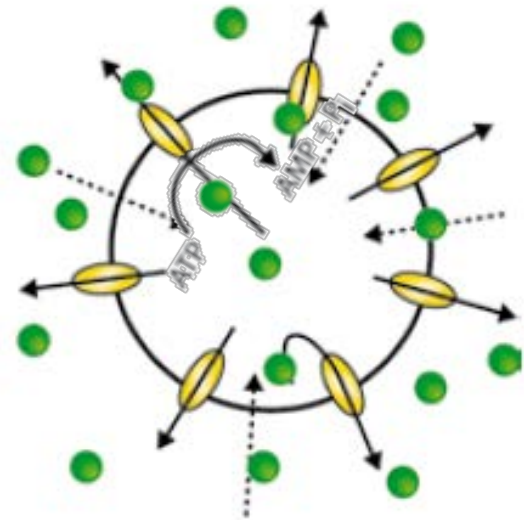


THE INHIBITORY POTENTIAL OF TB DRUGS ON ATP-BINDING CASSETTE TRANSPORTERS MRP1-5, P-GP, BCRP AND BSEP

Lindsey H.M. te Brake, Jeroen J.M.W van den Heuvel, David M. Burger, Jurriaan E. de Steenwinkel, Gerjo J. de Knegt, Reinout van Crevel, Frans G. Russel, Rob E. Aarnoutse, Jan B. Koenderink

ABC transporters

- ✓ ABC transporters actively transport substrates across the cell membrane with the use of ATP hydrolysis
- ✓ Located at pharmacological barriers and important in ADME of drugs
- ✓ Transporter interactions have a major impact on later phases of drug development
- ✓ *In vitro* high-throughput: “fail fast - fail cheap”



Aim

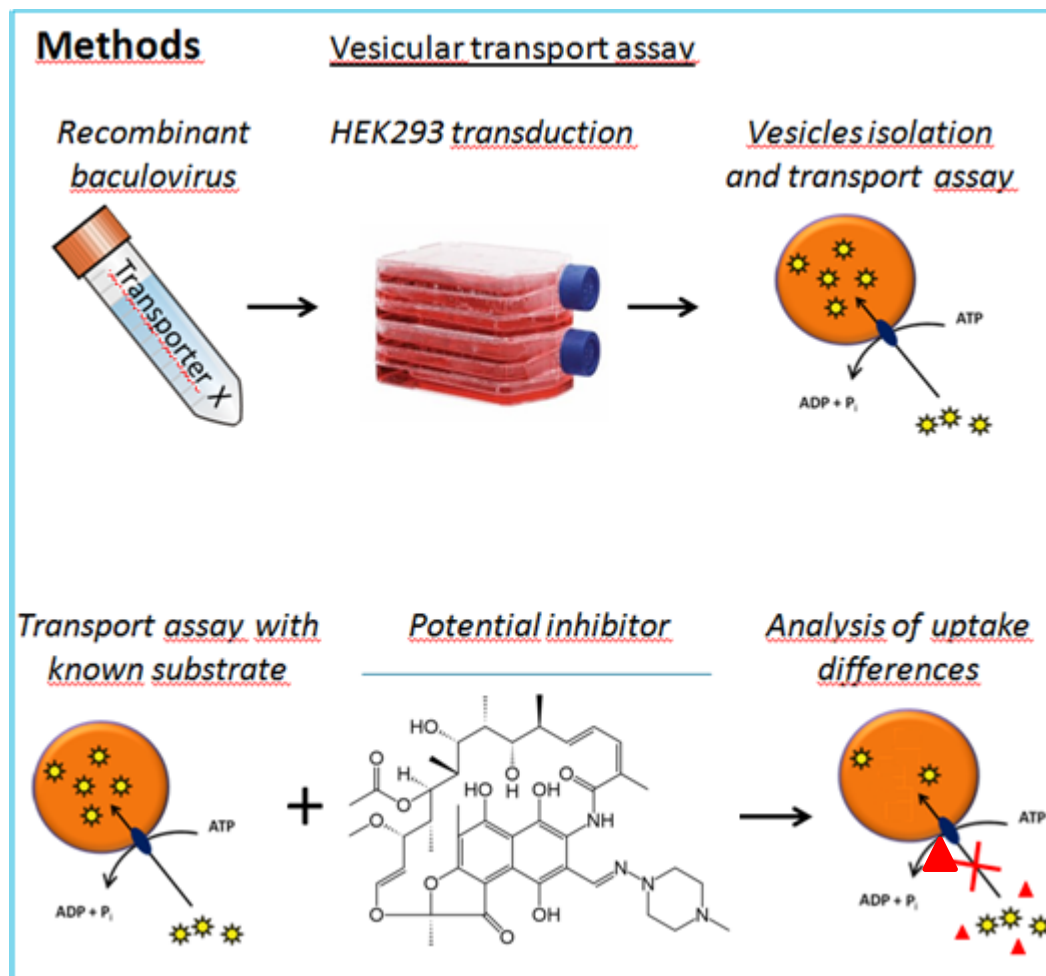
To study the inhibitory potential of a range of anti-TB drugs on the ABC transporters MRP1-5, BCRP, P-gp and BSEP *in vitro*, to obtain insight in the role of efflux transporters in drug-drug interactions

Vesicle assay

✓ Vesicles:
cell membrane
preparations containing
transporter(s)

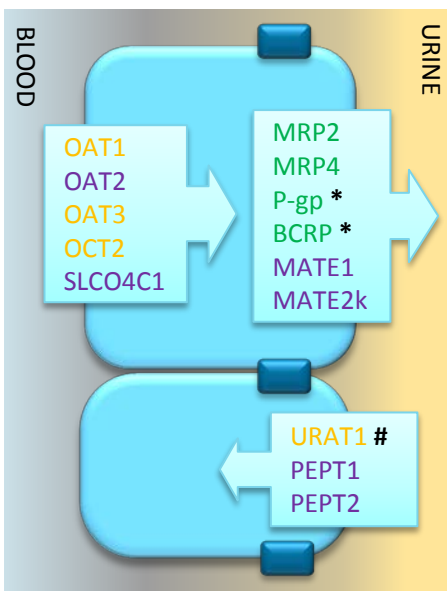
✓ Functional expression
in vesicles:

human ABCB1: MDR1 / Pgp
 human ABCB11: BSEP
 human ABCC1: MRP1
 human ABCC2: MRP2
 human ABCC3: MRP3
 human ABCC4: MRP4
 human ABCC5: MRP5
 human ABCG2: BCRP

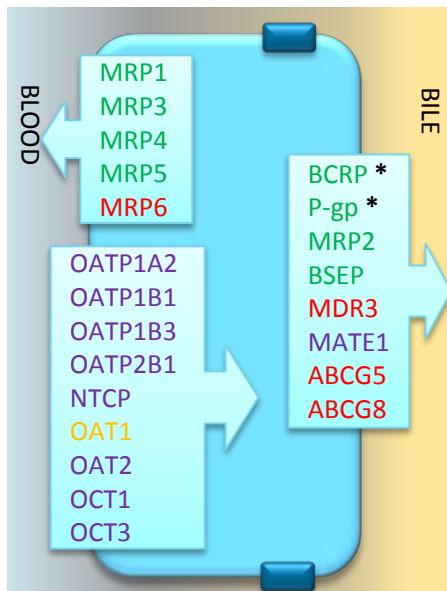


Transporters in different organs

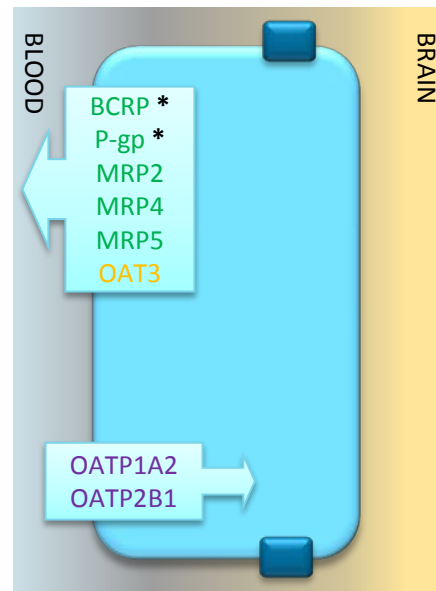
KIDNEY



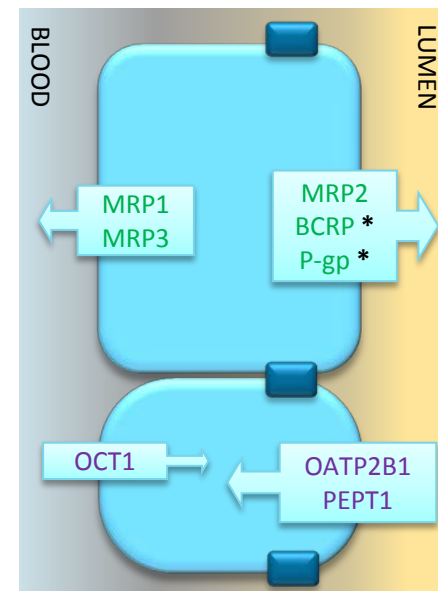
LIVER



BLOOD-BRAIN-BARRIER



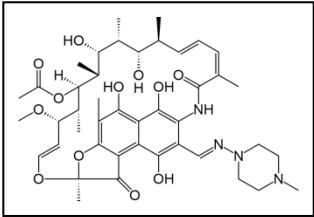
INTESTINE



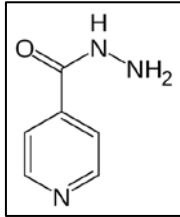
- ATP mediated transporter functional in vesicles
- ATP mediated transporter non-functional
- Non ATP mediated transporter functional in cell line
- Non ATP mediated transporter non-functional
- # Also functional in vesicles
- * Also functional in cell line

I. First line oral agents

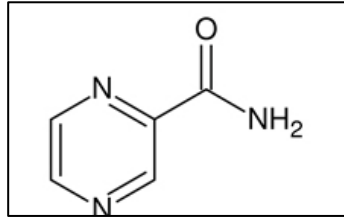
Rifampicin



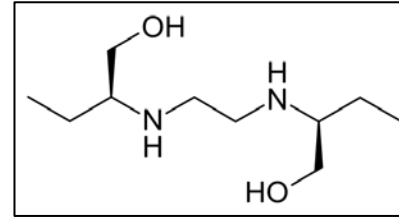
Isoniazid



Pyrazinamide

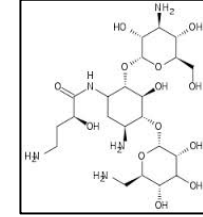


Ethambutol



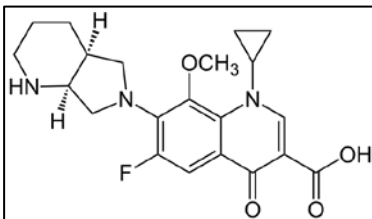
II. Injectable agents

Amikacin

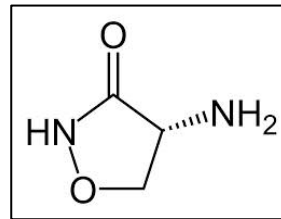


III. Fluoroquinolones IV. Oral bacteriostatic second line agents

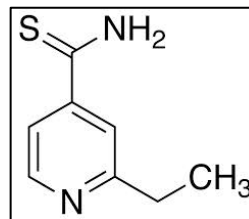
Moxifloxacin



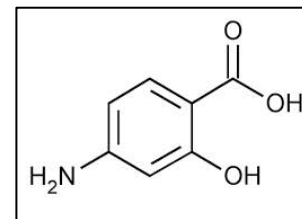
Cycloserine



Ethionamide

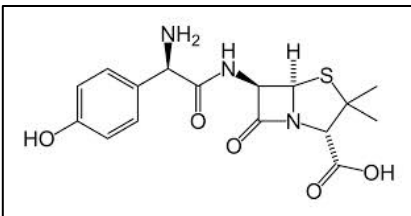


PAS

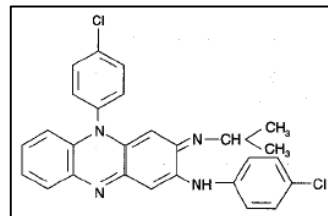


V. Agents with unclear role

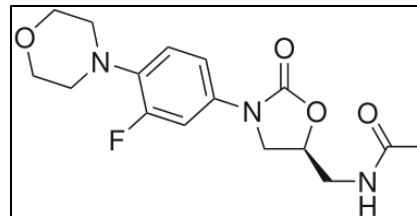
Amoxicillin



Clofazimine



Linezolid



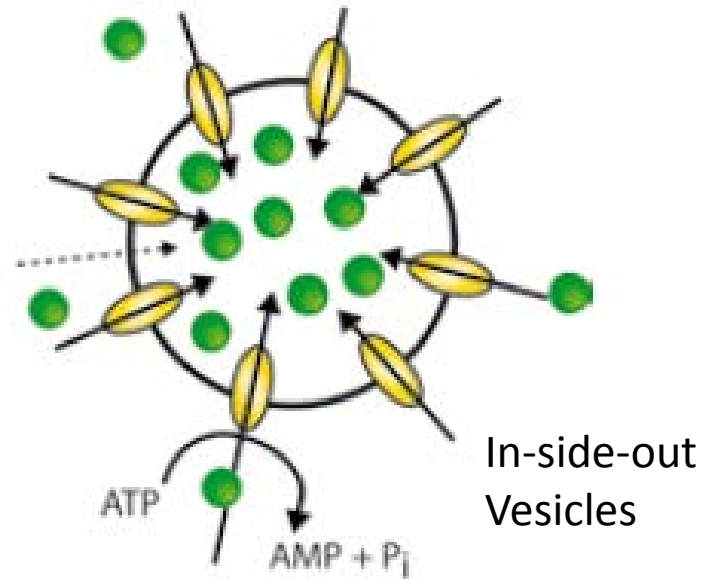
Agents experimental in TB treatment (EPI)

- ✓ Thioridazine
- ✓ Timcodar
- ✓ SQ109

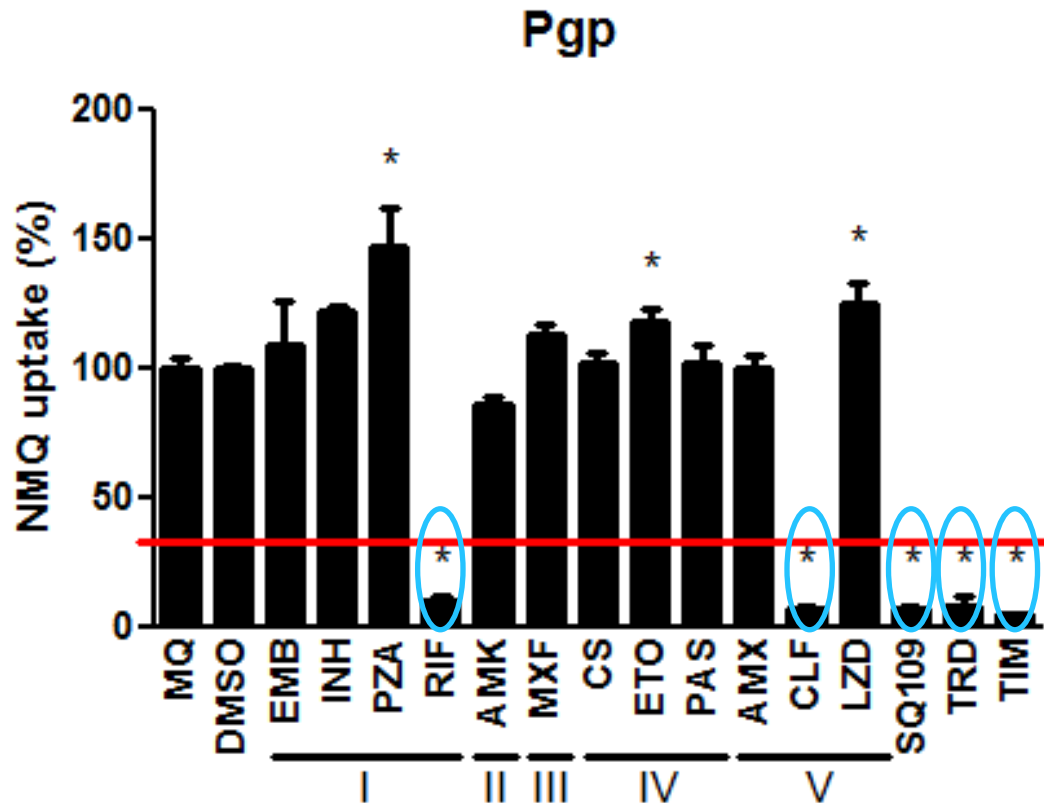
Vesicle assay (II)

Assay ingredients:

- ✓ Vesicles
- ✓ Model substrate (RA label)
- ✓ Potential drug inhibitors (200 μM)
- ✓ ATP

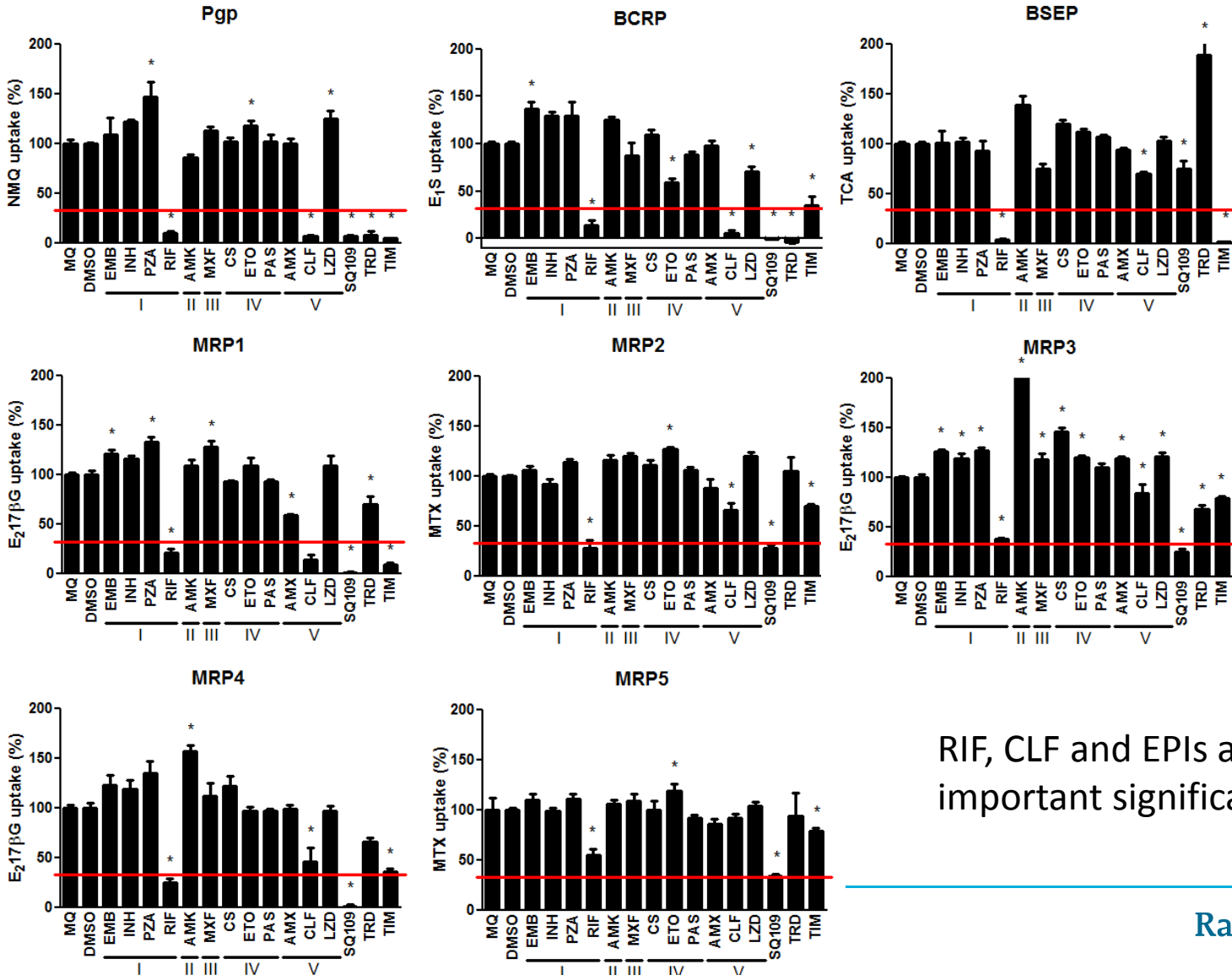


Inhibitory effects TB-drugs – high-throughput



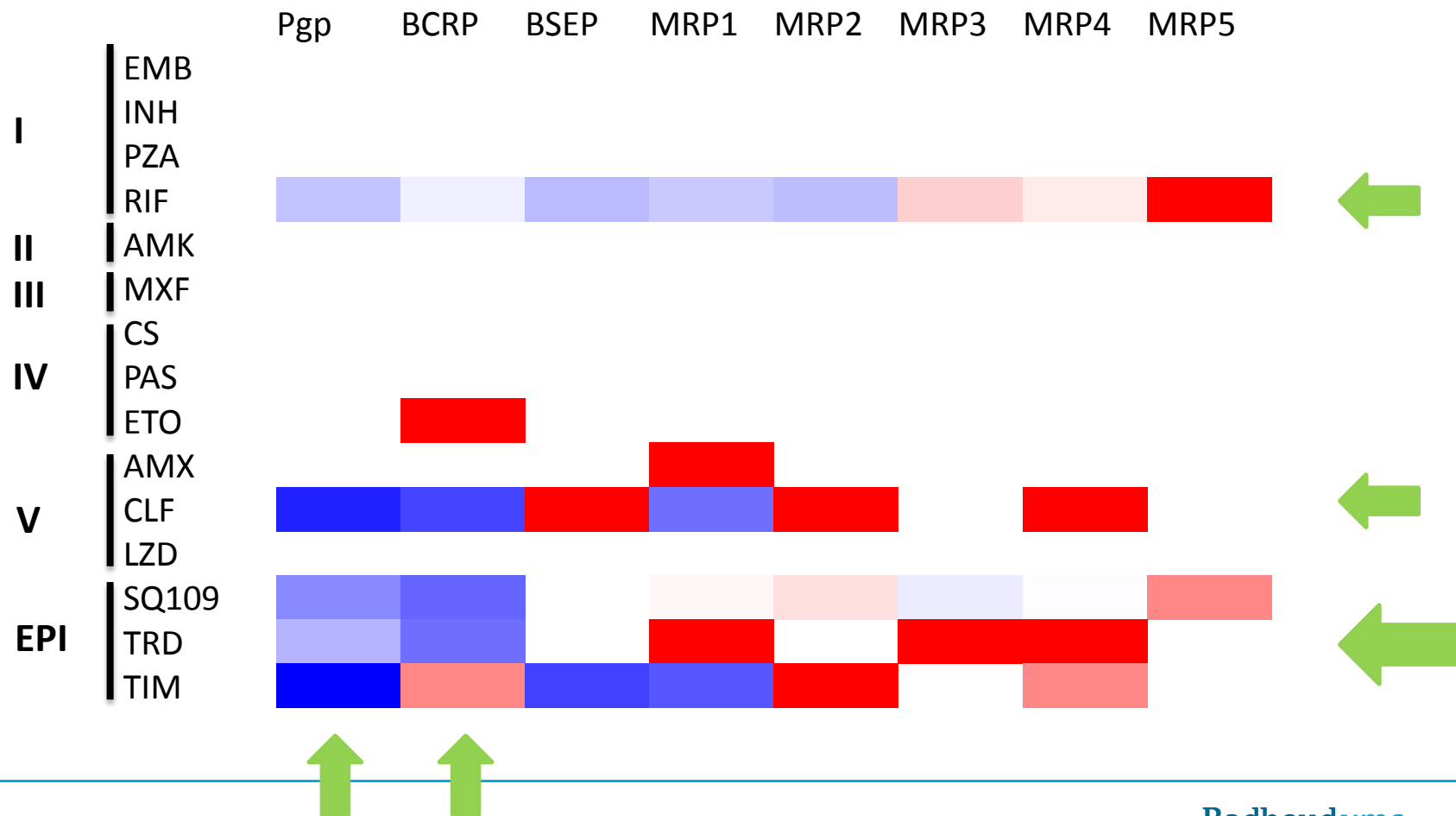
200 μ M solutions -> derive concentration-effect curves

Inhibitory effects TB-drugs – high-throughput

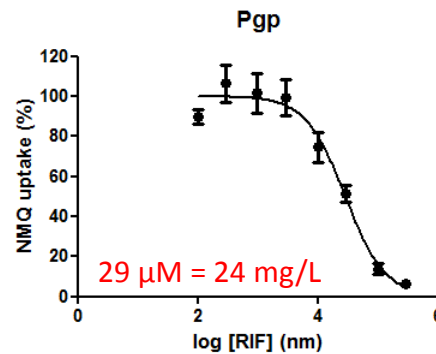
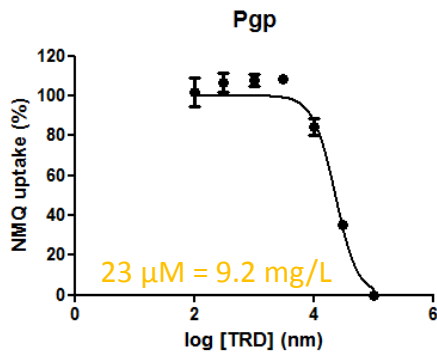
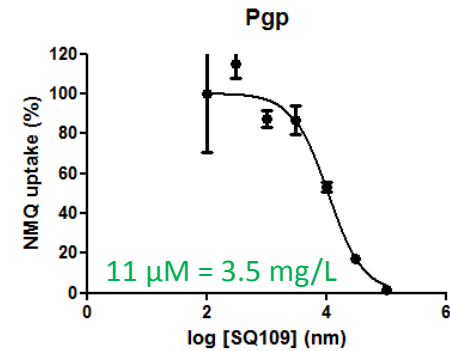
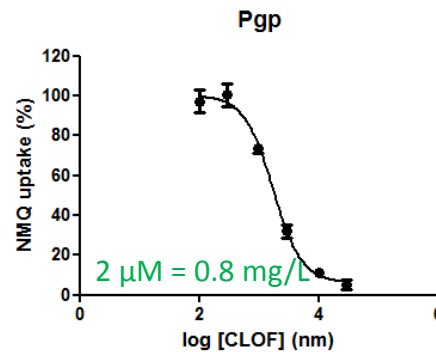
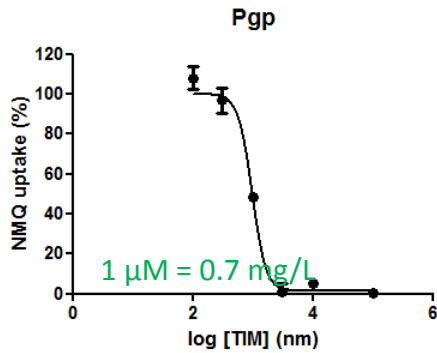


RIF, CLF and EPIs are most important significant inhibitors

IC50 – heat map



IC50 – P-gp



✓ Curve shift right -> higher IC50

✓ Free concentrations:
possible relevant/irrelevant

Conclusion

1. Supposedly mycobacterial EPIs (timcodar, SQ109 and thioridazine) inhibit a range of human ABC transporters at possibly clinical relevant concentrations
2. Clofazimine shows potent *in vitro* transporter inhibition. In clinical setting DDI are relatively unknown
3. Rifampicin shows inhibition at probably clinical irrelevant free concentrations

Conclusion (II)

✓ Vesicle assay can be used to screen for transport inhibition in an early stage of drug development, to predict DDI expectancy:

1. IC50 values represent free concentrations at target site
-> no *in vivo* protein binding and drug accumulation
2. No effect on expression levels
-> rifampicin induces expression *in vivo*
3. Mechanism of inhibition unknown (competitive vs direct inhibition)

Acknowledgements



RIMLS - Pharmacology/ Toxicology

- Jeroen van den Heuvel
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- Jan Koenderink



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- David Burger



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- Reinout van Crevel



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- Gerjo de Knegt
- Jurriaan de Steenwinkel