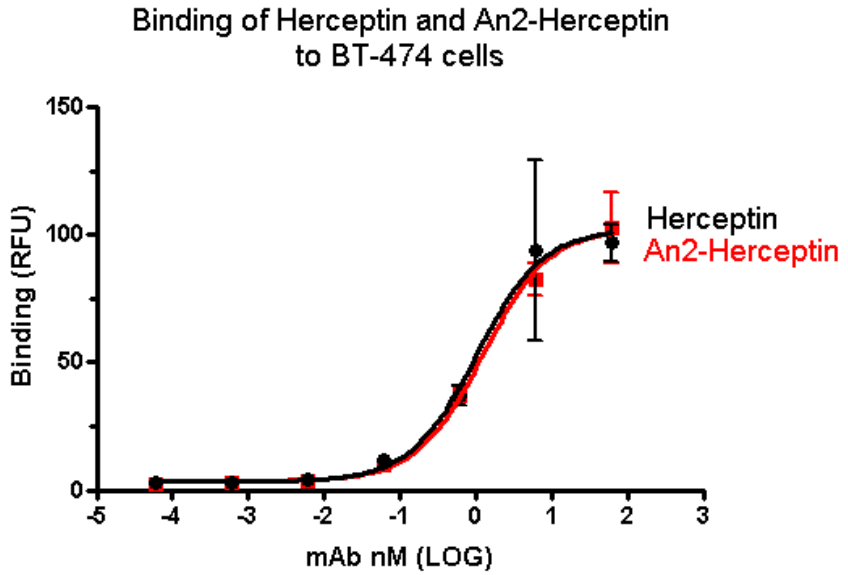
1. Update on Herceptin-Angiopep (ANG4043) status?

Binding of Herceptin and An2-Herceptin to BT-474 Breast Cancer Cells
FACS Analysis – (SATA Chemistry)



IC50
Herceptin : 1.01 ± 0.17 nM
An2-Herceptin : 1.23 ± 0.08 nM

The binding of An2-Herceptin is similar to that observed for the unconjugated Herceptin in BT-474 cells which overexpressed HER-2

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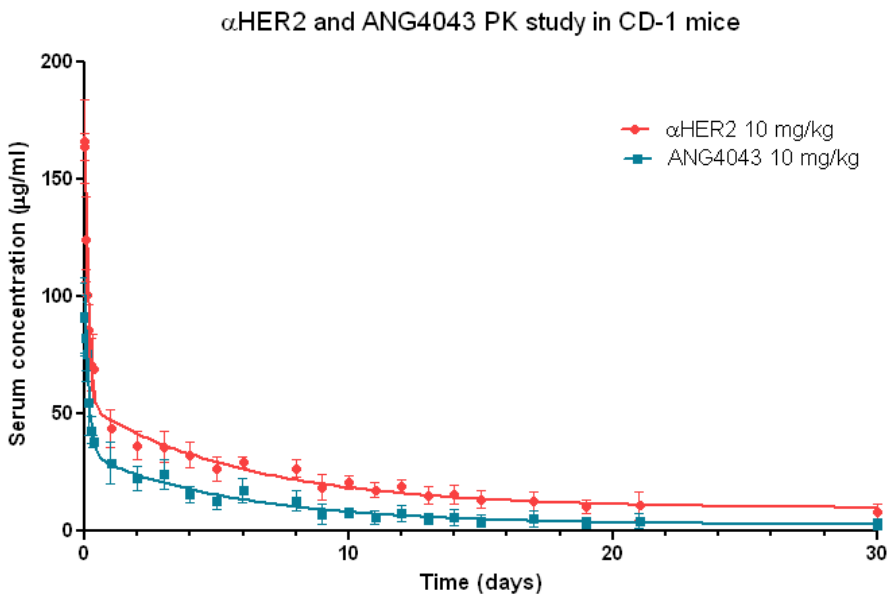
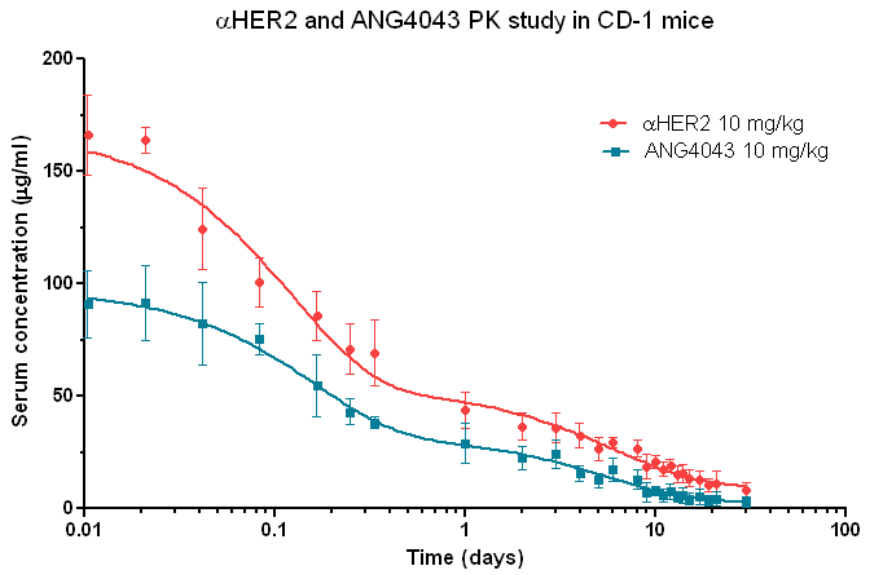
PK Study in C57 Mice (ELISA Assay)

Linear graph



Log graph



Note: healthy, non-tumor bearing mice were used

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Preliminary PK Parameters for ANG4043

(WinNonlin noncompartmental analysis)

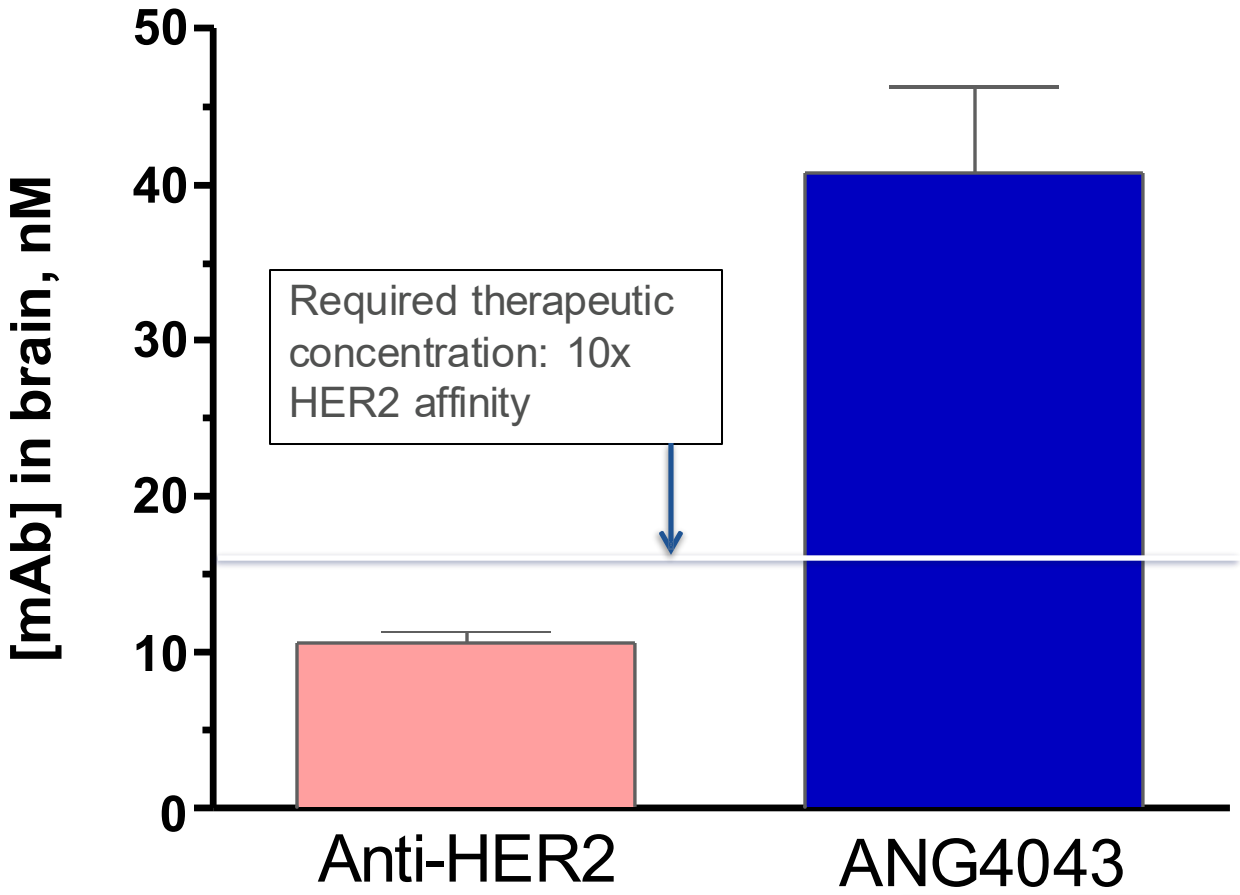
Parameters	αHER2	ANG4043
Cmax (μg/ml)	166	91.5
T1/2β (day)	11.4	8.5
AUC0-30 (day*μg/ml)	573.3	275.4
AUC0-∞ (day*μg/ml)	703.6	310.3
Cl (ml/day)	0.43	0.97
Vd (ml)	7.3	11.8
*MRT (day)	16.7	12.2

***Mean residence time (MRT):** The average total time molecules of a given dose spend in the body

- Increase in the Cl and in the Vd may explain lower Cmax, T1/2β and AUC
- Based on Herceptin monography, the half-life of ANG4043 is still within the range for Herceptin in mice 6-10 days
- The trastuzumab-DM1 (T-DM1) terminal half-life (t1/2,b) in mice: 5.2–5.6 days (Jumbe et al., 2010)

Therapeutic Levels of ANG4043 in Brain

(One hour after IV injection (10 mg/Kg))



Brain/Plasma Ratio

Anti-HER2: 1.2%

ANG4043: 7.9%

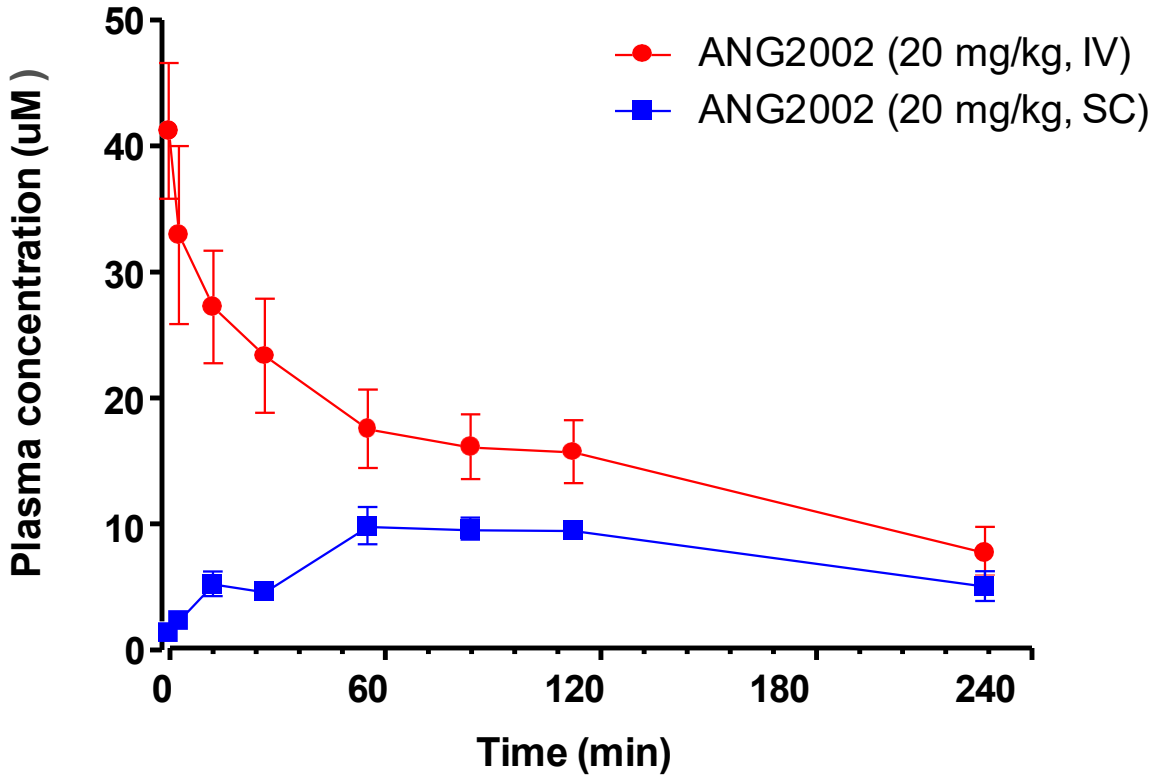
6.5 fold

2. Have you tested the subcutaneous pharmacokinetic profile of ANG4043, or other Angiopep-therapeutic protein conjugate?

- No, we have not tested ANG4043 SC.
- However, we have SC data with ANG-neurotensin (ANG2002) and ANG-exendin-4, details in the following slides (8-12)

2. Have you tested the subcutaneous pharmacokinetic profile of ANG4043, or other Angiopep-therapeutic protein conjugate?

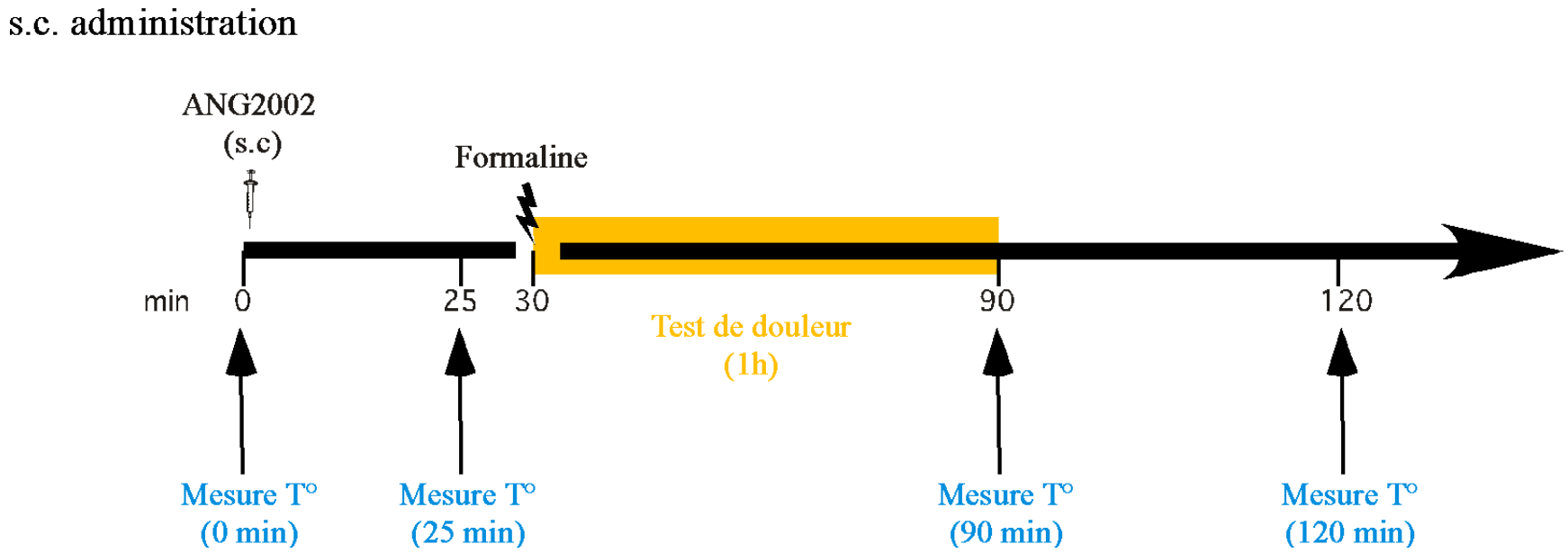
Plasma Concentration of ^{125}I-ANG2002 after IV and SC administration



Biodisponilibility after SC administration : 47%

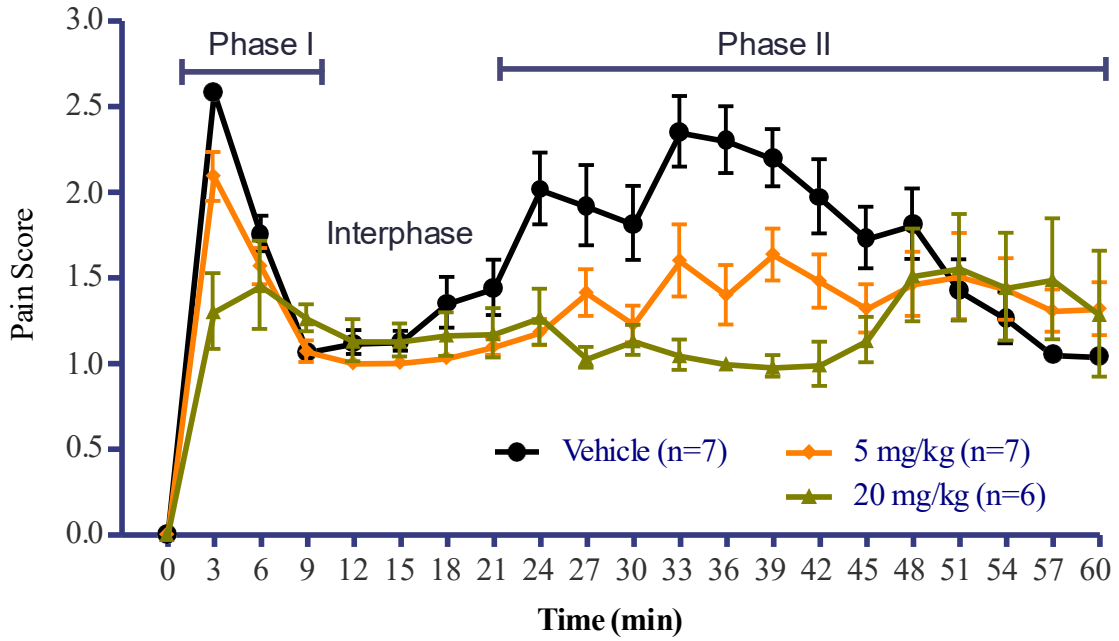
2. Have you tested the subcutaneous pharmacokinetic profile of ANG4043, or other Angiopep-therapeutic protein conjugate?

Experimental protocol for evaluation of subcutaneous ANG2002



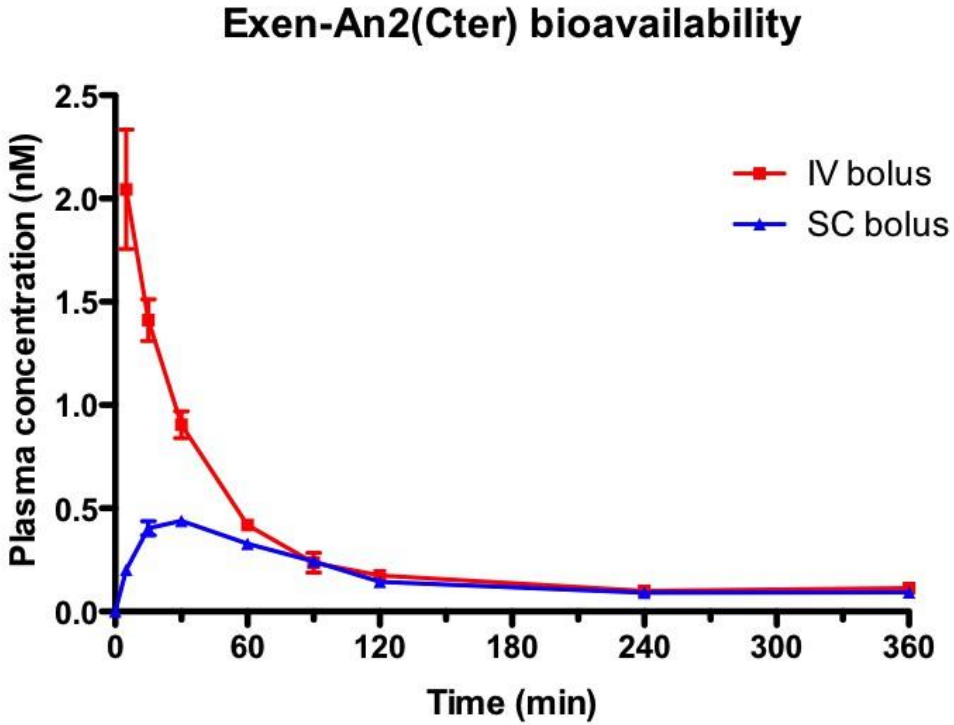
2. Have you tested the subcutaneous pharmacokinetic profile of ANG4043, or other Angiopep-therapeutic protein conjugate?

Effect of subcutaneous injection of ANG2002 in a model of inflammatory pain



2. Have you tested the subcutaneous pharmacokinetic profile of ANG4043, or other Angiopep-therapeutic protein conjugate?

Plasma Concentration after IV and SC injection of ANG-Exendin-4



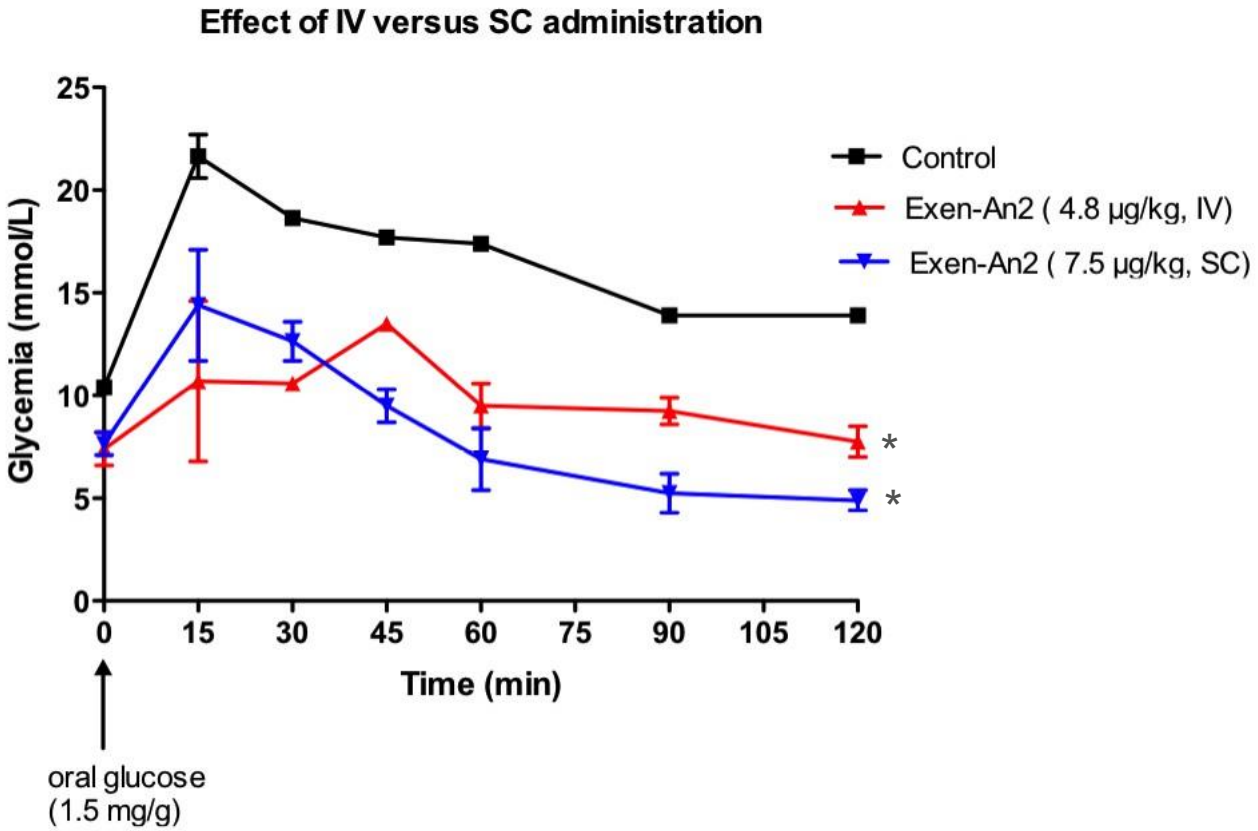
Estimated bioavailability after subcutaneous injection

$(AUC_{s.c.}/AUC_{i.v.})x100=(66/99)x100=66.6\%$

NB: Subcutaneous Exendin-4 bioavailability: 65-75% (Parkes et al., 2001)

2. Have you tested the subcutaneous pharmacokinetic profile of ANG4043, or other Angiopep-therapeutic protein conjugate?

Efficacy after IV and SC administration of ANG-Exendin-4: OGTT in ob/ob mice



NB: Exen-An2 was administered 30 minutes before oral glucose gavage

3. Have you generated any toxicity data on Ang4043, or other Angiopep-therapeutic protein conjugates? If so, does conjugation to the Angiopep alter the toxicity profile of the therapeutic protein?

- We have not generated preclinical toxicity data on ANG4043 or other proteins. However, we have dosed ANG4043 to the limit of solubility (30 mg/kg, 6-fold therapeutic dose) to mice (9 doses) without observing adverse events.
- While not a protein, ANG1005 is the An2-conjugate for which we do have preclinical toxicology testing.
- Toxicities were solely related to Paclitaxel or vehicle and none was attributed to Angiopep.

4. Have you investigated immunogenicity of Angiopep-therapeutic protein conjugates in primates compared to that of the therapeutic protein alone?

- No primate studies have been conducted with An2-therapeutic proteins.
- Immunogenicity of ANG1005, although not a protein, has been extensively evaluated in more than 350 patients, some of them received the product every 3 weeks for more than 20 cycles.
- No clinical or biological immunogenicity response has been observed.

5. Have you conducted a study to quantitatively determine the level of brain uptake?

- Yes. Please refer to answer to question 1 for details.

6. Have you done a similar study after multiple dosing? If so, does the uptake into brain change with repeated dosing?

- We have not yet performed multiple dose PK/brain distribution studies with ANG4043.
- Our multiple dose survival studies were followed by evaluation of ANG4043 in brain tumor.
- ANG4043 was detected in brain tumors from animal treated twice weekly up to 7 weeks.

7. Is there any evidence for a differential brain uptake of PDC conjugates of IgG, Fab, or other therapeutic protein formats?

- Initial conjugation attempts could produce variable results.
- Once the conjugation chemistry and linkers are optimized, the brain uptake is expected to be similar

8. **Regarding, in general terms, the optimization of the Angiopep conjugation, how do you determine the optimal:**
   - **Angiopep to use for each therapeutic protein, retaining both affinity to the target and LRP-1 transporter.**
   - **Peptide-to-therapeutic protein ratio.**
   - **Linker type.**

- For all of these variables, optimization of leads is based on empirical results. Having said that, our experience with mAb and other therapeutic protein lead-optimization programs has enabled us to streamline the process as we have our preferred linkers, Angiopeps, and reaction conditions. Our research operating plan typically begins with evaluation of *in vitro* target affinity.
- With the approaches that we currently use, we rarely observe reduction in target affinity. Next evaluated is rate of brain uptake; this is the parameter that is most likely to vary when the above variables are changed. We then measure brain/plasma ratio at longer time points to ensure that exposures are adequate.
- Conjugate generation continues during these evaluations until the appropriate brain exposures are achieved.

9. What experience do you have in optimizing these parameters? How long, and how much resource, is typically required for this optimization?

- Angiopep-drug conjugate programs have been conducted at Angiochem since 2006, with 6 years spent on Angiopep-biologics, so a large amount of know-how has been accumulated. The time that it takes to fully optimize a lead has decreased greatly since our first attempts.
- Currently, typical optimization time is 6-9 months. For example, our latest research collaboration, we have reached our target in 7 months. Resource required would be dependent on the number of conjugates to be produced and tested, and the nature of the outcome to be measured.
- In short, it would depend on the research operating plan.

10. We understand that the Angiopep conjugation is not site-specific, and is via chemical linkers with varying properties (cleavage characteristics and mechanisms, chemistry…). Is this correct?

- Yes, however, site-specific conjugation is achievable with methodology that we are currently using for two programs.

11. Is the optimum of these parameters highly variable between antibodies (or other therapeutic proteins)?

- The optimal mix of characteristics does vary from one protein to another, however, we have found a set of linkers and chemistries that work well.
- Typically, testing of the parameters in this set results in successful lead generation.

12. Have you any experience with site-specific conjugation (eg. Conjugation to engineered cysteine residues)? If so, does this confer any advantage for delivery or other conjugate properties?

- Yes, we do have experience with site-specific conjugation and have observed advantages with this method with one specific protein, however, we are unable to share that data as it is part of a confidential collaboration.
- The collaboration does not preclude us from using the same approach for other targets.

13. Do you have any updated information on the applicability of Angiopep conjugates to oral dosing?

- We do not have the required expertise for oral peptide delivery, however, we could evaluate these parameters in a collaboration.