following intravenous administration.
- Distribution of IDUA to somatic organs is comparable whether the enzyme is administered alone or as the AGT-181 fusion protein.

Mol Pharm 2017

Efficacy of TfRMAb-IDUA in MPS I mouse model

(Molec. Pharm. 2011)

DISEASE	DEFICIENT ENZYME	STANDARD OF CARE
Hurler Syndrome (MPS I)	a-L-iduronidase (IDUA)	Aldurazyme® enzyme replacement therapy (ERT) administered weekly by IV
STUDY DESIGN	**DOSE**	**CONCLUSION**
Mouse with MPS I (age: 6 months)	TfRMAb-IDUA (1 mg/kg) administered 2 times per week by IV for 8 weeks	Reductions in: • Lysosomal inclusion bodies in brain • Glycosoaminoglycans in peripheral organs • Immune response

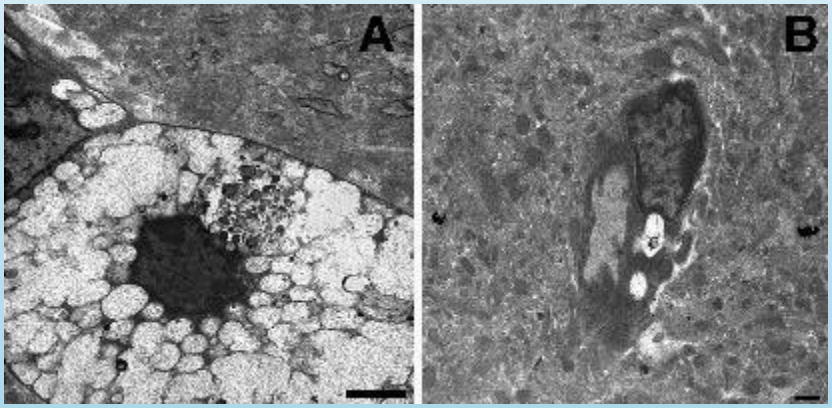


Efficacy of TfRMAb-IDUA in MPS I mouse model

(Molec. Pharm. 2011)

Organ	Organ GAG (µg/mg protein)	
	Saline	TfRMAb-IDUA
Liver	77.8 ± 7.4	< 2.5****
Spleen	49.6 ± 11.6	9.9 ± 3.1**
Kidney	46.6 ± 6.2	37.2 ± 3.0
Heart	35.1 ± 3.8	22.6 ± 3.2*
* p < 0.05, ** p < 0.01, **** p < 0.001		

Saline TfRMAb-IDUA



Electron Microscopy of Reduced Lysosomal Inclusion Bodies Following TfRMAb-IDUA Treatment

Group	Number of Multi-vacuolated Brain Cells/100 Brain Cell Nucleoli
Saline	18.5 ± 1.1
AGT-m181	5.0 ± 1.6 (p<0.005)

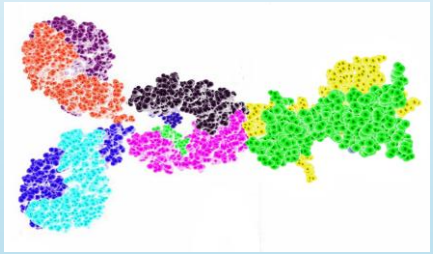
- GAG levels in peripheral tissues reduced comparable to control
- Lysosomal inclusions bodies in the brain reduced 73%

Reduction in Brain Heparan Sulfate with Systemic Administration of an TfRMAb-Sulfamindase Fusion Protein in the MPS Type IIIA Mouse

(Mol Pharm 2018)

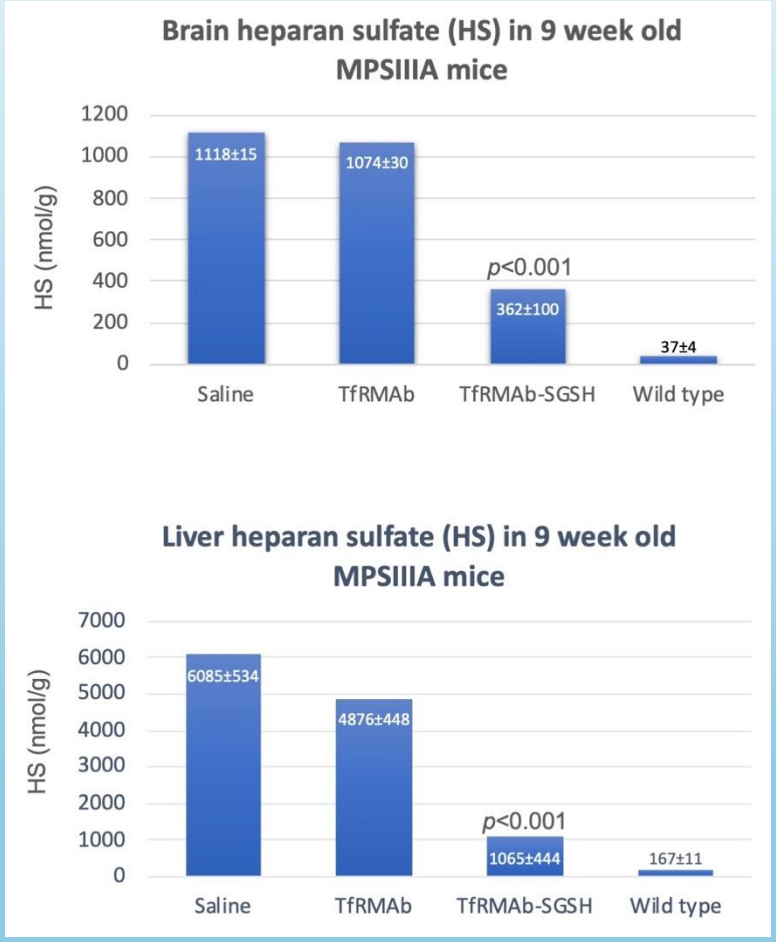
Therapeutic Agent

Genetically engineered TfRMAb that is specific for the mouse TfR.



Human SGSH, minus the enzyme signal peptide, was fused to the C-terminus of each heavy chain

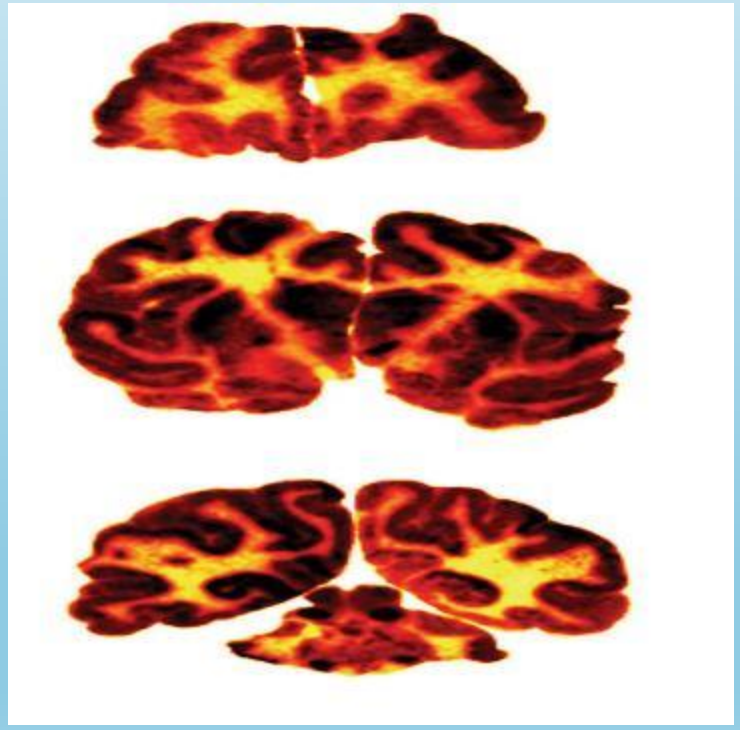
- 2 weeks old MPSIIIA mice (JAX) were treated 3 x week x 6 weeks by IP 5 mg/kg of the TfRMAb-SGSH fusion protein or the isotype control . The mice were euthanized 1 week after the last dose.
- Haparan sulfate (HS) was measured in brain and liver by LC-MS following enzymatic digestion into disaccharides using HS disaccharide standards.
- The 30-fold elevation in HS in brain is reduced 70% by the chronic treatment of these mice for 6 weeks with systemic injections. HS is also elevated in liver and treatment with the AGT-m184 fusion protein reduces hepatic HS by 85%.



Distribution to Brain Tissue in Rhesus Monkey

- Autoradiography demonstrates the extensive distribution of drug into all regions of the brain (at 2 hours after IV injection)
  - Autoradiograph of HIRMAb-IDS versus IDS (the enzyme domain of the IgG-enzyme fusion protein)

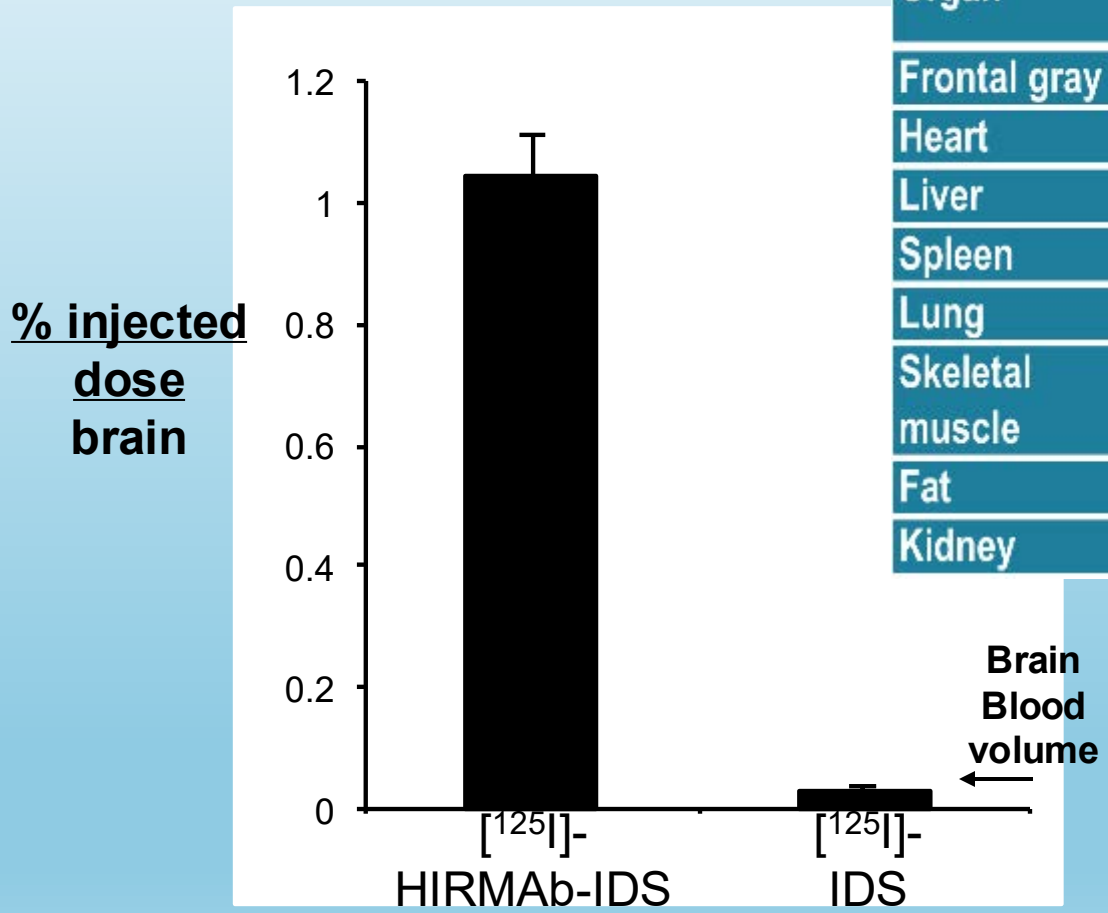
IDS-alone



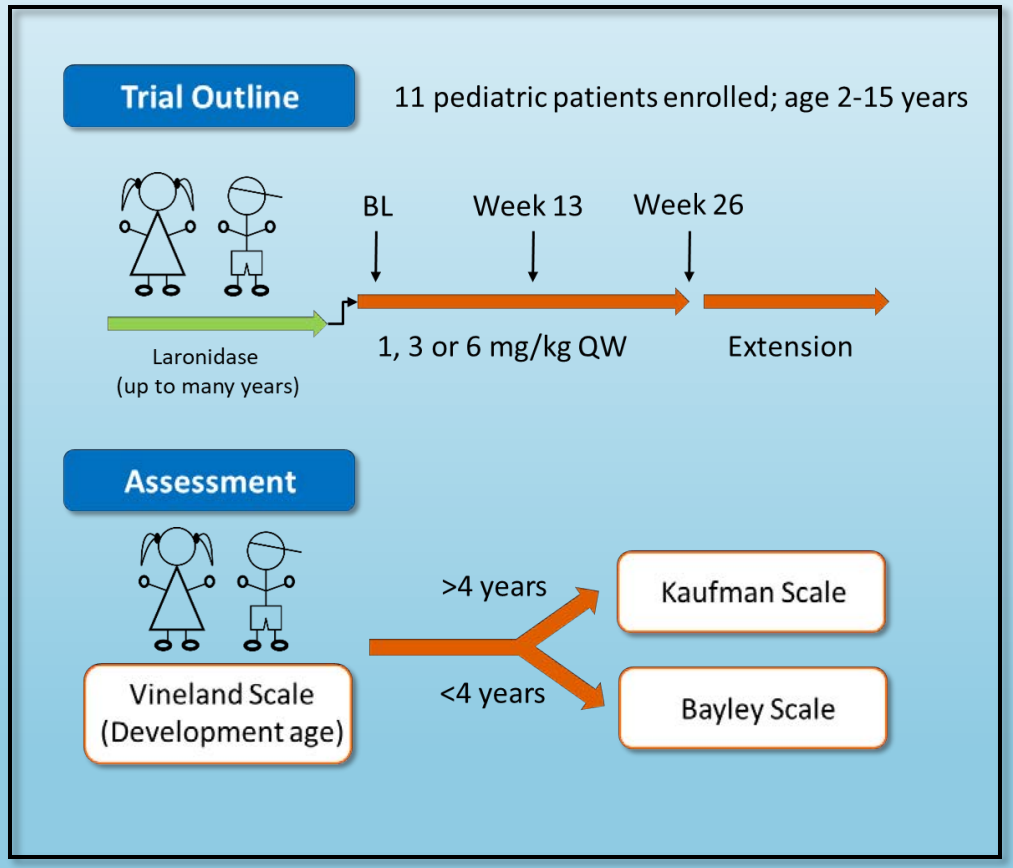
HIRMAb-IDS

Brain uptake & peripheral biodistribution in the Rhesus monkey: *HIRMAb-IDS vs IDS*

Organ	125I-AGT-182	125I-IDS	182/IDS ratio
	Organ Uptake (%ID/100g)		
Frontal gray	1.03 ± 0.07	0.030 ± 0.005	34
Heart	1.32 ± 0.11	1.11 ± 0.20	1
Liver	33.23 ± 4.52	27.76 ± 0.66	1
Spleen	19.24 ± 0.24	12.36 ± 0.16	2
Lung	2.97 ± 0.13	3.35 ± 0.19	1
Skeletal muscle	0.23 ± 0.05	0.20 ± 0.05	1
Fat	0.25 ± 0.02	0.27 ± 0.03	1
Kidney	14.0 ± 0.69	20.97 ± 1.54	1



Valanafusp alpha (HIRMAb-IDUA) Proof of Concept Clinical Trial Design in Pediatric MPS I Patients



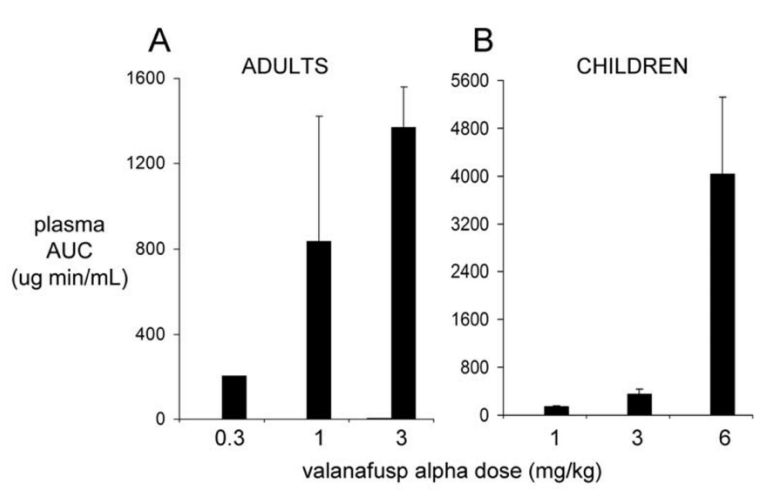
- Pediatric MPS I patients who had been on laronidase were immediately switched to AGT-181 and treated with weekly infusions of 1, 3 or 6 mg/kg for 6 months.
- All patients who completed the 6-month study remained in the extension arm for another 6 months.

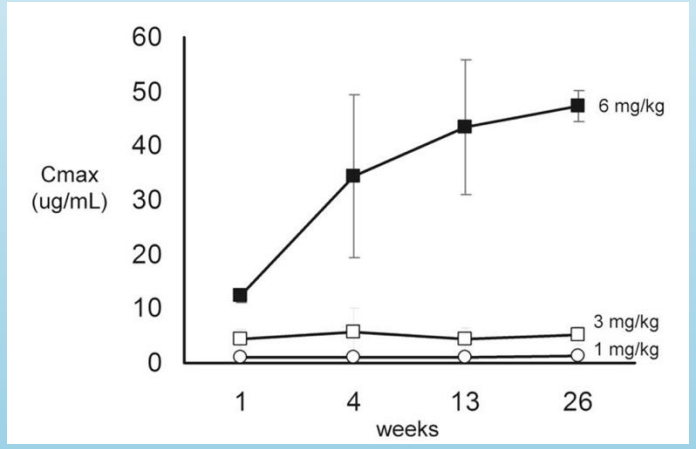
Endpoints investigated

- Safety, and pharmacokinetic properties.
- Total urinary GAGs.
- Liver and spleen volume (MRI).
- Brain volumetrics (MRI and MRI DTI).
- Shoulder ROM.
- Age-appropriate neurocognitive testing.

Plasma Pharmacokinetics of Valanafusp alpha (HIRMAb-IDUA) in Patients with Mucopolysaccharidosis Type I (BioDrugs 2018)

Plasma AUC is non-linear at 6 mg/kg and Cmax at 6 mg/kg increases over time





- 3 mg/kg is a safe dose to conduct the pivotal trial
- Increased Cmax over time may be explained by down-regulation of the M6P receptor

Drug Related Adverse Events in Phase II

Infusion Related Reactions (IRR)

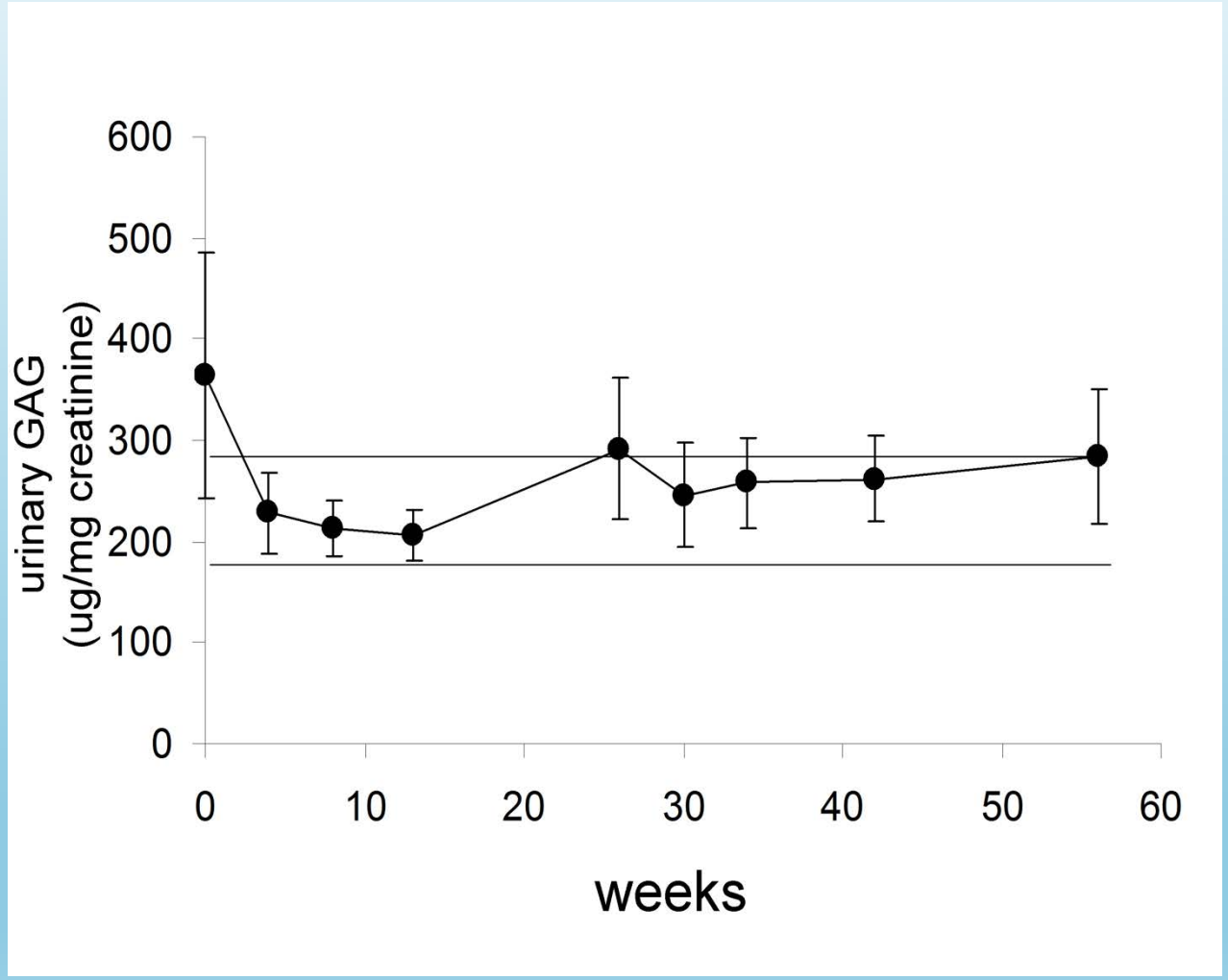
- 10 IRRs with >570 infusions
- incidence of 1.7%
- 60% of IRRs were observed in a single patient not previously on ERT; tolerance to drug developed by 10th week

Transient hypoglycemia

- overall incidence of 5.9%
- transient and resolved within 10-20 min following snack or glucose sachet
- 62% of all episodes at high dose of 6 mg/kg
- incidence of 2.8% at 1-3 mg/kg infusion dose
- mean glucose =101 ± 20 mg/dl over course of 52 weeks and >3,000 measurements

OJRD 2018

Stabilization of Urinary GAGs in Pediatric Subjects with MPSI on Valanafusp alpha (HIRMAb-IDUA) for 52 Weeks



mean ± SEM of uGAG in pediatric subjects on laronidase (Wraith et al, 2007)

OJRD 2018

Somatic Improvement in Pediatric Subjects with MPSI on Valanafusp alpha (HIRMAb-IDUA) for 52 Weeks

parameter	Change at 52 weeks from baseline*
Liver volume	Decreased 23 % ($p<0.0005$)
Spleen volume	Decreased 26 % ($p<0.0005$)
Shoulder flexion (w26)	Increased 10.9 degrees ($p<0.01$)
Shoulder extension (w26)	Increased 9.5 degrees ($p<0.01$)

*9/11 patients were already in ERT before the study

OJRD 2018

Cognitive Data in Trial Valanafusp alpha (HIRMAb-IDUA) in Context With The Natural History of MPS I

Study	Patients	DQ Change per year
Krivit et al. 1999	Severe untransplanted MPS I	-20.5
Valanafusp alpha (HIRMAb-IDUA)	Severe untransplanted MPS I treated with AGT-181	-0.6
Valanafusp alpha (HIRMAb-IDUA)	MPS I attenuated treated with AGT-181	+7

Conclusion: A dramatic change in stabilization of DQ is observed in valanafusp alpha (HIRMAb-IDUA) treated pediatric patients compared to the natural history of untransplanted untreated MPS IH patients

Completed and in progress clinical trials with brain penetrating IgG-fusion proteins in LSD

Indication	Therapeutic Domain	IgG-Fusion Protein	ID
Hurler syndrome (MPS I)	Iduronidase (IDUA)	HIRMAb-IDUA (valanafusp alpha, AGT-181)	Phase I/II completed NCT02371226,NCT02597114, NCT03053089,NCT03071341
Hunter syndrome (MPS II)	Iduronate-2-sulfatase (IDS)	HIRMAb-IDS (AGT-182)	Phase I completed NCT02262338
Hunter syndrome (MPS II)	Iduronate-2-sulfatase (IDS)	HIR-Fab-IDS (GNR-055)	Phase I/II in progress, NCT05208281
Hunter syndrome (MPS I)	Iduronidase (IDUA)	hTfR-Fab-IDUA (lepunafusp alfa, JR-171)	Phase I/II in progress, NCT04227600, NCT04453085
Hunter syndrome (MPS II)	**Iduronate-2-sulfatase (IDS)**	**hTfRMAb-IDS (pabinafusp alfa, JR-141)**	**Phase I/II completed, NCT03128593, NCT03359213 Phase III in progress, NCT03568175, NCT04573023 Approved in Japan**
Hunter syndrome (MPS II)	**Iduronate-2-sulfatase (IDS)**	**TfR transport vehicle, ETV:IDS (tividenofusp alpha, DNL310)**	**Phase I/II/III in progress, NCT04251026, NCT05371613**
Sanfilippo A (MPSIIIA)	Sulfamidase (SGSH)	hTfR-Fab-SGSH (postnafusp alfa, JR-441),	Phase I/II in progress, NCT06095388
Sanfilippo A (MPSIIIA)	Sulfamidase (SGSH)	TfR transport vehicle, EVT:SGSH (DNL126)	Phase I/II in progress, NCT06181136
Sanfilippo B (MPSIIIB)	N-acetyl-alpha-D-glucosaminidase (NAGLU)	hTfR-Fab-NAGLU (JR-446)	Phase I/II in progress, NCT06488924

From Boado RSC Pharmaceutics 2025 (DOI: 10.1039/d5pm00204d)

Conclusions

- A broad range of brain-penetrating IgG-fusion proteins for LSD have been engineered, validated in animal models, and currently being evaluated in human clinical trials, with pabinafusp alfa being the first of this class of biologicals approved by a regulatory agency in Japan.

- Pending further drug development, other members of the brain-penetrating IgG fusion protein family are positioned to become a new generation of pharmaceutical drugs for the treatment of human lysosomal storage disorders.

Thank You!