Conjugation of the brain penetrant Angiopep-2 peptide to therapeutic anti-HER2 mAb (ANG4043) and Antibody-Drug Conjugates for the treatment of HER2-Positive brain metastases

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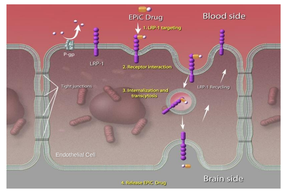
BACKGROUND

Angiochem is a clinical-stage biotechnology company discovering and developing new breakthrough drugs that are uniquely capable of crossing the blood brain barrier (BBB) to treat brain diseases. These new drugs have the potential to address significant medical needs, many of which cannot be effectively addressed due to the fundamental physiological challenge the BBB presents.

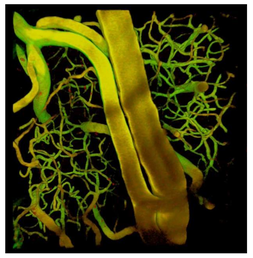
Angiopep Technology



Using LRP1 receptor-mediated transcytosis has a number of inherent biochemical advantages for drug transport across the BBB, including high capacity, rapid turnover, recognition of numerous ligands, and limited down-regulation. We have created peptides (Angiopeps), including Angiopep-2 (An2), using a library based on binding sequences of known LRP-1 ligands.

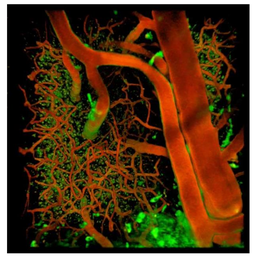
Angiopeps cross the BBB

Intravital imaging in mouse brain after iv Cy5.5-Angiopep injection



Immediately after injection

Following iv administration of Cy5.5-labeled Angiopep (green) and Dextran Texas Red, yellow color in vessels results from presence of both Dextran Texas Red and Angiopep.



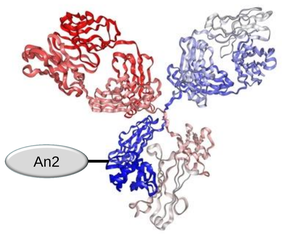
24hrs post-injection

Dexran Texas Red is again administered iv to define blood vessels. Green Angiopep signal occurs outside of red vessels, indicating parenchymal distribution.

HER2+ Brain Metastases

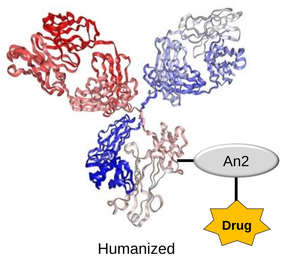
- A critical unmet medical need.
- In US and Europe, 465,000 new diagnoses of breast cancer per year.
- Breast cancers overexpressing HER2 represent ~116,000 patients.
- About 33% of them will develop brain metastases.
- Herceptin does not cross the BBB and therefore can only address peripheral metastases.

ANG4043



Humanized Anti-HER2 mAb

An2-mAb-drug conjugate



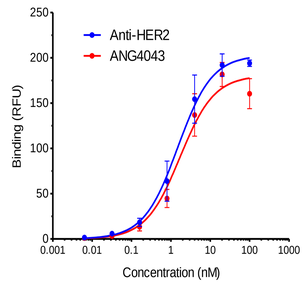
Humanized Anti-HER2 mAb

- ANG4043 is a chemical conjugate of the anti-HER2 mAb with the peptide Angiopep-2 (An2).
- Docetaxel and maytansine were conjugated to An2-anti-HER2 mAb

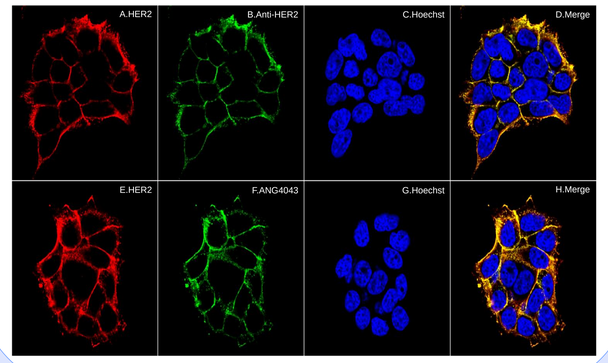
An2-mAb conjugates retain their affinity for HER2 receptor

- Affinity for the HER2 receptor of the mAb is unaffected by incorporation of An2.
- The anti-proliferative potency of ANG4043 is similar to that of anti-HER2 mAb.
- ANG4043 plasma PK is also similar to native mAb.

Binding analysis of anti-HER2 and ANG4043 on BT-474 cells

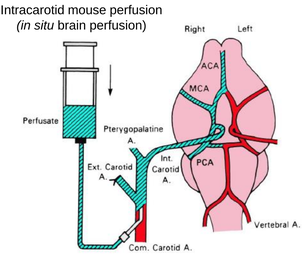


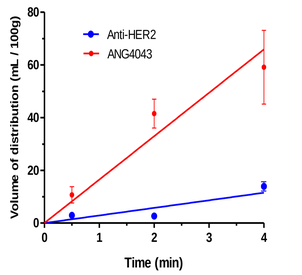
Confocal microscopy analysis after 1h incubation of anti-HER2 and ANG4043 on BT-474 cells



Rate of entry into the brain (BBB permeability)

Intracarotid mouse perfusion (*in situ* brain perfusion)



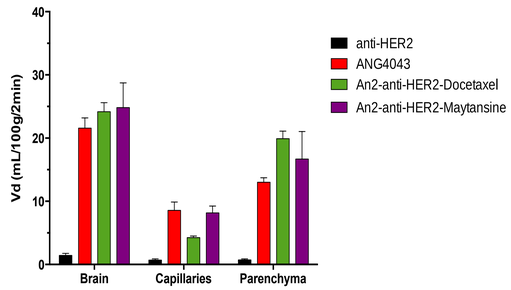


Initial transport rate (Kin) measured by *in situ* brain perfusion in mouse brain demonstrates that Angiopep-2 Drug Conjugates better penetrate the brain than unconjugated drugs. ANG4043 Kin is similar to that of An2-paclitaxel conjugate (ANG1005) which is currently in Phase 2 clinical trials for primary and secondary brain tumors.

Anti-Cancer Drugs	BRAIN K_{in} (ml/s/g)	K_{in} Ratio (ANG vs Ctl)
Anti-HER2	4.8×10^{-4}	---
An2-anti-HER2 (ANG4043)	2.7×10^{-3}	5.6
Paclitaxel	6.9×10^{-4}	---
An2-Paclitaxel (ANG1005)	4.4×10^{-3}	6.4
Doxorubicin	2.8×10^{-4}	---
An2-Doxorubicin (ANG1007)	3.7×10^{-3}	13

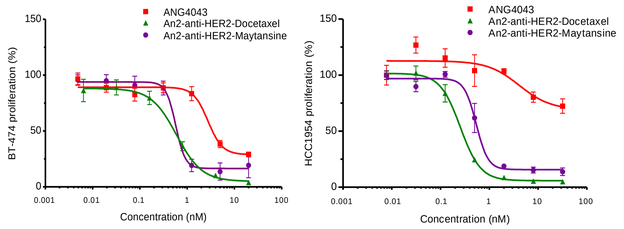
An2-anti-HER2-drug conjugates

Brain uptake of An2-HER2 derivatives (*in situ* brain perfusion)



Brain uptake of [^{125}I]-An2-anti-HER2-drug conjugates was measured by *in situ* brain perfusion and compared to that of [^{125}I]-ANG4043 and unconjugated [^{125}I]-anti-HER2 at 2 minutes. Brain capillary depletion was performed to assess the brain distribution between the brain capillaries and brain parenchyma.

Effect on cell proliferation of sensitive and resistant cells to Trastuzumab



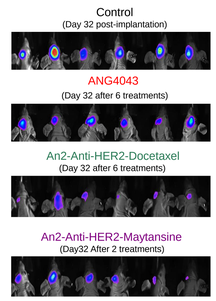
Compilation of proliferation assay - IC_{50} (nM)

Compounds	Sensitive BT-474 cells	Resistant HCC-1954 cells
Anti-HER2	3.6 ± 1.6	---
ANG4043	3.7 ± 1.7	---
An2-anti-HER2-Docetaxel	0.6 ± 0.4	0.2 ± 0.1
An2-anti-HER2-Maytansine	0.9 ± 0.3	0.5 ± 1.1

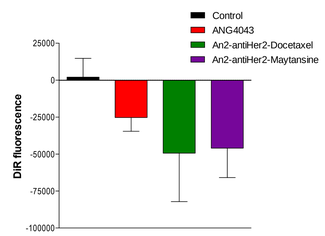
Effect of ANG4043 and An2-anti-HER2-drug conjugates on BT-474/HCC1954 cell proliferation. Cancer cells were incubated for 5 days with the drugs. [^{3}H]-Thymidine incorporation assay was then performed for IC_{50} evaluation.

An2-drug conjugates reduce tumor size

Near Infrared Imaging of fluorescent DiR BT-474 cells



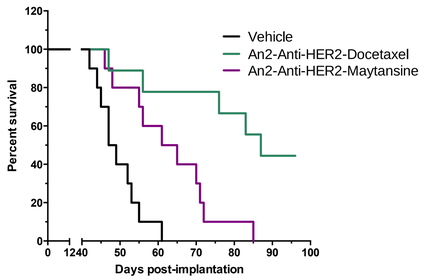
Effect of treatments on fluorescence DiR intensity



Size reduction of HER2-positive tumors with An2-mAb derivatives. BT-474 tumor cells were pre-loaded with a fluorescent dye (DiR), prior to intracranial implantation in mice. Mice were treated twice weekly with ANG4043 (15 mg/kg), An2-anti-HER2-Docetaxel (15 mg/kg) or An2-anti-HER2-Maytansine (15 mg/kg/once every 2 weeks) beginning on day 12. NiR imaging was performed on day 32.

An2-anti-HER2-drug conjugates increase mice survival

- Day 1: Intracranial implantation of BT-474 tumor cells in mice.
- Day 12: Treatments started:
  - Vehicle
  - An2-Anti-HER2-Maytansine (15 mg/kg/once every 2 weeks
  - An2-Anti-HER2-Docetaxel (15 mg/kg/twice a week)



Treatment	Median survival (days)	Increase (%)
Vehicle	48	-
An2-anti-HER2-Docetaxel	87	+ 81%
An2-anti-HER2-Maytansine	63	+ 31%

Early data also indicate that An2-anti-HER2-Docetaxel was better tolerated and more efficacious against BT-474 brain tumors than An2-anti-HER2-Maytansine.

Conclusions

- **Two anticancer drugs (Docetaxel and Maytansine) have been conjugated to An2-anti-HER2.**
- **The brain-penetrant An2-anti-HER2 mAb derivatives target intracranial tumors and shrink tumor size in mice, indicating that therapeutic concentrations have been achieved in brain.**
- **Both new An2-anti-HER2-drug conjugates showed increases in BBB permeability when compared to unconjugated anti-HER2 mAb and in anti-proliferative activities against cell lines sensitive and resistant cell lines to Trastuzumab.**
- **This reduction in tumor size translates to a significant increase in survival in mice with intracranial HER2+ (BT-474 cell) tumors.**
- **These new brain penetrant An2-anti-HER2-drug conjugates (Docetaxel and Maytansine) improved mouse survival.**
- **An2-anti-HER2-Docetaxel showed the strongest potency against HER2-positive BT-474 brain tumors.**
- **These results suggest that the conjugation of Angiopep-2 to anti-HER2 mAb confers higher brain penetration leading to therapeutic efficacy for the treatment of HER2-positive breast cancer brain metastases and that the Angiopep-2 platform may be beneficial for targeting other CNS diseases.**

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