Strategy for creating molecules that cross the blood-brain barrier using LRP1 targeting:

Using the Angiopep-2 Peptide to Create a GFP Fusion Protein

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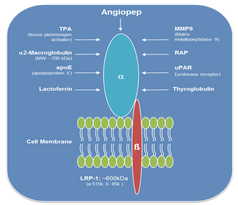
Introduction

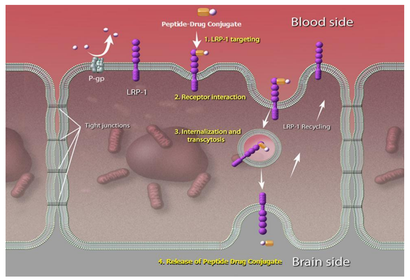
The blood-brain barrier (BBB) is a major obstacle to treatment of CNS disorders with biologics such as enzyme replacement therapy.

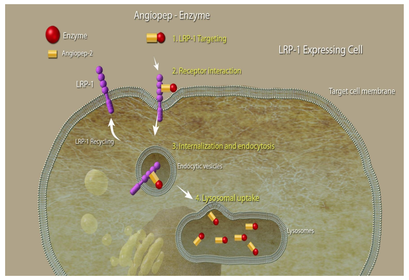
One strategy for creating brain-penetrant biologics is to target BBB receptors, such as the low-density lipoprotein receptor-related protein-1 (LRP1). This receptor has a number of inherent biochemical advantages for drug transport across the BBB. These include high capacity, rapid turnover, recognition of numerous ligands, and limited down-regulation.



In endothelial cells as at the BBB, LRP1 functions is to bring ligands into and across the cell via receptor-mediated transcytosis.

In non-endothelial cells, LRP1 functions as a scavenging receptor, directing ligand transport into the lysosome via receptor-mediated endocytosis.

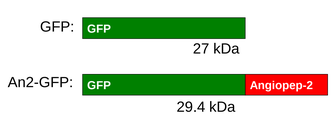


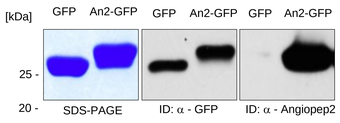


We have created peptides (Angiopeps), including Angiopep-2 (An2) using a library based on LPR-1 binding sequences of known LRP-1 ligands. These peptides can be introduced, by chemical conjugation or recombinant fusion, to small molecules and biologics, thus forming NCEs that are brain-penetrant **Peptide-Drug Conjugates.**

Experimental Methods

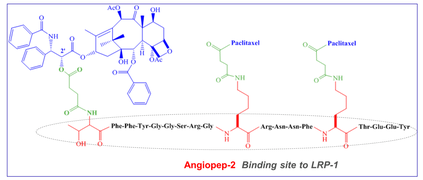
An2-GFP cDNA was constructed as shown below. Purified An2-GFP and GFP were used for iv injection to adult CD-1 mice. Sixty minutes following An2-GFP injection, the mice were sacrificed by cardiac perfusion with saline. Brains were frozen in isopentane, cooled to -40°C and cut into 6 mm sections. The images were generated by direct fluorescence or indirect immunohistochemical staining using anti-GFP IgGs. Immunostaining was visualized using an Alexa-488 labeled secondary antibody, shown by green fluorescence. Nuclear staining was performed with Hoechst dye. GFP fluorescence was observed under UV illumination: Reichert (filter B1: BP 450-495; DS 510; LP 520) for single green fluorescence or Omega Opticals (filter XF56, triple band) for double blue (Hoechst) and green fluorescence.





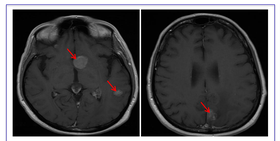
Clinical Proof of Concept: ANG1005 (An2-paclitaxel)

Conjugation of paclitaxel, which does not cross the BBB, and An2 has created a brain-penetrant chemo-therapeutic New Chemical Entity that crosses the BBB.



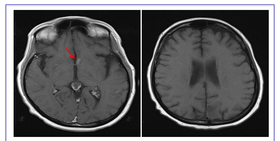
Phase 1 and Phase 2a trials completed in patients with brain tumors:

- Tolerability profile similar to paclitaxel
- Efficacy in high dose group including MTD, Phase 1:
  - Brain Mets (n=21): 71% with tumor shrinkage or stabilization
  - Recurrent Glioma (n=28): 61% tumor shrinkage or stabilization, including 2 complete responders
- Efficacy at 550 mg/m^2, Phase 2a:
  - BC Brain Mets (n=80): 61% tumor shrinkage or stabilization



Baseline

3 lesions: left caudate, left temporal lobe & left subinsaler cortex
T1 Axial Post



79%↓ in tumor size (Wk 24)
After 8 cycles of ANG1005

Out of the 3 lesions, only 1 lesion seen (left caudate)
T1 Axial Post

ANG1005 development continues with two Phase 2 clinical trials:

- Phase 2a in recurrent glioma - started October 2013
- Phase 2b in breast cancer with brain metastasis – starting Q1 2014

Lysosomal Storage Diseases: On Going & Future Studies

In-House

- MPS1 Program
  - Created multiple fusion IDUA proteins
  - On-going lead optimization of chemical conjugates
  - IDUA activity in brains of IDUA-/- mice following iv dosing has been demonstrated.

On-Going Collaborations

- GSK
- Genzyme

Conclusions

While other receptor-mediated transcytosis systems have shown preclinical data, the LRP-1 strategy is the only one with clinical validation.

Clinical validation

- CNS responses seen in breast cancer brain metastases (Ph2), glioma (Ph1/2), brain metastases from various cancers (Ph1/2)

Pre-clinical validation

- Enzymes, monoclonal antibodies , peptides
- All show increased transport into the CNS when linked to Angiopeps,

Preclinical Proof of Concept: Green Fluorescent Protein (GFP)

Uptake of An2-GFP in brain endothelial cells (in vitro)

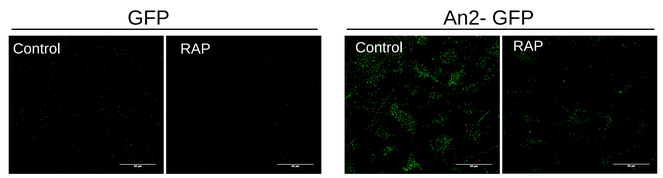


Figure 1. Uptake (1hr) of GFP and An2-GFP in human brain Endothelial cells. Presence of RAP (1μM), an LRP-1 inhibitor, blocked An2-GFP internalization.

Brain-penetrant An2-GFP (in vivo)

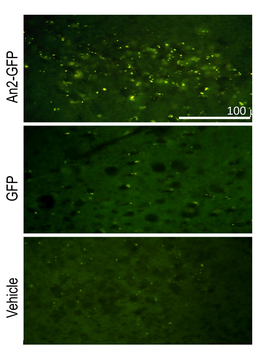


Figure 2. 60 minutes following tail vein administration of native GFP (15 mg/kg) to mouse produces brain fluorescence that is limited to capillaries (arrows), while administration of the An2-GFP fusion protein results in fluorescence in parenchyma. These results suggest that GFP does not readily cross the BBB, but addition of the An2 peptide creates a brain-penetrant GFP.

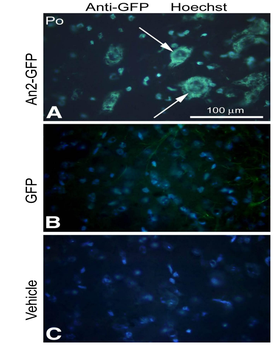


Figure 3. GFP Immunostaining (green arrows) show a cytoplasmic immunofluorescence pattern, suggesting that An2-GFP is internalized into neuronal cell bodies. Native GFP injection shows no green immunostaining. Cell nuclei are stained in blue with Hoechst dye. Sections are from pontine region. Magnification x460.

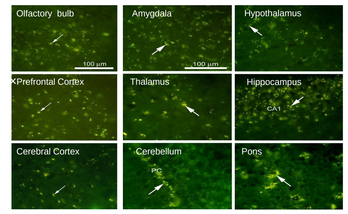


Figure 5. Sixty minutes following i.v. bolus injection, An2-GFP green fluorescence is found to be present in whole brain. Punctate labelling displays a neuronal perinuclear distribution pattern.

Brain Structure	Fluorescence Intensity
Olfactory bulb	+++
Amygdala	+++
Hypothalamus	++
Prefrontal Cortex	++
Thalamus	++
Hippocampus	+++
Cerebral Cortex	++
Cerebellum	++
Pons	+++++

Table 1. Regional distribution of GFP fluorescence 60 min An2-GFP post-injection.

CNS Distribution of An2-GFP

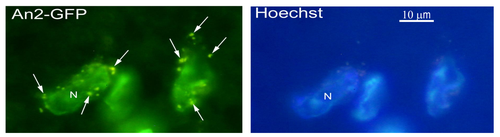


Figure 4. An2-GFP fluorescence (left) in the cortex observed with large-spectrum green fluorescence filter at high magnification. Punctate staining (arrows) indicates cellular internalization. Hoechst staining (right) shows location of nuclei.