ABSTRACT # 864

ANG4043: A new brain-penetrant peptide-mAb conjugate that reduces tumor growth in a HER2-positive orthotopic tumor model.

Jean E. Lachowicz, Michel Demeule, Christian Ché, Sasmita Tripathy, Jean-Christophe Currie, Anthony Régina, Tran Nguyen, Simon Lord-Dufour, Jean-Paul Castaigne.

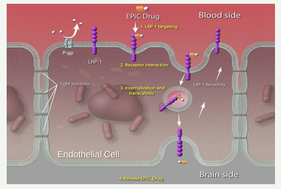
Angiochem Inc., 201 President Kennedy Avenue, PK-2880, Montréal, QC, Canada H2X 3Y7

Angiochem BEYOND BARRIERS

Abstract

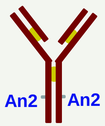
While monoclonal antibodies to receptor tyrosine kinases such as HER2 have been demonstrated to reduce tumor size and increase survival, these agents achieve little to no brain penetration, making them ineffective against metastatic brain tumors. The blood-brain barrier (BBB), efficient at restricting entry of proteins such as mAbs into the brain, is comprised of capillary endothelial cells with tight junctions and efflux pumps. Entry of nutrients, hormones, and other required molecules is accomplished by processes such as receptor-mediated transcytosis. As low-density lipoprotein receptor-related protein 1 (LRP1) is known to perform this function in BBB endothelial cells, we have created a family of peptides (Angiopeps) designed for LRP1 recognition. These proprietary Angiopeps can be used to create novel Peptide-Drug Conjugates that successfully cross the BBB by receptor-mediated transcytosis. Here we describe chemical conjugation of Angiopep-2 (An2) to a mAb against HER2. This Peptide-Antibody Conjugate, ANG 4043, displays HER2 binding affinity and in vitro cytotoxic potency similar to that of native anti-HER2. ANG4043 demonstrates a high rate of entry into the brain, consistent with achieving therapeutic concentrations. The plasma half-life of ANG4043 is similar to that of Anti-HER2 in mouse. Mice with intracranially implanted BT-474 cells showed reduced brain tumor size when dosed with ANG4043 compared to controls. Overall, these data demonstrate that a brain-penetrant Peptide-Antibody Conjugate is efficacious in a mouse HER2-positive tumor model. These results extend the validation of An2 conjugation beyond small molecules and peptides to include larger molecules such as therapeutic mAbs for development of new brain-penetrant antitumor therapeutics.

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.

Angiochem's proprietary platform targets low-density lipoprotein receptor-related protein-1 (LRP1). This endogenous transcytosis system 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 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.



Overexpression of the receptor tyrosine kinase HER2 (ErbB2, human epidermal growth factor receptor 2) is characteristic of 20-30% of breast cancers, with 50% of HER2+ breast cancers leading to brain metastasis. A humanized monoclonal antibody to HER2 (trastuzumab, Herceptin, Genentech) is currently in clinical use for treating HER2+ tumors. While success has been achieved in treating such cancers in peripheral tissues, mAbs do not readily cross the BBB, thus limiting their utility for metastatic brain tumors. Conjugation of anti-HER2 mAb with stable linkers to An2, we have created a peptide-mAb conjugate that is recognized by LRP1 and crosses from plasma to brain by receptor-mediated transcytosis. Here we report brain entry and reduction in brain tumor size by a peptide-anti-HER2 conjugate, ANG4043.

Methods

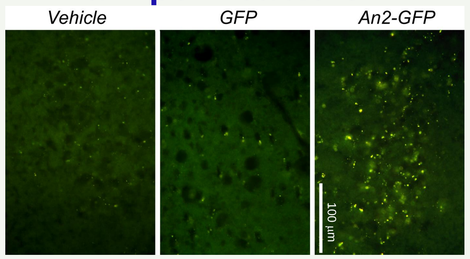
1. Preparation of An2-GFP and An2-anti-HER2 (ANG4043) conjugates. An2-GFP was prepared as a recombinant fusion protein between An2 and GPF. ANG4043 was prepared by chemical conjugation of An2 to the mAb via linker attachment.

2. Binding of anti-HER2 and ANG4043 to BT-474 cells. BT-474 cells (10^6 per tube) were incubated with with increasing concentrations of ANG4043 or anti-HER2 mAb in ice-cold Binding Buffer (Hepes/NaCl/$CaCl_2$) for 30 minutes at 4°C followed by washing and incubation with an α-Human-AlexaFluor488 secondary antibody. Cells were extensively washed with ice-cold BB and analyzed by flow cytometry (10,000 gated events per condition).

3. Pharmacokinetics in plasma. Adult mice were administered 10 mg/kg [^{125}I]ANG4043 via tail vein. At timed intervals, plasma was collected and ^{125}I quantified by gamma counting.

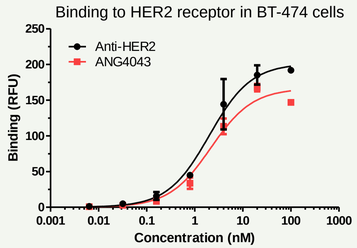
4. Imaging of mice in tumor cells For near infrared (NiR) in vivo imaging studies, ANG4043 and anti-HER2 antibody were labeled with a NiR reactive dye Cyto750-NHS ester (Cytodiagnostics, Burlington, ON) according manu-facturer's protocol and separated from the unreacted dye by gel filtration. Mouse imaging was performed 24 hrs after mAb iv dosing using the in vivo Xtreme imaging system from Carestream. For imaging of implanted tumor cells, BT-474 cells were preloaded with DiR, a lipophilic, membrane-intercalating dye, before intracranial implantation.

Technology Validation: Brain-permeable GFP



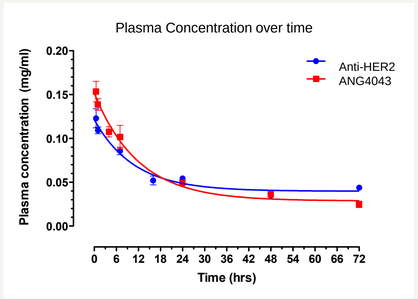
Tail vein administration of GFP (15 mg/kg) to mouse produces brain fluorescence similar to that of vehicle administration, while administration of the An2-GFP fusion protein demonstrates a high level of fluorescence. These results suggest that GFP does not readily cross the BBB, but addition of the An2 peptide creates a brain-penetrant GFP.

An2-anti-HER2 conjugate

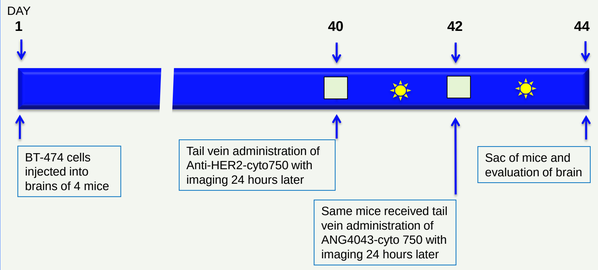


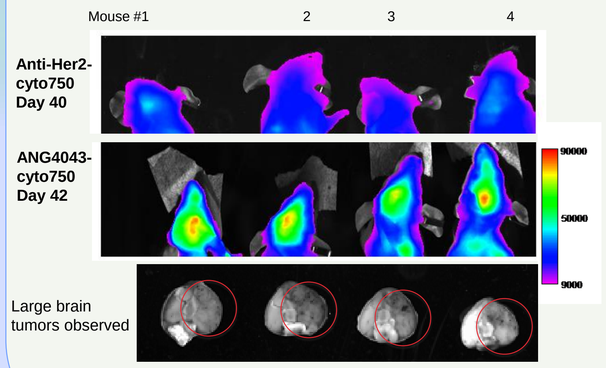
	(IC_{50} nM)
Anti-HER2	0. 2 +/- 0.13
ANG 4043	0.3 +/- 0.09

ANG 4043 retains binding activity and has similar plasma pharmacokinetics compared with native Anti-HER2 mAb.

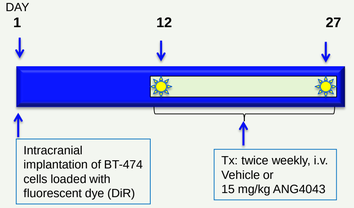


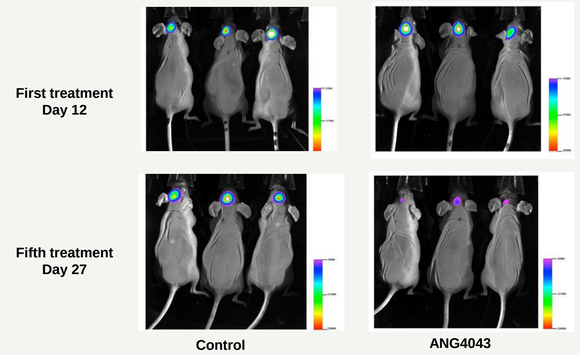
ANG4043 Enters Brain and Targets Tumors

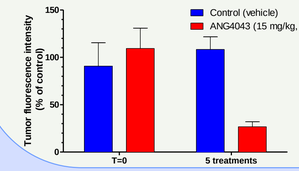




ANG4043 Reduces Tumor Mass







Tumor fluorescence was reduced 75% after five treatments of ANG4043.

Conclusions

- **The peptide An2, which is recognized by LRP-1 receptors on BBB endothelial cells, can be conjugated to proteins to confer brain permeability. This strategy is illustrated by brain fluorescence from systemic An2-GFP administration.**
- **Conjugation of An2 to anti-HER2 mAb (similar to trastuzumab/Herceptin) does not affect its binding affinity or plasma pharmacokinetics.**
- **Limited brain permeability of anti-HER2 mAb is illustrated by systemic administration of fluorescent mAb to mice with intracranial BT-474 tumors. In contrast, systemic administration of ANG4043 (An2-mAb) produces a strong fluorescent signal in brain in the same mice, indicating that ANG4043 enters the brain to recognize HER2 receptors.**
- **BT-474 cells preloaded with a lipophilic fluorescent dye, DiR, produce a fluorescent signal. After five twice-weekly iv treatments, ANG4043 significantly reduced the intensity and area of fluorescence, indicating that ANG4043 reduces tumor cell size in this model of breast cancer brain metastasis.**