Utilization of the Angiopep platform to enable brain penetration of therapeutic mAbs or Antibody-Drug Conjugates for treatment of brain tumors

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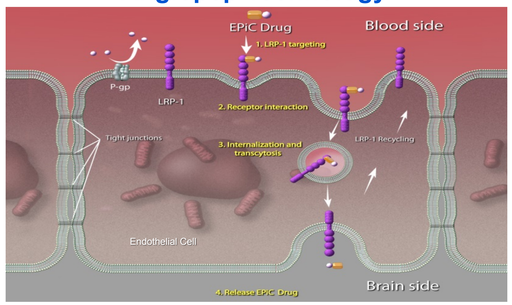
INTRODUCTION

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 50% of them will die from brain metastasis.
- **Herceptin does not cross the BBB and therefore can only address peripheral metastasis**.

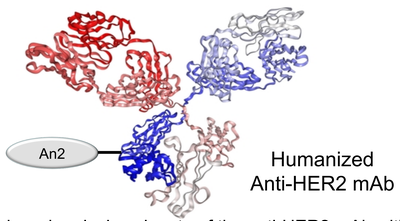
Angiopep Technology



Chemotherapy for malignant brain tumors often has limited efficacy, largely due to restricted blood–brain barrier (BBB) permeability for chemotherapeutic drugs. Angiochem's proprietary Angiopep peptides target the low-density lipoprotein receptor-related protein (LRP) receptor. This endogenous transcytosis system has a number of inherent biochemical advantages for drug transport across the BBB, including high expression, rapid turnover, numerous ligands of varying sizes, and limited down-regulation.

ANG1005 is the first compound developed using our peptide-drug conjugate platform for the treatment of primary and secondary brain cancers. Phase 1/2 clinical trials in humans have been completed. ANG1005 reached therapeutic concentrations in brain tumors and produced significant antitumor responses in patients with primary gliomas or secondary brain metastases. These peptides can be introduced to small molecules and biologics, thus forming NCEs that are brain-penetrant Peptide-Drug Conjugates.

ANG4043

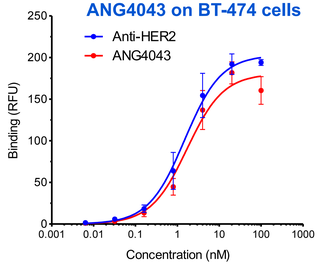


- ANG4043 is a chemical conjugate of the anti-HER2 mAb with the peptide Angiopep-2 (An2).
- ANG4043 is not a biosimilar.

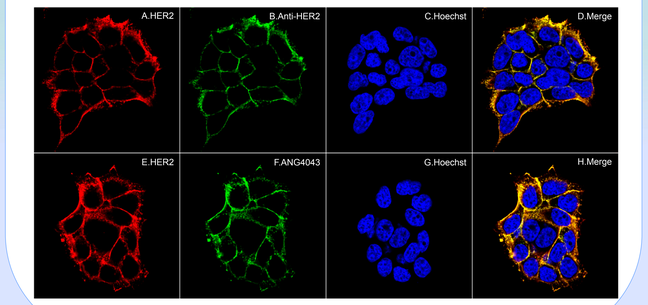
ANG4043 retain anti-HER2 principal characteristics

- 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

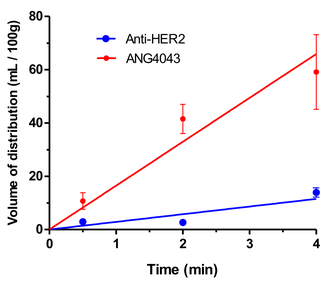


Confocal microscopy analysis after 1h incubation of anti-HER2 and ANG4043 on BT-474 cells



Rate of brain permeability in vivo

In situ brain perfusion



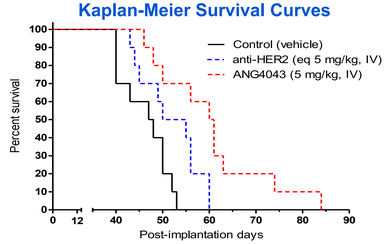
Anti-Cancer Drugs	BRAIN K_{in} (ml/s/g)	K_{in} Ratio (ANG vs Ctl)
Anti-HER2	4.8×10^{-4}	---
An2-anti-HER2 (ANG4043)	2.7×10^{-3}	5.6
Paclitaxel	6.9×10^{-4}	---
An2-Paclitaxel (ANG1005)	4.4×10^{-3}	6.4
Doxorubicin	2.8×10^{-4}	---
An2-Doxorubicin (ANG1007)	3.7×10^{-3}	13

Initial transport rate (Kin) measured by in-situ brain perfusion in mouse brain demonstrates that Angiopep-2 Anticancer Drugs Conjugates better penetrate the brain than unconjugated drugs

ANG4043 Increases Survival

- Day 1: Intracranial implantation of BT-474 tumor cells in mice.
- Day 12: Treatment begins: twice weekly, i.v.
- Comparison study (n=10)
  - Vehicle
  - Anti-HER2 mAb (5 mg/kg)
  - ANG4043 (5 mg/kg equivalent)

Kaplan-Meier Survival Curves

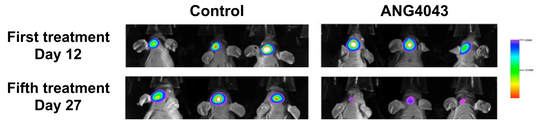


Summary Table for Two Survival Studies

	Control	Anti-Her2 (5 mg/kg)	ANG4043 (5 mg/kg)	ANG4043 (15 mg/kg)
Mean	46 days	52 days	64 days	80 days
% increase vs. control		+13%	+39%	+74%
P value		p=0.028	p=0.0012	p=0.0003
			p=0.019	

ANG4043 Reduces Tumor Size

Near Infrared Imaging of fluorescent DiR BT-474 cells



Size reduction of HER2-positive tumors with ANG4043. BT-474 tumor cells were pre-loaded with a fluorescent dye (DiR), prior to intracranial implantation in mice (day 1). Mice were treated twice weekly with ANG4043, 15 mg/kg, beginning on day 12. NiR imaging was performed on day 27, at which point 5 treatments had been administered.

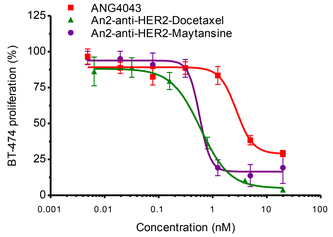
Angiopep2-anti-HER2-drug Conjugates

Two anticancer drugs (docetaxel and maytansine) have been conjugated to An2-anti-HER2 derivatives

Effect on BT-474 cell proliferation

Proliferation assay in BT-474

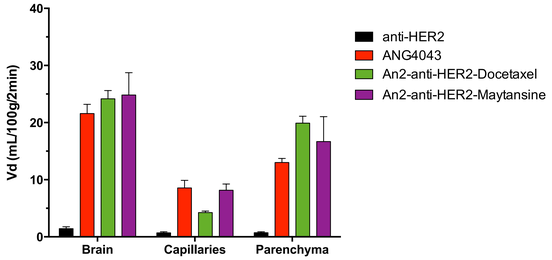
5 days treatment follow by 4h Thymidine -^{3}H incorporation



IC50 (nM)	
ANG4043	2.76 ± 1.13
An2-anti-HER2-Docetaxel	0.60 ± 1.09
An2-anti-HER2-Maytansine	0.57 ± 1.18

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

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 for 2 minutes. Brain capillary depletion was performed to assess the brain distribution between the brain capillaries and brain parenchyma.

Conclusions

- **The brain-penetrant An2-anti-HER2 mAb conjugate, ANG4043, targets intracranial tumors and shrinks tumor size in mice, indicating that therapeutic concentrations have been achieved in brain.**
- **This reduction in tumor size translates to a significant increase in survival in mice with intracranial HER2+ (BT-474 cell) tumors.**
- **Two anticancer drugs (docetaxel and maytansine) have been conjugated to An2-anti-HER2.**
- **Both drugs showed increased anti-proliferative activities and BBB permeability when compared to unconjugated anti-HER2 mAb.**
- **These new brain penetrant An2-anti-HER2-drug conjugates (An2-ADCs) could further improve mouse survival observed with ANG4043.**

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