Quantitative Targeted Absolute Proteomics (QTAP) analysis of transporters for the validation of an *in vitro* Blood Brain Barrier model

Mouhssin Oufir[1,3] | Jordan Goncalves[2,3]| Grégoire Harichaux[2,3] |Yann Courbebaisse[2,3,4] | Isabell Seibert[5]| Henriette E. Meyer zu Schwabedissen[5]| Fabrice Viviani[3] and Matthias Hamburger[1]

[1]Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland
[2]Bertin Pharma, 10, Avenue Claude Guillemin, F-45071 Orléans cedex 2, France
[3]Oncodesign, 25-27 rue du Quebec, F-91140 Villebon-sur-Yvette, France
[4]Adocia, 115 avenue Lacassagne, F-69003 Lyon, France
[5]Biopharmacy, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland

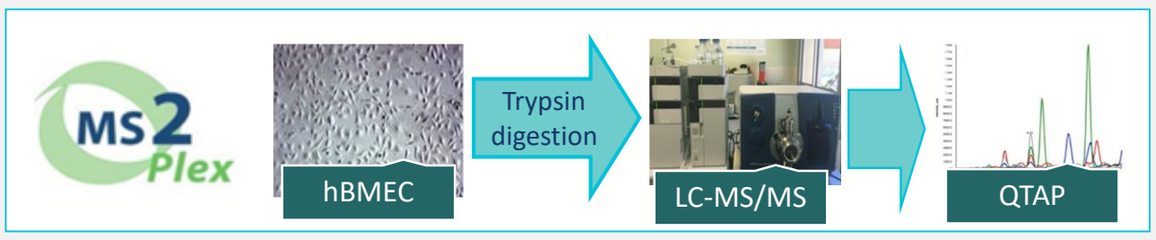
Vector of innovation.

Scan QRCode to receive the poster!

1 CONTEXT & OBJECTIVES

We selected the human brain microvascular endothelial cell line (hBMEC)[1] as a suitable cell line for an *in vitro* BBB model due to the barrier tightness and paracellular permeability in a 24-well mono-culture format[2]. The BBB permeability of several structurally diverse drugs was correctly predicted using this model, with the exception of quinidine, a known substrate for P-gp/MDR1[3].

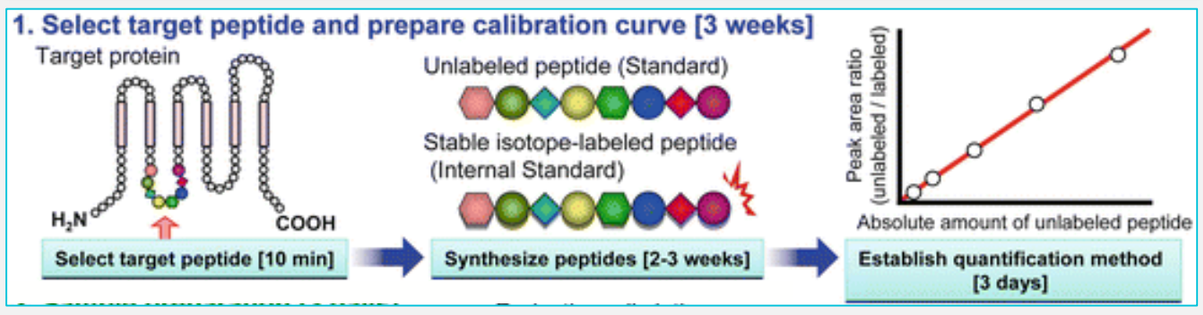
We used **Quantitative Targeted Absolute Proteomics (QTAP)** based multiplexed MRM[4-5] to evaluate the protein expression levels of ten selected transporters in the hBMEC BBB model. Classical techniques of Real-time PCR and Western blots were used for reference.

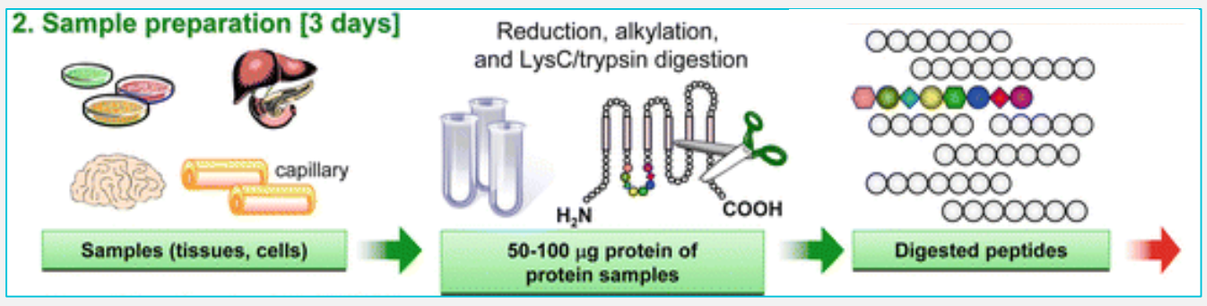


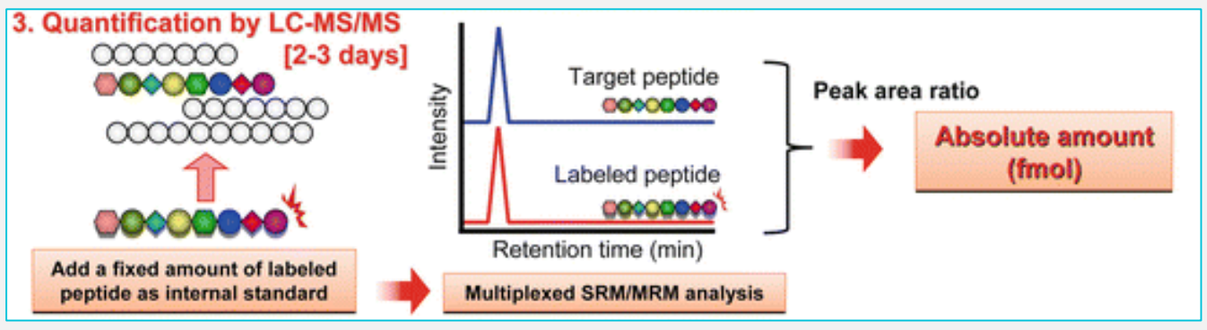
Why QTAP?[7]

- MS: highly sensitive MS with wide dynamic range
- Purpose : identification and quantification of target proteins
- Sensitivity : covers low-abundance proteins (~0.1 fmol/µg protein)
- Quantification: accurate absolute & relative quantification
- Coverage: identify & quantify only target proteins

Basic workflow of quantitative targeted absolute proteomics (QTAP)[6]







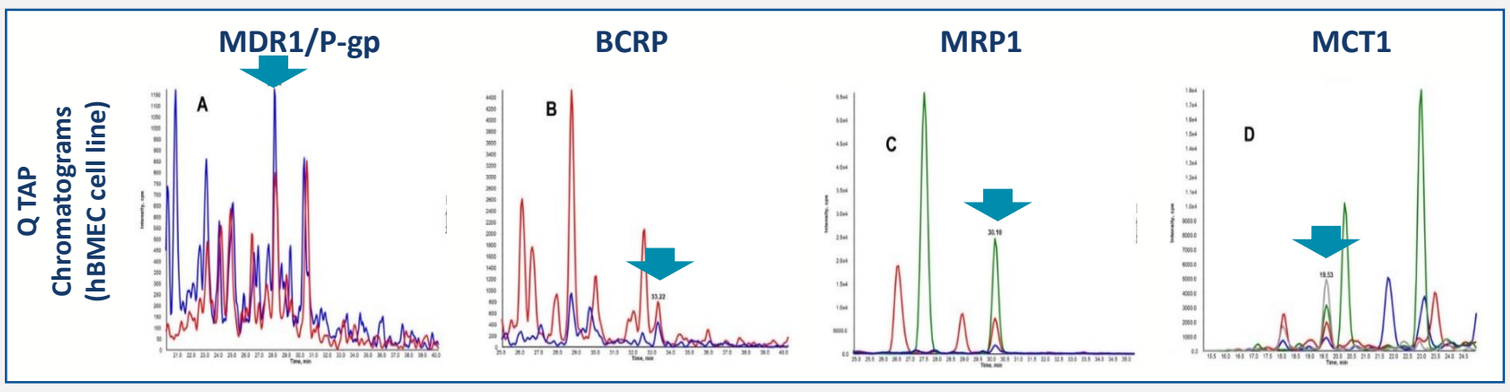
2 RESULTS

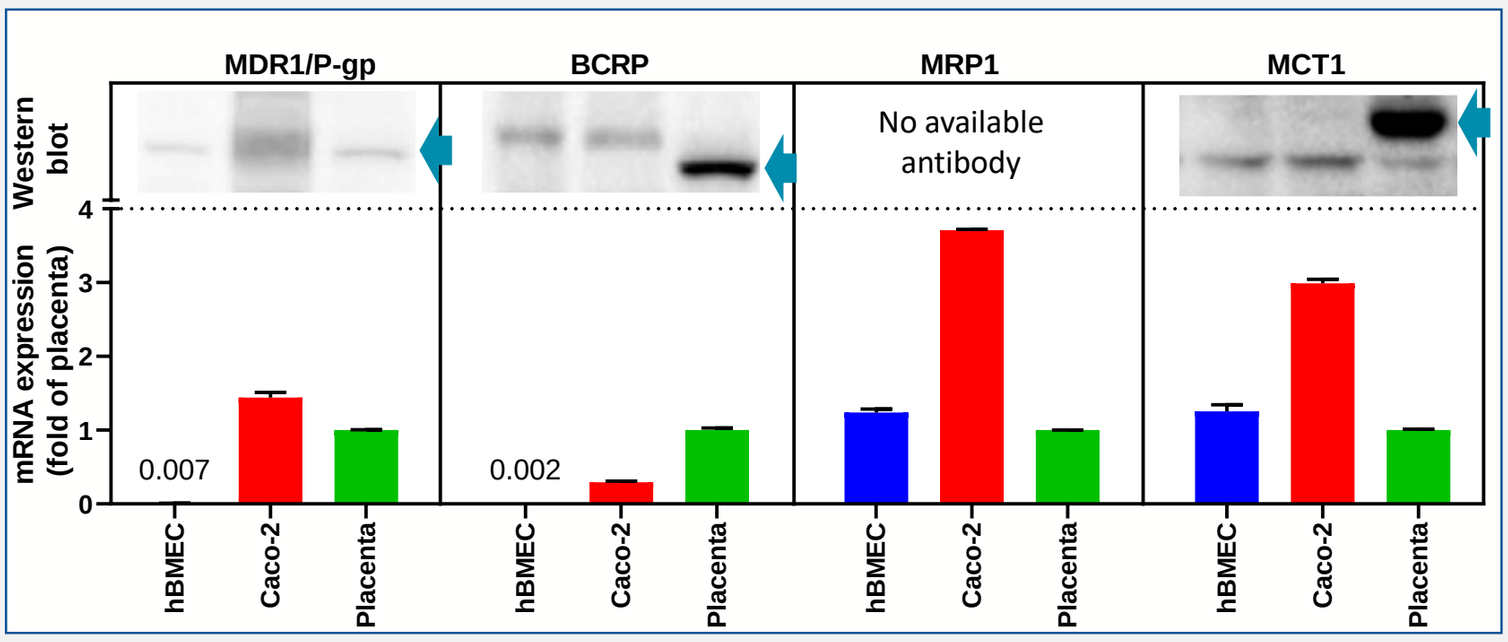
Of the ten transporters selected for validation by QTAP, six [MRP2 (ABCC2), MRP3 (ABCC3), OATP1A2 (SLCO1A2), OATP2B1 (SLCO2B1), OAT3 (SLC22A8), and OCTN2 (SLC22A5)] were below the limit of quantification (0.13 fmol/µg protein) in hBMEC (Sciex Triple Quad 5500).

Two ATP binding cassette (ABC) transporters MDR1 (P-gp) and BCRP, which are highly recommended by regulatory guidances[8-9], were detectable but not quantifiable in hBMEC cells. Only levels of MRP1 and MCT1 were quantifiable using QTAP. Grey, green, red and blue profiles represent four different specific MRM transitions of unlabeled peptide

Neither MDR1 (P-gp), BCRP nor MCT1 were significantly detectable by Western Blot analysis in hBMEC cells. However, expression of these transporters was observed in Caco-2 cells and the placenta.

Using RT-PCR, the quantitation of MDR1 (P-gp) and BCRP expression (normalized to placenta) showed very low transcription of these efflux transporters in hBMECs. In contrast, transcription of the efflux transporter/uptake transporter MCT1 was detected.



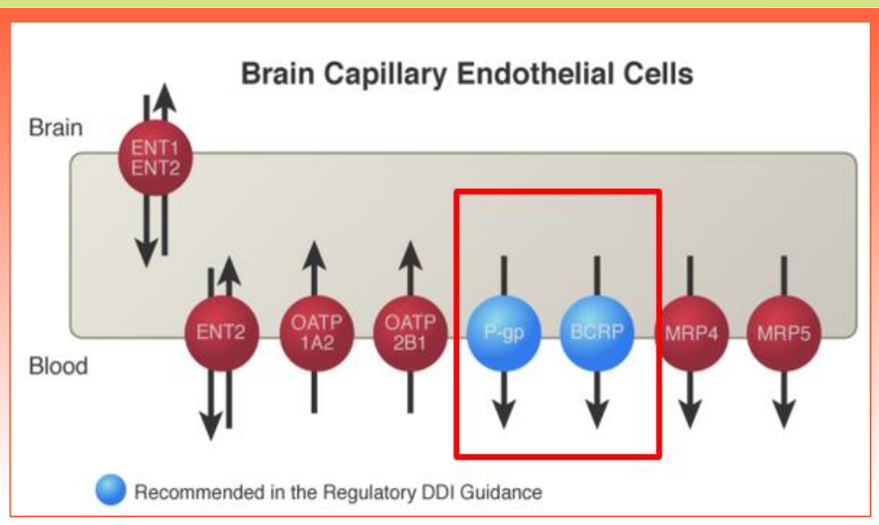


3 CONCLUSION

QTAP, RT-PCR and Western blot all demonstrated that the two ABC transporters, MDR1 (P-gp) and BCRP, involved in active transport are weakly expressed in the hBMEC cell line.

The FDA[8] and EMA[9] guidelines both cite these two transporter proteins as being highly relevant do drug-drug interactions (DDI). Thus, our results reveal a major limitation for the use of the hBMEC cell line as *in vitro* human BBB model.

With its high sensitivity, QTAP is also able to quantify all CYPs and UGTs of interest. Oncodesign holds the exclusive license for this powerful technology.



[1] Stins MF et al., Microb. Pathog. 2001
[2] Eigenmann DE et al., Fluids Barriers CNS 2013
[3] Eigenmann DE et al., Anal. Bioanal. Chem. 2016
[4] Uchida Y et al., J. Neurochem. 2011
[5] Ohtsuki S et al., Mol Pharm 2012
[6] Uchida Y et al., Drug delivery to the brain, 2013
[7] Uchida Y et al., Fluids Barriers CNS, 2013
[8] U.S. Department of Health and Human Services, FDA. Guidance for industry. Drug interaction studies. February 2012
[9] European Medicine Agency, Committee for Human Medicinal Products. Guideline on the investigation of drug interactions. 21 June 2012