In vitro ADME studies aiming at characterization of molecular mechanisms behind drug pharmacological or toxicological actions

(ヒト薬効・毒性発現機序解明およびその評価能向上を目指した*in vitro*薬物動態研究)

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Study focuses

1.

Identification of ribavirin uptake systems in human hepatocytes and characterization of their roles in ribavirin antiviral actions

In vitro ADME studies aiming at characterization of molecular mechanisms behind drug pharmacological or toxicological actions

2.

Identification of cancer-type OATP1B3 and its potential application to cancer therapy

3.

Establishment of new immortalized human brain cells for development of *in vitro* human BBB models

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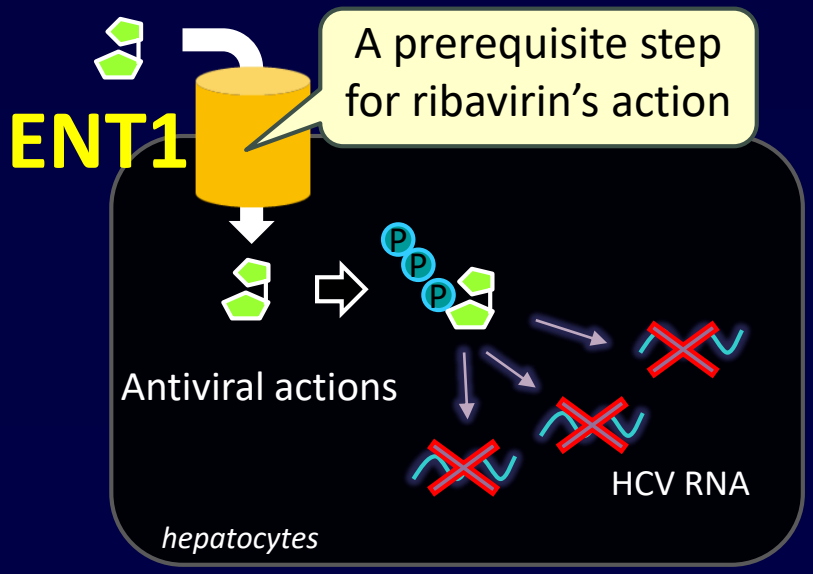
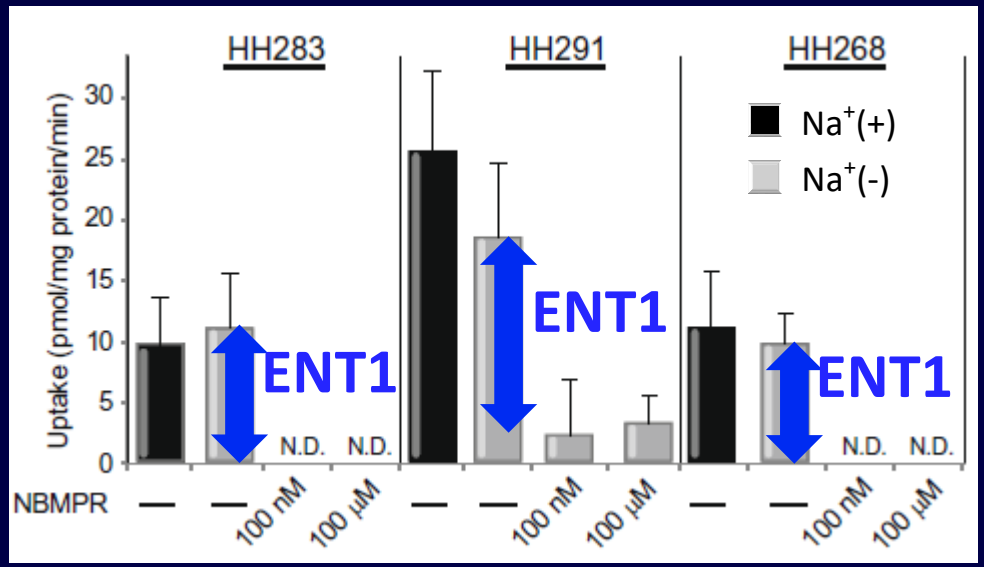
Identification of ribavirin uptake transporter in human hepatocytes

Ribavirin is a nucleoside analogue used for anti-hepatitis C virus (HCV) therapy (RBV)

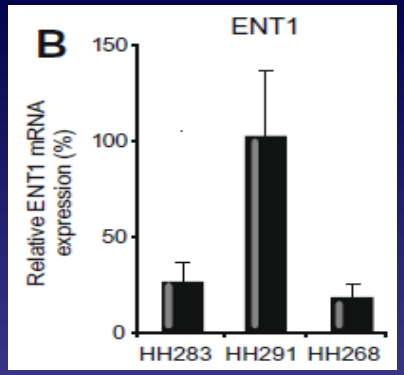


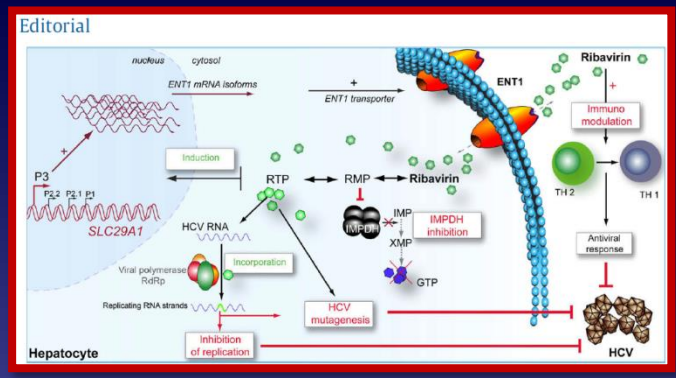
RBV uptake by human hepatocyte lines



Equilibrative nucleoside transporter 1 (ENT1) is a primary RBV uptake transporter in human hepatocytes.

ENT1 mRNA level





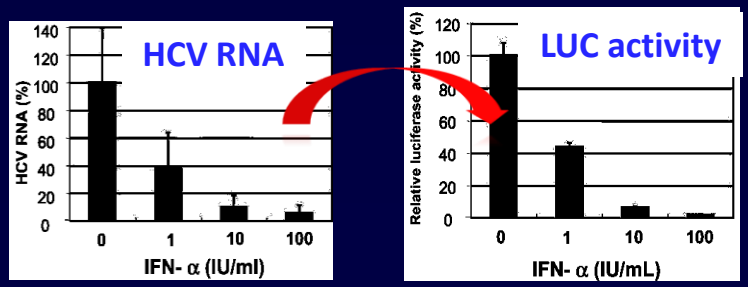
Fukuchi, Furihata, et al. J Hepatol 2010;52:486-92.

Critical role of ENT1 in antiviral action of RBV in HCV-model cells

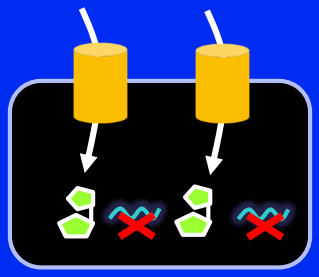
HCV-replication model cells (OR6 cells)

Ikeda, et al. Biochem Biophys Res Comun 2005;329:1350-9.

The renilla luciferase activity level correlates well with the HCV replication activity level in OR6 cells

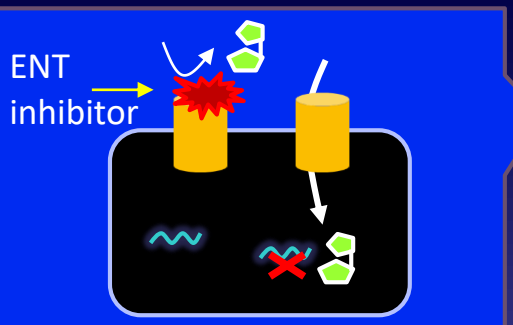


No ENT1 inhibition



HCV replication model cells

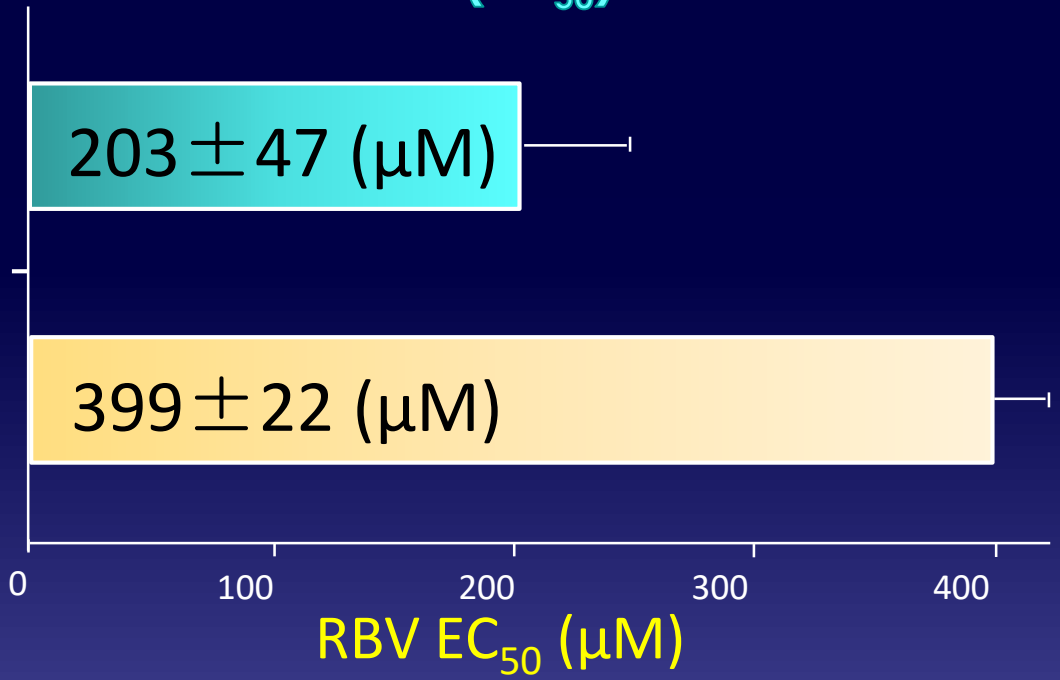
RBV uptake **100%**



Partial ENT1 inhibition

RBV uptake **50%**

Concentration-dependent RBV activity (EC_{50})



Iikura, Furihata, et al. Antimicrob Agent Chemother 2012;56:1407-13.

Association of an SNP in the ENT1 gene with clinical outcome

Collaborative work with Dr. Tsubota

Baseline profile of the patients recruited for the association study

Number (race): 526 (517 Japanese, 4/3/2 Chinese/Mongol/Korean)
HCV genotype: 1b
Regimen: Peg-INF/RBV combination therapy

SNPs in the *ENT1* gene analyzed in this study

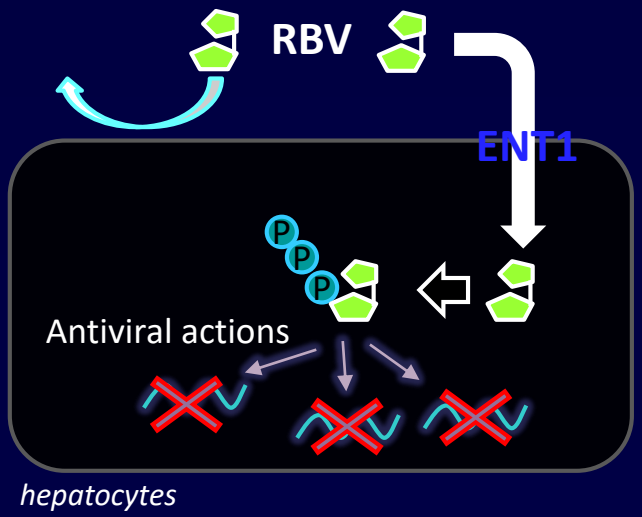
14 SNPs located in the 5'-upstream, intronic, or 3'-untranslated regions (indicated by red arrowheads)

Association of SNP rs6932345 with the rate of the sustained virological response (SVR, the therapeutic goal)

	Genotype (MAF)	*P* value on multivariate analysis	Odds ratio (95% CI)
rs6932345	**AA vs AC/CC (0.196)**	**0.03**	**1.85 (1.06-3.21)**

Tsubota, Shimada, Yoshizawa, Furihata, et al. Liver Int 2012;32:826-36.

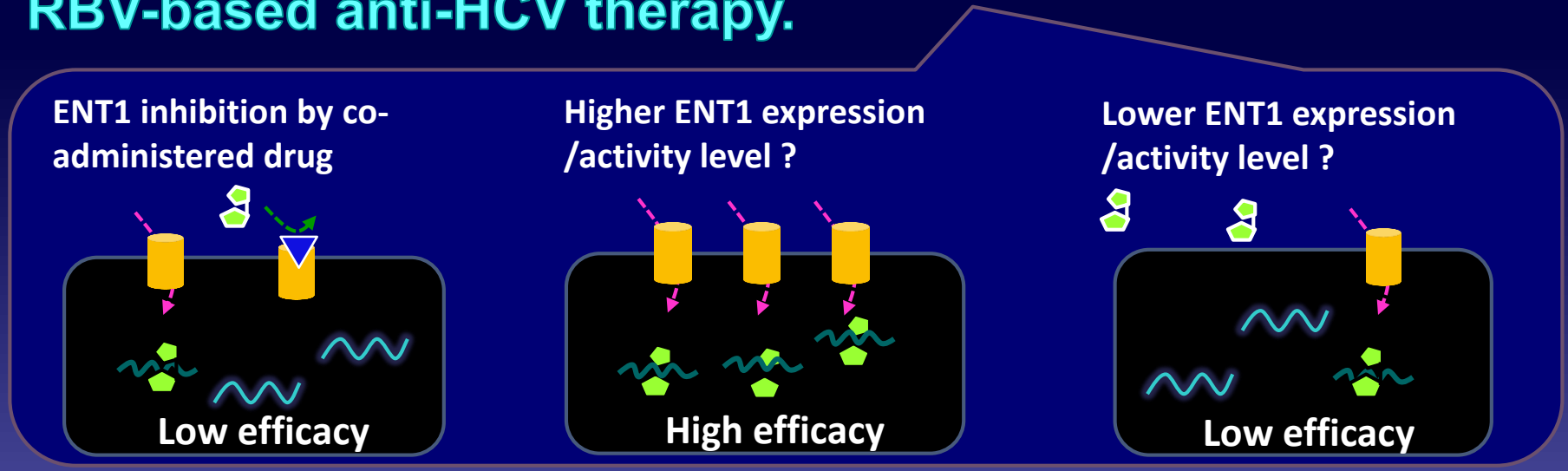
Summary & Perspective -1-



ENT1 is a primary RBV uptake transporter in human hepatocytes.

Accordingly, ENT1 plays a critical role in RBV's antiviral action.

It can be assumed that hepatic ENT1 activity level is a factor that determines treatment efficacy of RBV-based anti-HCV therapy.



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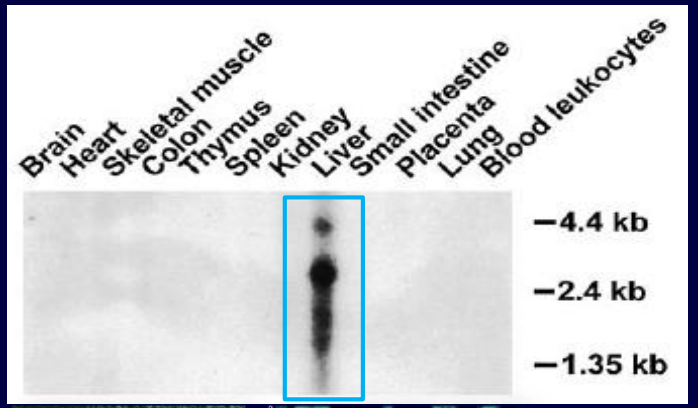
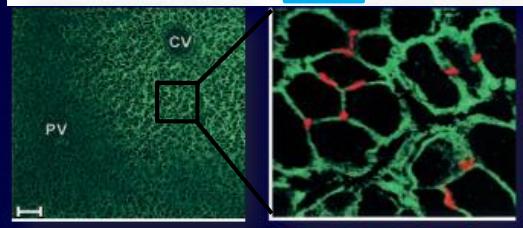
Organic anion transporting polypeptide 1B3 (OATP1B3)

OATP1B3

- ✓ is initially identified as a liver-specific transporter expressed at hepatocyte sinusoidal membrane.
- ✓ can transport various drugs into hepatocytes.
- ✓ is subsequently reported to be expressed in various cancer tissues.

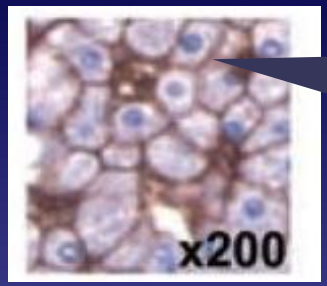


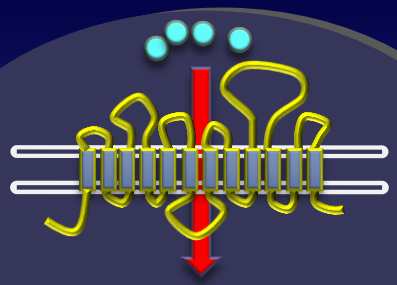


Konig et al., JBC 2000;275:23161-8

It had long been taken for granted that OATP1B3 expressed in cancer tissues was identical to that expressed in the liver.

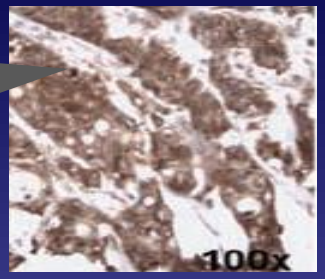
Normal liver





The same protein!
The same function!

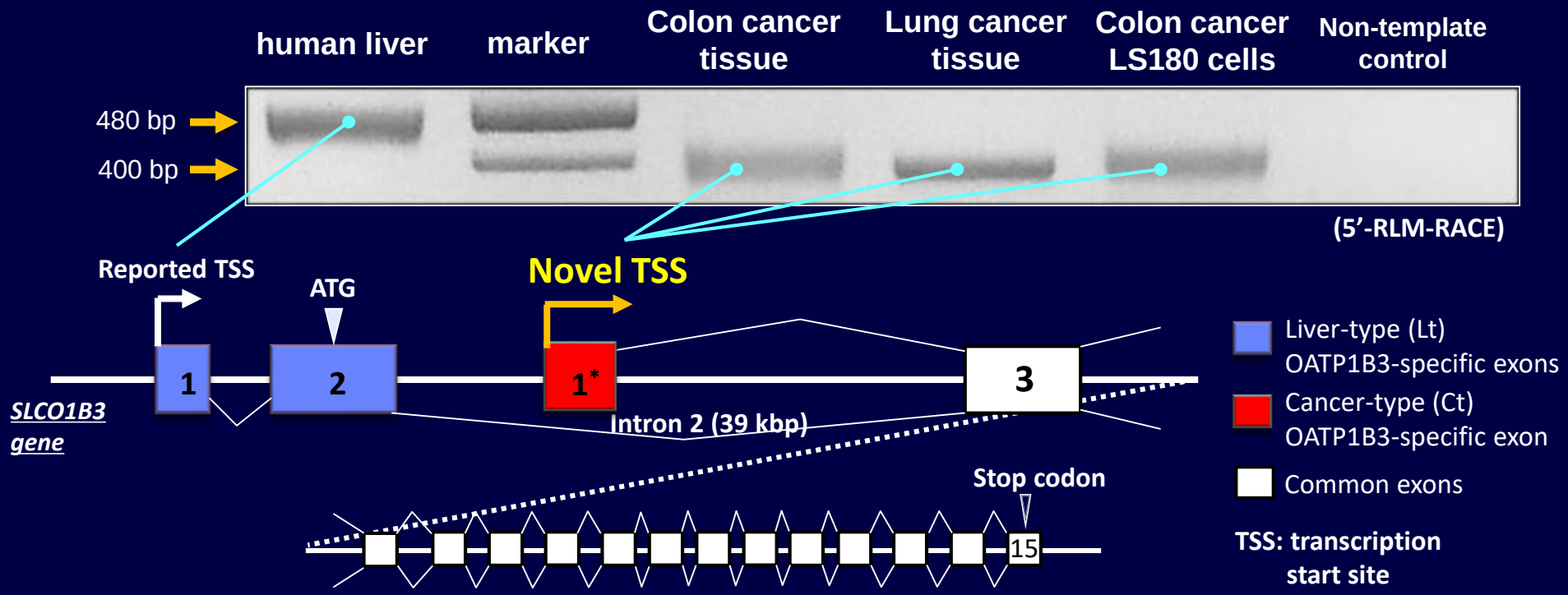
Colon cancer



Lee et al. Cancer Res. 2008;68:10315-23.

Identification of cancer-type OATP1B3 in human cancer tissues

Cancer-type OATP1B3 (Ct-OATP1B3)



The new isoform identified in cancer is hereafter referred to as

Cancer type (Ct)-OATP1B3 mRNA

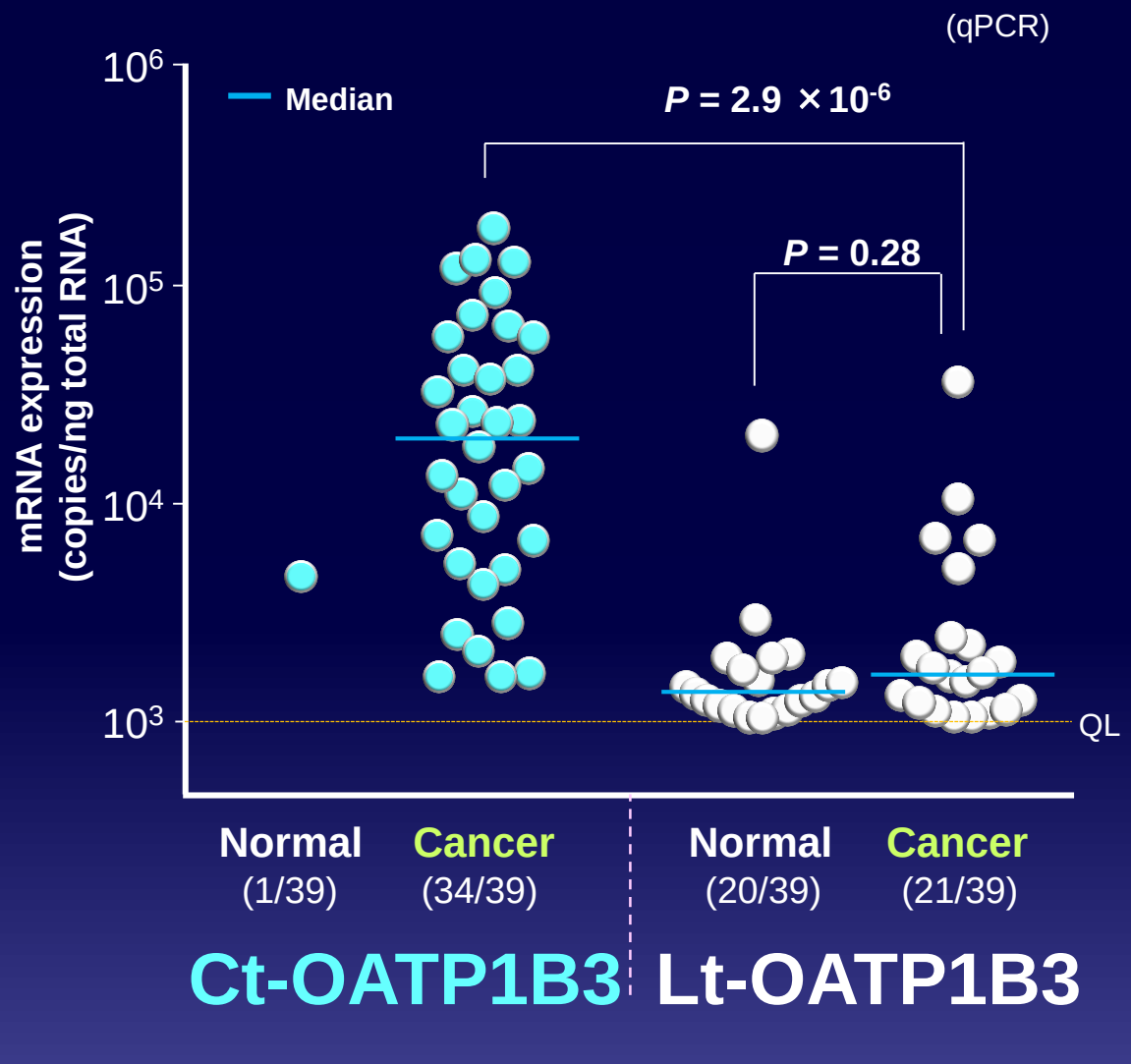
The known isoform identified in the liver is hereafter referred to as

Liver type (Lt)-OATP1B3 mRNA

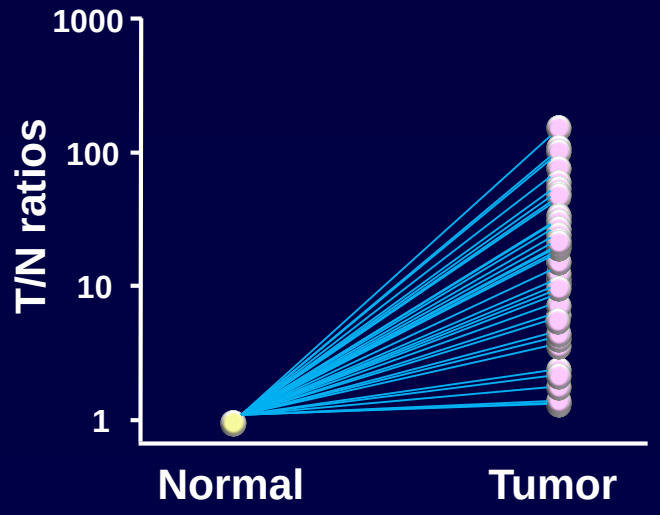
Nagai, Furihata et al. (2012) BBRC 418:818-823.

Cancer-restricted expression profile of ct-OATP1B3

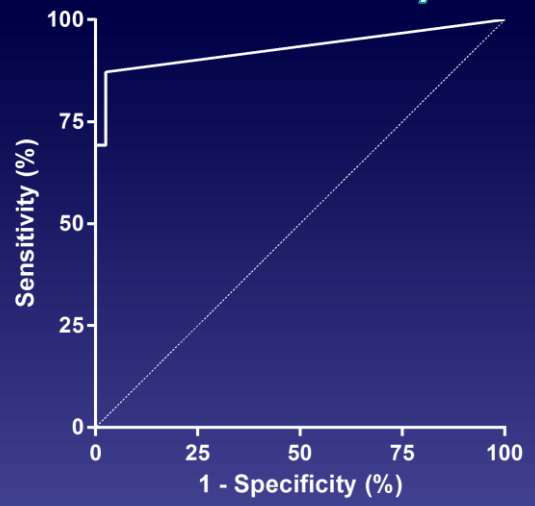
OATP1B3 mRNA expression profile in 39 matched-pairs of colon tissues



T/N ratios of Ct-OATP1B3 mRNA levels in individual patients



ROC curve analysis for evaluation cancer specificity and selectivity

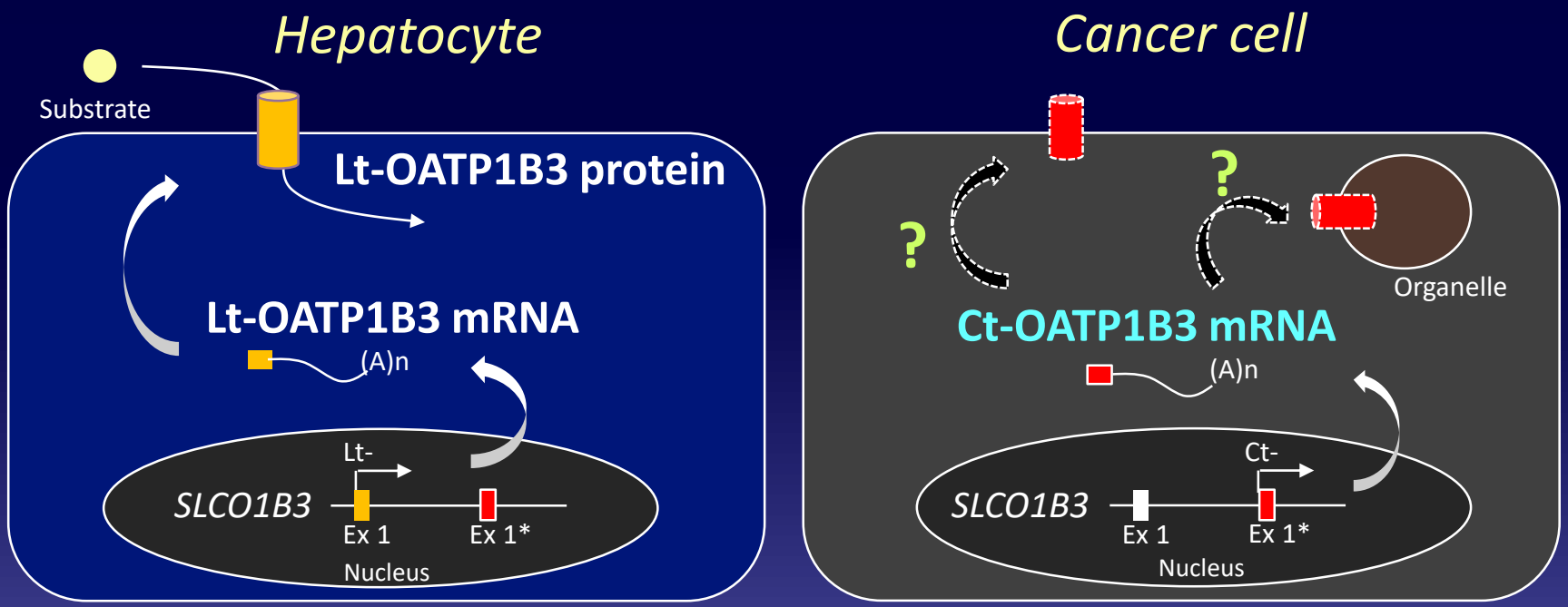


Sun, Furihata et al. Clin Transl Med 2014:3:37

Summary & Perspective -2-

Ct-OATP1B3, which is a variant isoform of Lt-OATP1B3, is considered to be a *bona fide* cancer-associated OATP1B3 isoform.

Identification of Ct-OATP1B3 is likely to revise the long-standing study premise, which is thus expected to open up new avenues in cancer-related OATP1B3 studies.



Furihata et al. Curr Drug Metab 2015;16:474-85.

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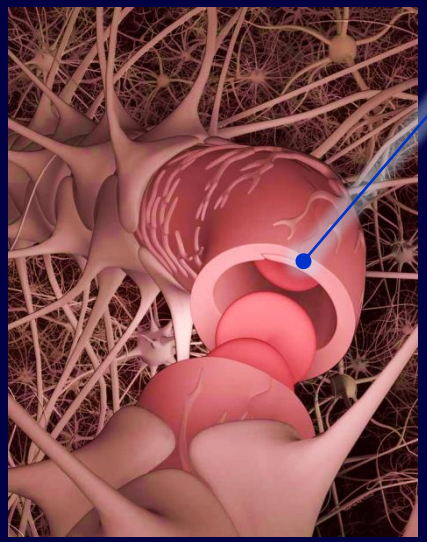
Blood-brain barrier and temperature-sensitive immortalized cells

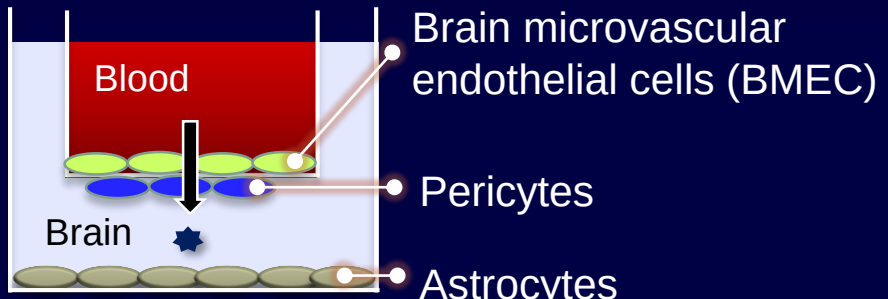


Wong AD et al. Frontiers in Neuroenginering 2014

Blood-brain barrier (BBB): A primary obstacle to drug penetration into the brain

In vitro BBB models can be applied to the different stages of CNS drug development.

In vitro BBB model



Primary human cells

are highly functional, but they show limited proliferation ability, rapid senescence, scarcity, and lot-to-lot variations, which

Immortalized human cells

generally show infinite proliferation ability, human gene functions, stable phenotype, and cell-type specific functionality, which

impede ↘ ↙ **allow**

researchers to take various trial-and-error approaches in drug development.

Establishment of immortalized human BMEC

Establishment of human BMEC/conditionally immortalized clone β (HBMEC/ciβ)

Primary human BMEC

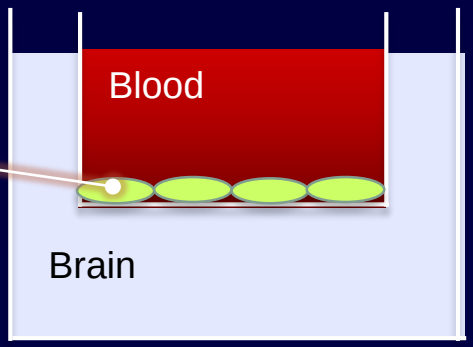
Immortalized human BMEC

Lentiviral transduction

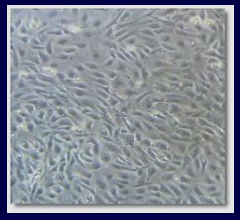
Temperature-sensitive simian virus 40 large T antigen gene (tsSV40T)

Human telomerase catalytic subunit gene (hTERT)

In vitro BBB model



Morphology



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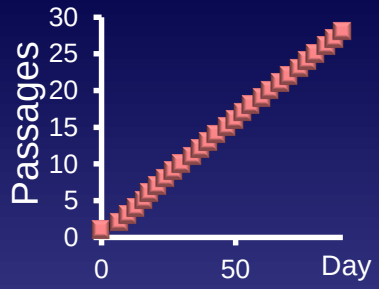
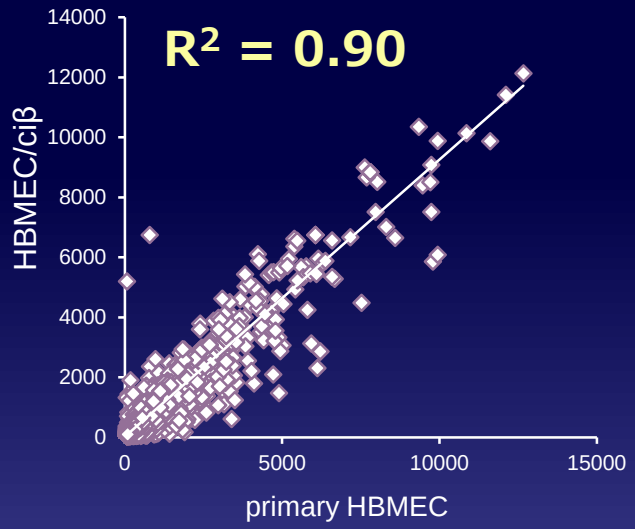
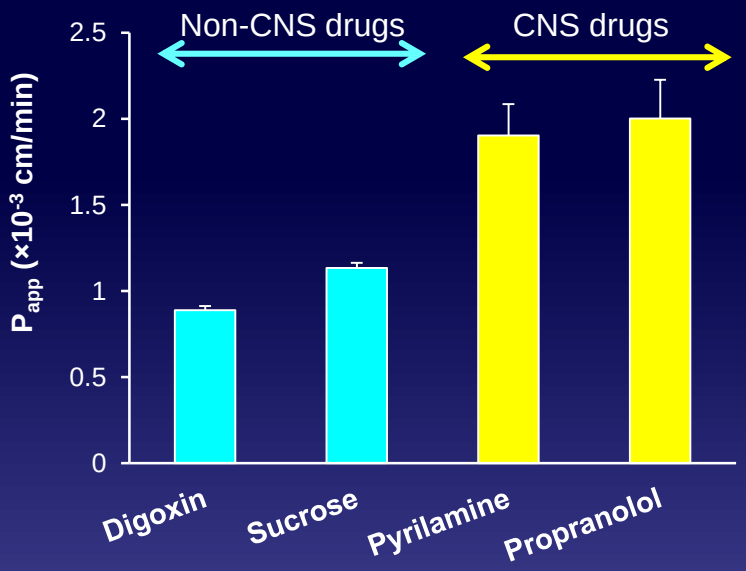


Global mRNA expression profile



Permeability analysis

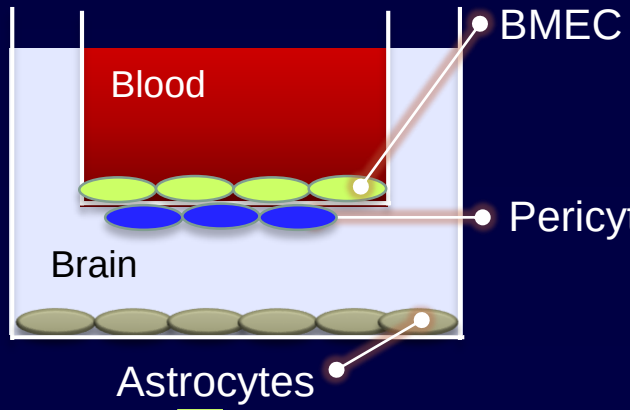


Kamiichi, Furihata et al. Brain Res 2012:1488:113-22
Furihata et al. Fluids Barriers CNS. 2015:5;12:7.

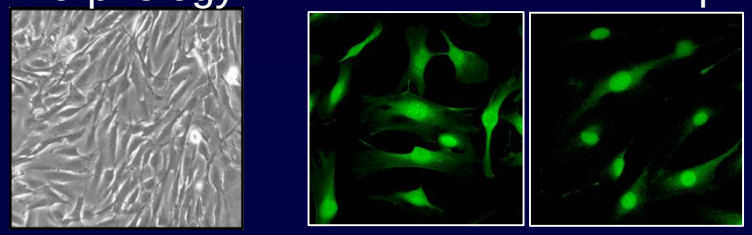
Furihata et al. unpublished.

Establishment of immortalized human astrocytes and pericytes

In vitro BBB model

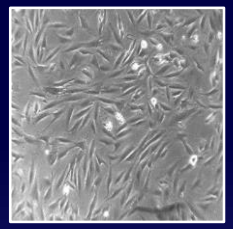


Establishment of human brain pericytes/ conditionally immortalized clone 37 (HBPC/ci37)

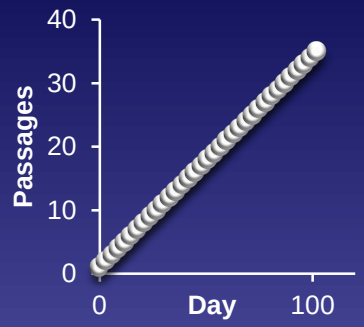


Furihata et al. unpublished.

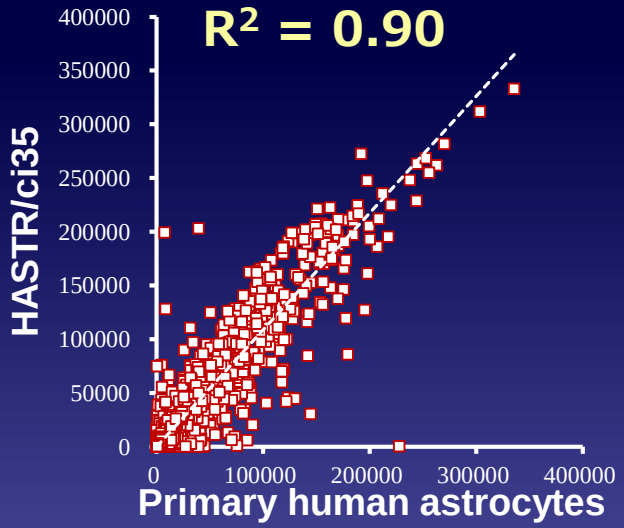
Establishment of human astrocyte/conditionally immortalized clone 35 (HASTR/ci35)



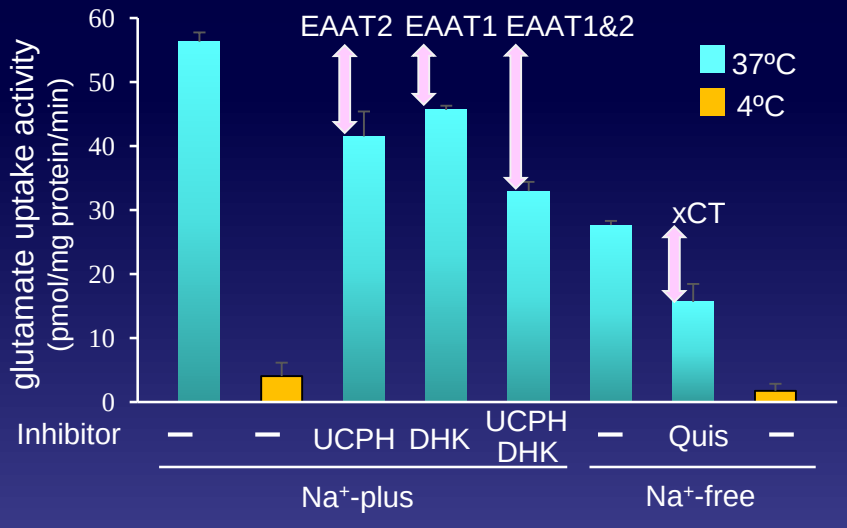
Successive passages



Global mRNA expression profile



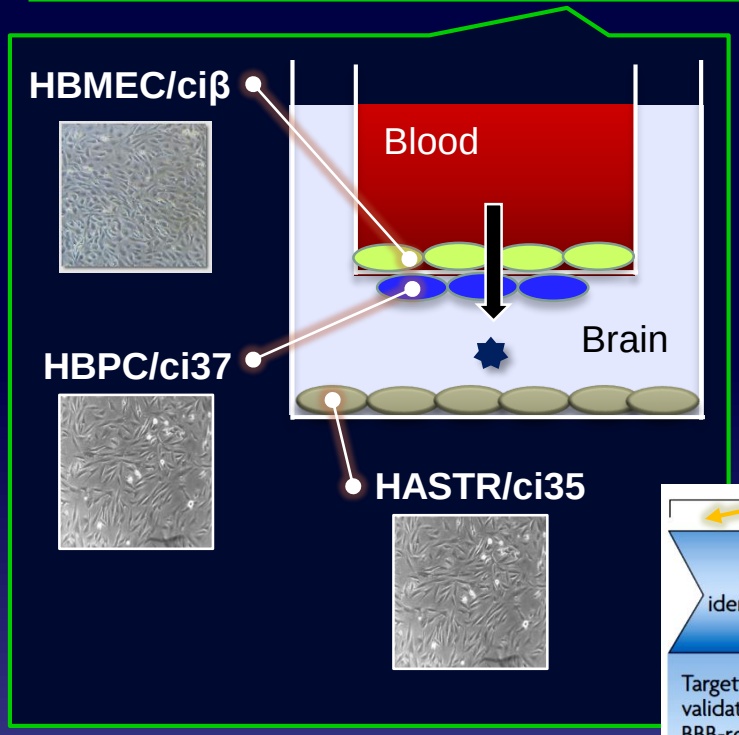
Glutamate uptake activity



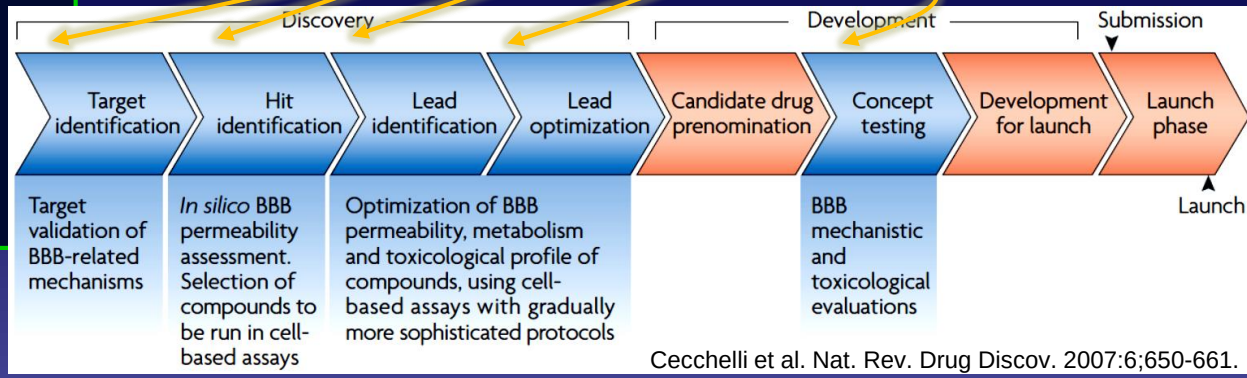
Furihata et al. J Neurochem. 2015, in press.

Summary & Perspective -3-

We have established HBMEC/ciβ, which are highly proliferative and possess BBB properties. Furthermore, we have also established HASTR/ci35 and HBPC/ci37, as part of our ongoing efforts to develop an immortalized cell-based tri-culture *in vitro* BBB model.



Such models will be expected to provide easy-to-use, scalable, versatile, simplified working system in CNS drug development.



Cecchelli et al. Nat. Rev. Drug Discov. 2007:6;650-661.

Acknowledgements

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