Trinity College Dublin
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Cell culture models of the air-blood barrier

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Workshop on Drug Transporters in the Lungs 2016

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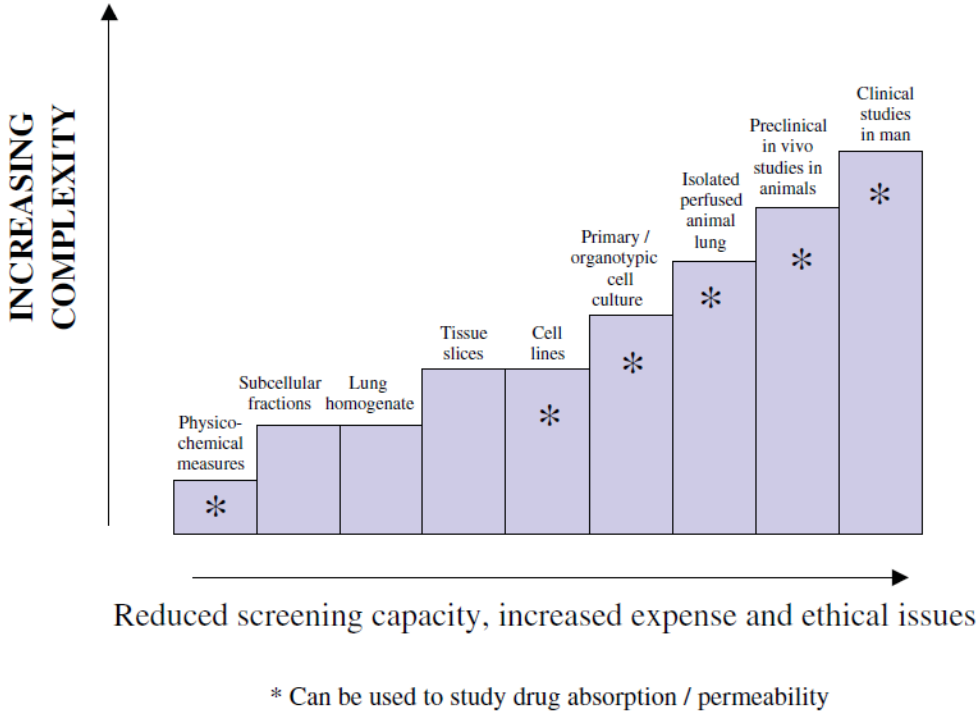
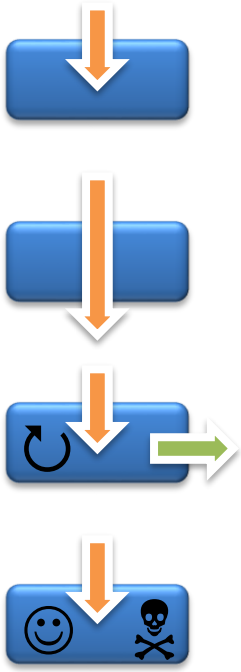
Pre-clinical systems for pulmonary drug disposition studies



Tronde *et al.* in Ehrhardt, Kim (eds.) Drug Absorption Studies, 2008

In vitro models - applications

- Uptake studies
- Transport studies
- Metabolism studies
- Irritation and toxicity studies

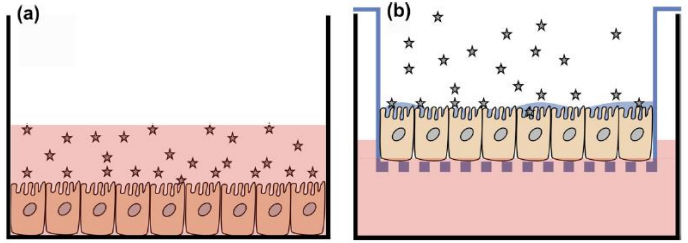


In vitro models – important questions

- Human or animal origin?
  - Animal cells to support *in vivo*/*ex vivo* data
- Freshly isolated cells in primary culture or continuously growing cell lines?
  - Cost, effort and dedifferentiation of primary cells
  - Cancer cells or immortalised cells?
- Monoculture or co-culture?

In vitro models – additional considerations

- Need for thorough characterisation
- GCCP: Genotyping, mycoplasma tests
- Choice of culture conditions
  - Air-liquid interface (a) *vs.* submersed culture (b)
  - Medium and supplements
  - Extracellular matrix
  - Time in culture
  - Oxygen pressure
  - Etc., etc., etc.

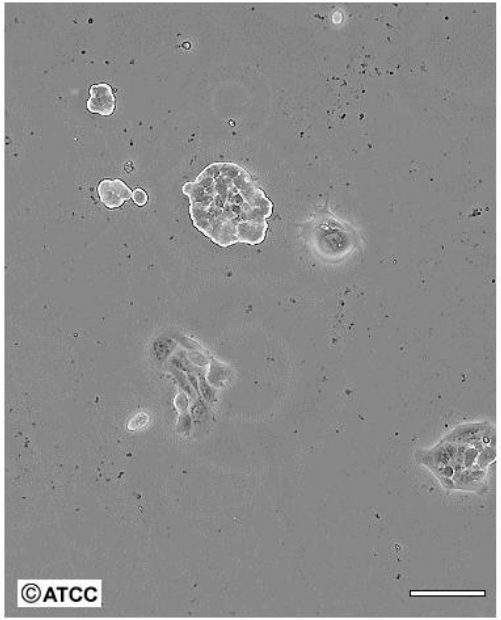


The ideal *in vitro* model of lung epithelial barrier

- Cellular phenotype of the barrier *in situ*
- Confluent, polarised cell layer(s)
- Mixed cell types
- Cilia (trachea/bronchi/bronchioles)
- Mucus (trachea/bronchi/bronchioles)
- Surfactant (bronchioles/alveoli)
- Membrane vesicles/invaginations (alveoli)
- Drug transporters
- Metabolic enzymes

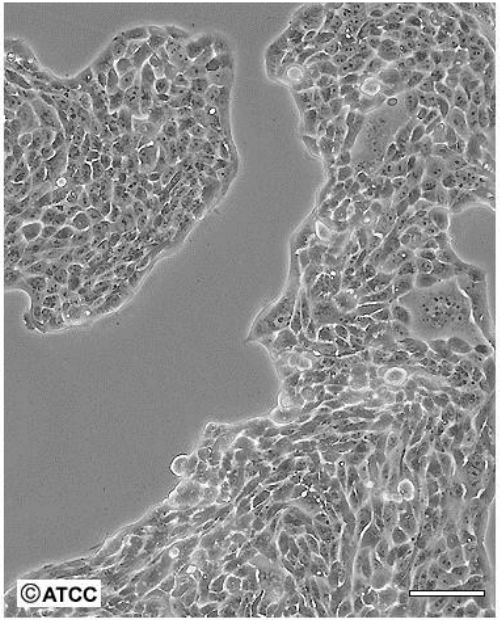
Calu-3 (ATCC HTB-55)

ATCC Number: **HTB-55**
Designation: **Calu-3**



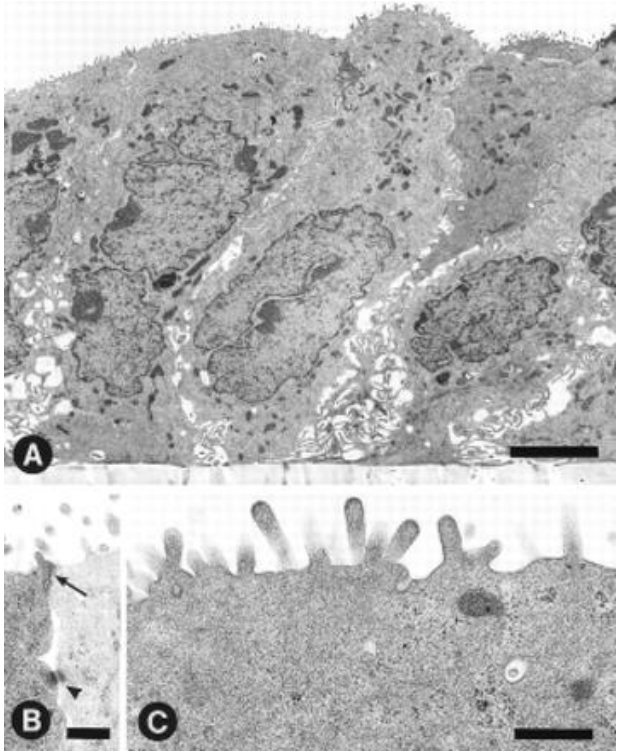
Low Density

Scale Bar = 100µm



High Density

Scale Bar = 100µm



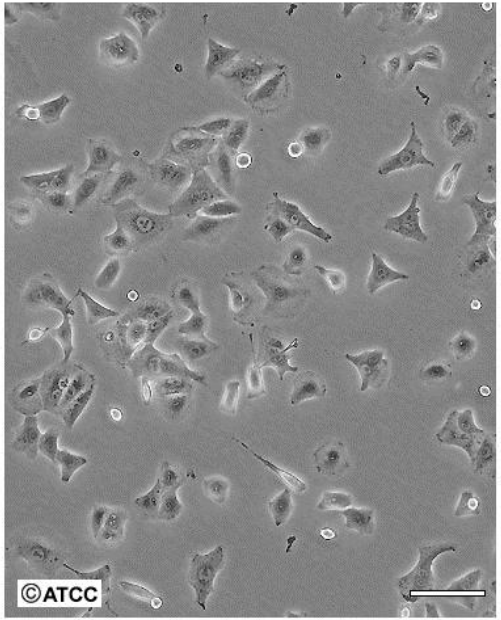
Loffing *et al.* (1998) *Am J Physiol*

Calu-3 (ATCC HTB-55)

- 25 years, Caucasian male
- Cell line was derived by J Fogh (<1975) from metastatic site: pleural effusion
- Advantages: Commercially available, polarised, AIC possible, BSL1
- Disadvantages: Lung adenocarcinoma; sub-epithelial gland cell phenotype, no cilia
- The stemline chromosome number is hypotriploid with the 2S component occurring at 1.4%. Normal chromosomes 1, 13, 15 and 17 are absent and the X is disomic. No Y chromosome detected.

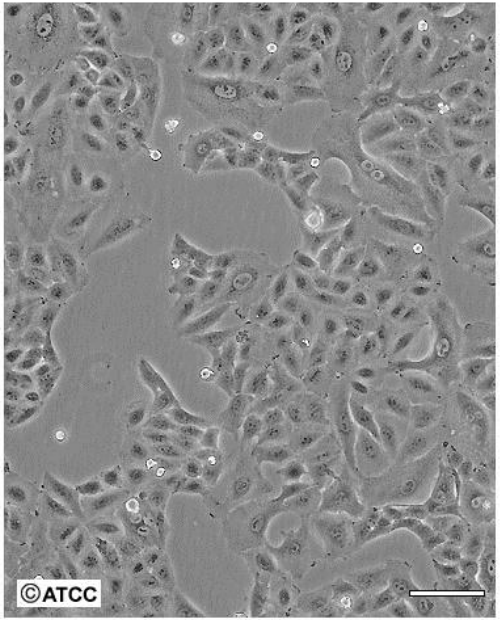
A549 (ATCC CCL-185)

ATCC Number: **CCL-185**
Designation: **A-549**



Low Density

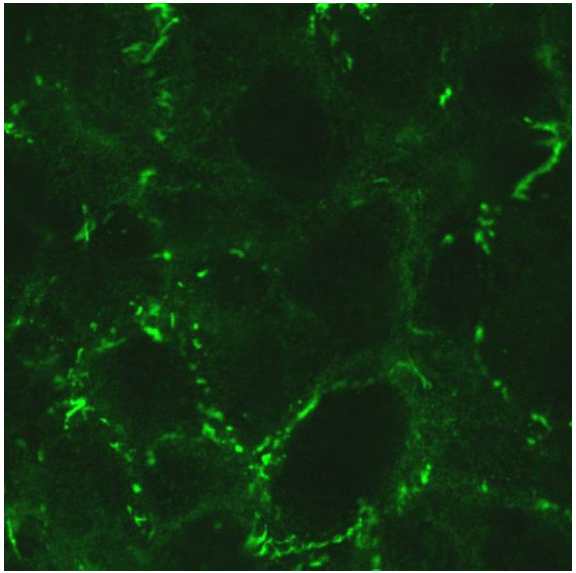
Scale Bar = 100µm



High Density

Scale Bar = 100µm

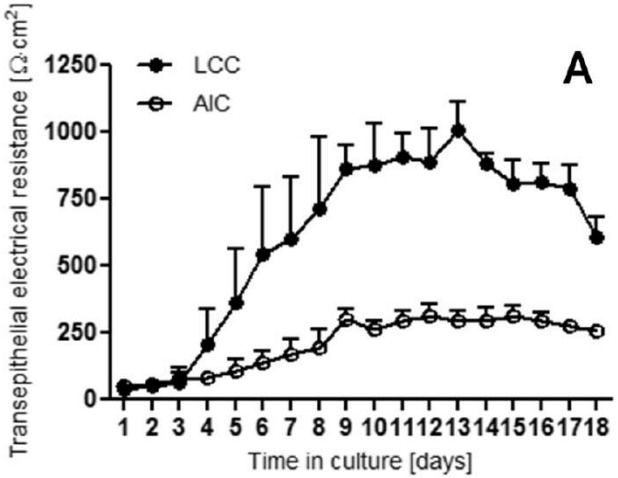
Occludin

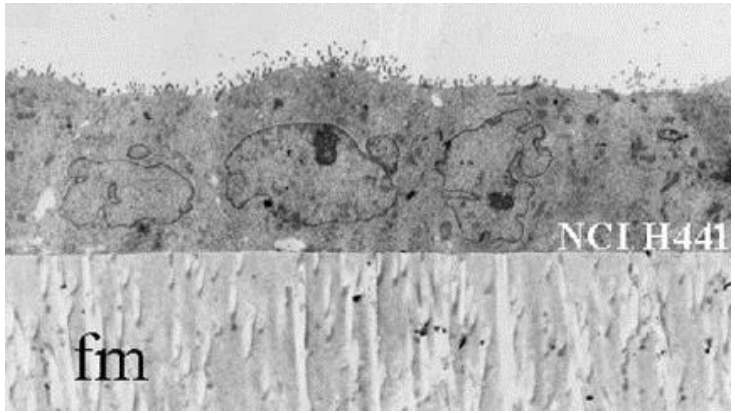


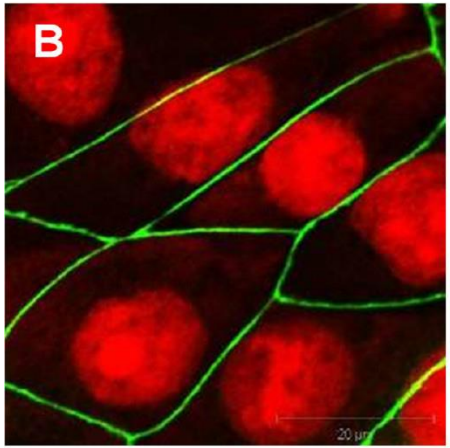
A549 (ATCC CCL-185)

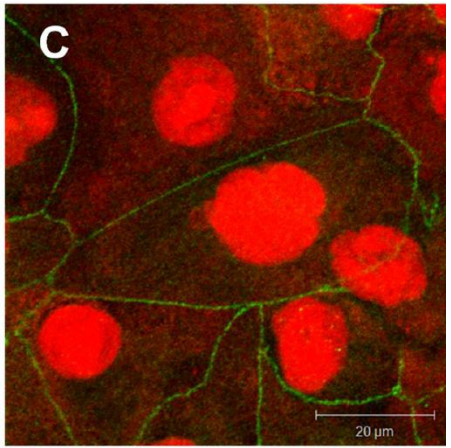
- 58 years, Caucasian male
- This line was initiated in 1972 by DJ Giard through explant culture of lung carcinomatous tissue
- Advantages: Commercially available, BSL1, ATII cell phenotype, AIC possible?
- Disadvantages: Lung adenocarcinoma; derived from metastatic site: pleural effusion, non-polarised
- This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65 and 67 chromosome counts also occur at relatively high frequencies; the rate with higher ploidies is low at 0.4%. Most cells have two X and two Y chromosomes. However, one or both Y chromosomes are lost in 40% of 50 cells analysed. Chromosomes N2 and N6 have single copies per cell; and N12 and N17 usually has 4 copies.

NCI-H441 (ATCC HTB-174)









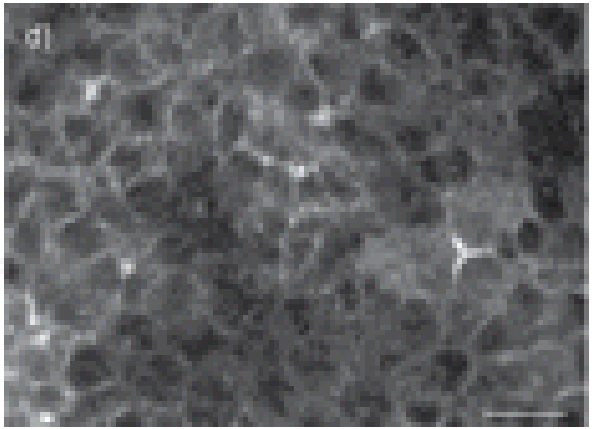
Salomon *et al.* (2014) *Mol Pharm*

NCI-H441 (ATCC HTB-174)

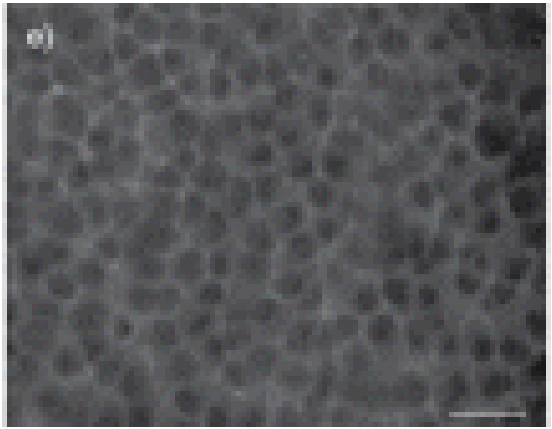
- The NCI-H441 cell line was derived by AF Gazdar and associates in 1982 from the pericardial fluid of a male patient with papillary adenocarcinoma of the lung.
- Advantages: Commercially available, polarised, AIC possible, BSL1
- Disadvantages: Lung adenocarcinoma
- This is a hyperdiploid human cell line. The modal chromosome number is 52 (range 44 -59). The rate of cells with higher ploidies is 4.9%. Structurally normal N13 and N14 are absent and N1, N6, N9, N12, N17, F and G chromosomes are single. A single copy each of the X and Y chromosome was detected.

NCI-H292 (ATCC CRL-1848)

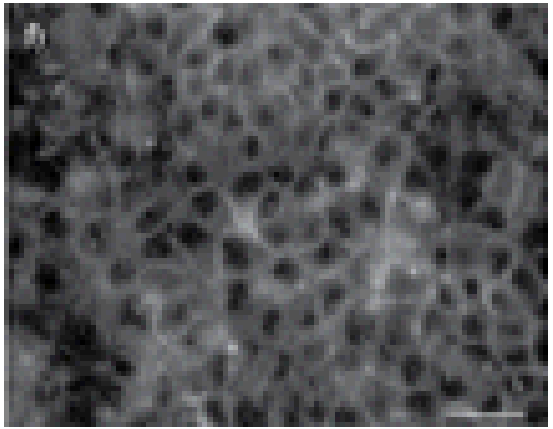
E-Cadherin



ZO-1



F-actin



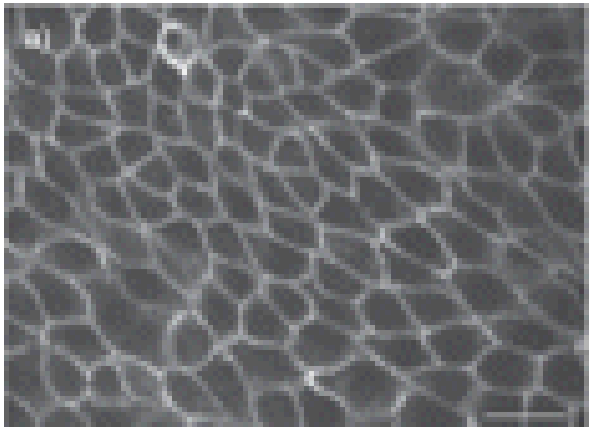
Heijink *et al.* (2010) *Eur Respir J*

NCI-H292 (ATCC CRL-1848)

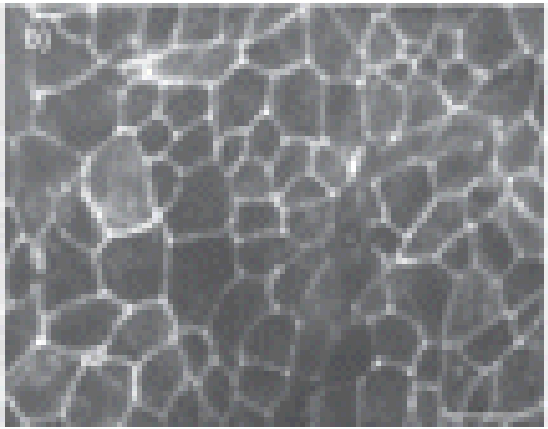
- 32 years, black female
- This line was derived by AF Gazdar (<1983) from a lymph node metastasis of a pulmonary mucoepidermoid carcinoma. The cells were isolated in a chemically defined medium (HITES) and later adapted to growth in media supplemented with serum.
- Advantages: Commercially available, BSL1, AIC possible, mucoepidermoid (bronchial?) phenotype
- Disadvantages: Non-polarised
- This is a human cell line with near-diploid chromosome counts. The modal chromosome number is 47. The rate of cells with a higher ploidy count is 3.9%. Normal N1 and N6 are absent. There are two normal X chromosomes. No other abnormalities detected.

16HBE14o-

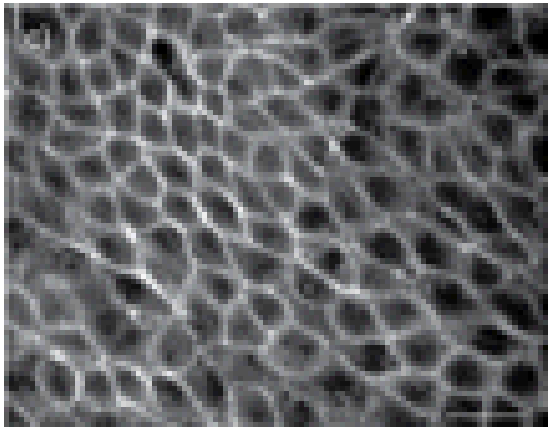
E-Cadherin



ZO-1



F-actin



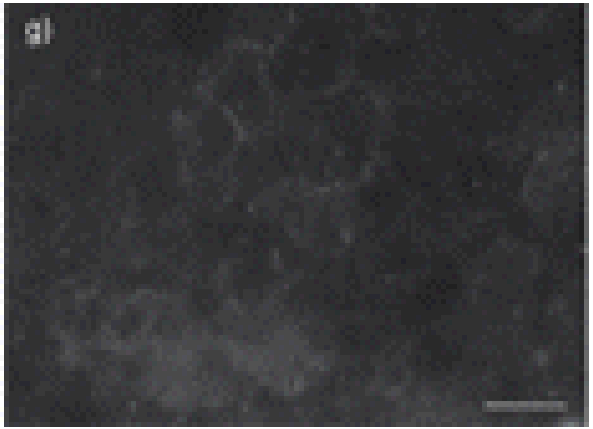
Heijink *et al.* (2010) *Eur Respir J*

16HBE14o-

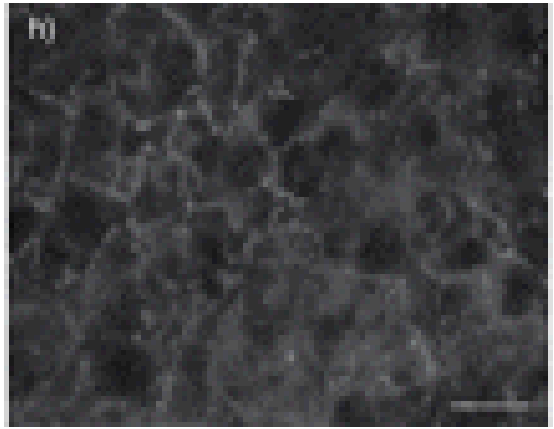
- Cells were isolated from normal human bronchial epithelium (1st bifurcation) obtained from a 1-year old heart-lung transplant patient. The cells were infected with a SV40 T antigen containing a replication defective pSVori- plasmid and cloned. 1988
- Advantages: Ciliated under AIC?
- Disadvantages: BSL2, polarised only when LCC, unclear supply situation (NB. DC Gruenert passed away in early 2016)

BEAS-2B (ATCC CRL-9609)

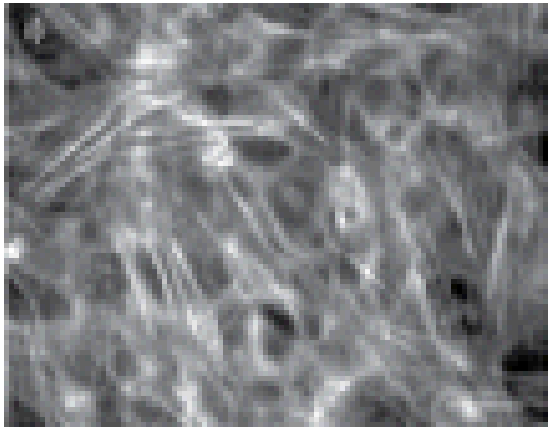
E-Cadherin



ZO-1



F-actin



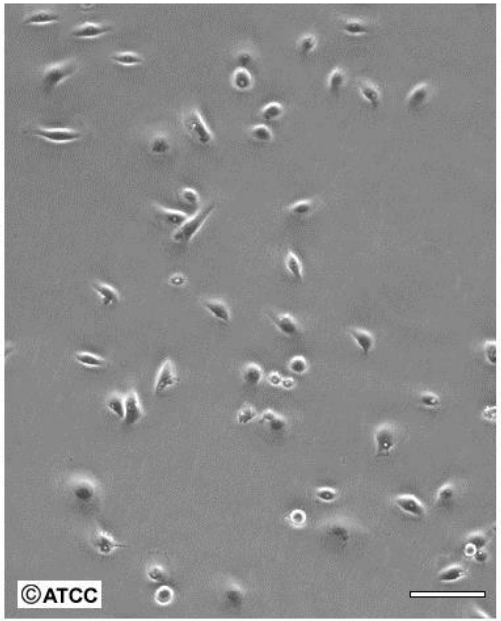
Heijink *et al.* (2010) *Eur Respir J*

BEAS-2B (ATCC CRL-9609)

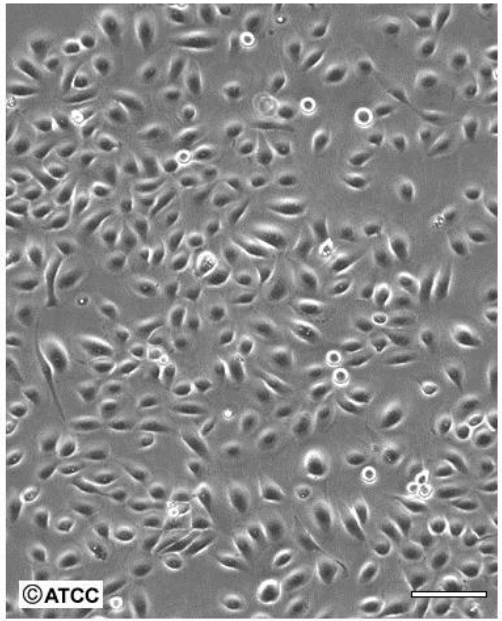
- Epithelial cells were isolated from normal human bronchial epithelium obtained from autopsy of non-cancerous individuals. The cells were infected with an adenovirus 12-SV40 virus hybrid (Ad12SV40) and cloned.
- Advantages: Commercially available, AIC possible?
- Disadvantages: BSL2, non-polarised
- “M-FISH results showed that the BEAS-2B cells in an unstable karyotype, chromosome number nearly diploid-based, but there are a large number of cross swap their chromosome structure.”

NuLi-1 (ATCC CRL-4011)

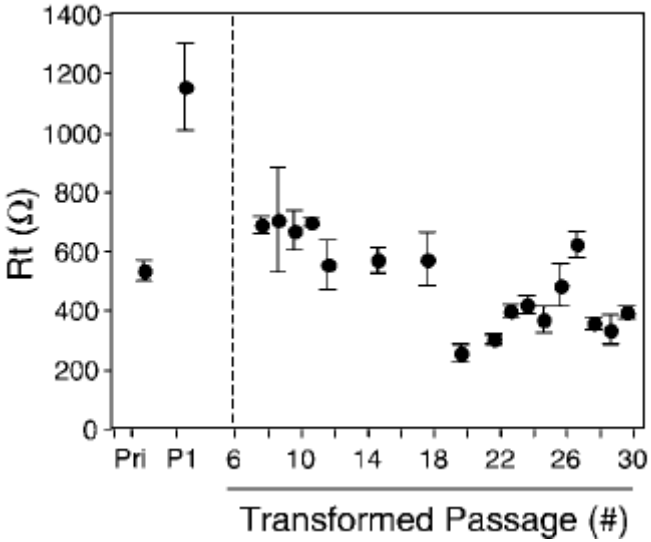
ATCC Number: **CRL-4011**
Designation: **NuLi-1**



Low Density Scale Bar = 100µm



High Density Scale Bar = 100µm

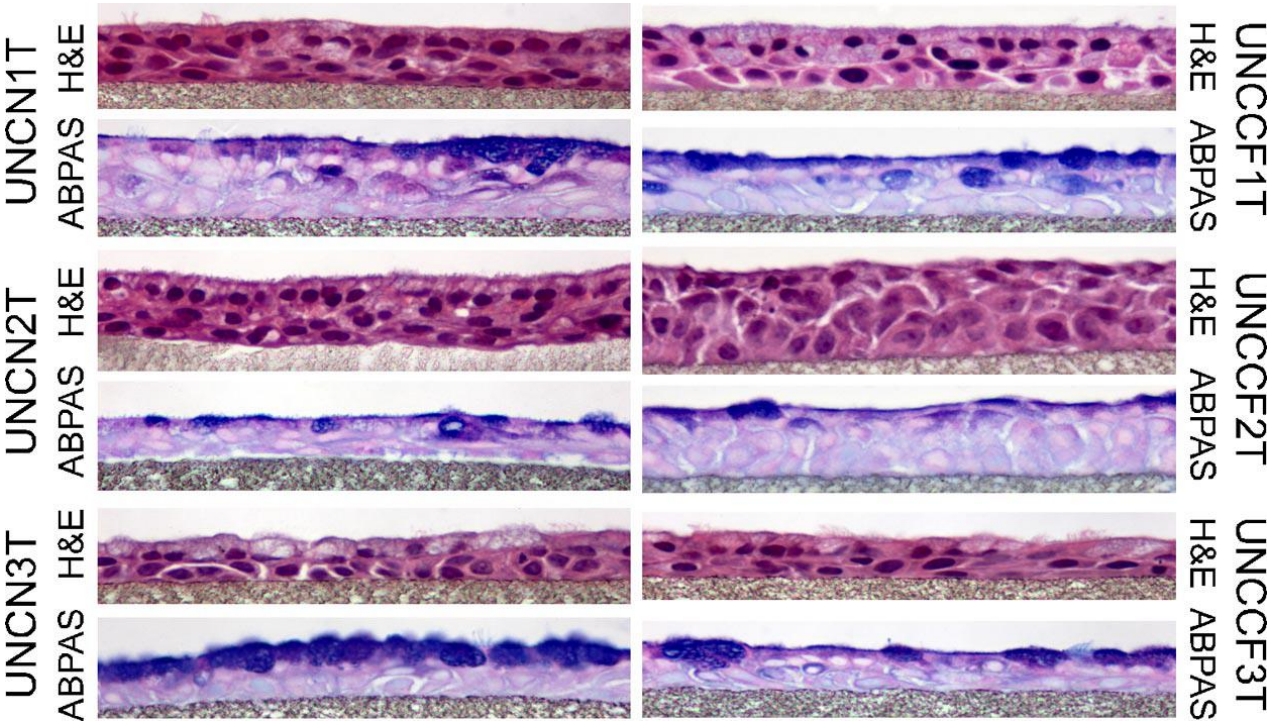


Zabner *et al.* (2003) *Am J Physiol*

NuLi-1 (ATCC CRL-4011)

- 36 years, Caucasian male
- NuLi-1 was derived in 2000 from normal lung of a 36-year-old patient by dual retroviral infection with HPV-16E6/E7-LXSN (hTERT)
- Advantages: Commercially available, AIC possible
- Disadvantages: BSL2
- ATCC. This is a near-diploid human cell line of male origin with a polyploidy rate of 24%. There are copies of karyotypically normal X and Y-chromosomes present in most of the cells analysed. Overall, some of the cells contained chromosomal abnormalities, with the most consistent being trisomy 5 and 20.

UNCN1T, 2T, 3T

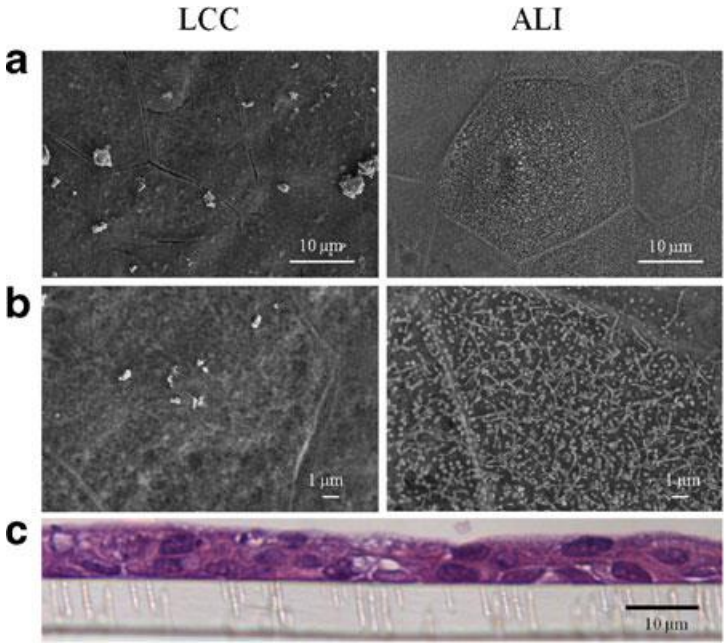


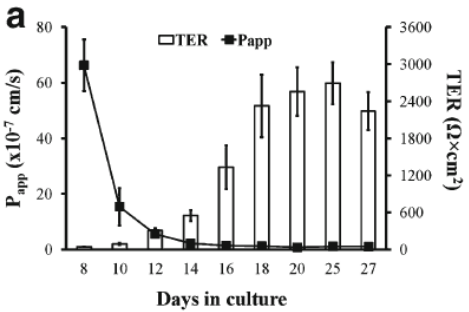
Fulcher *et al.* (2009) *Am J Physiol*

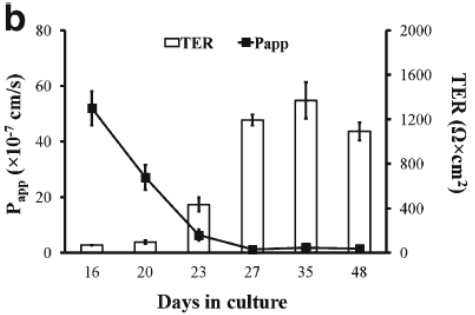
UNCN1T, 2T, 3T

- HIV-1-based lentiviral vectors expressing Bmi-1/hTERT. 2009
- Advantages: AIC possible, ciliated
- Disadvantages: BSL2, not characterised for drug absorption studies, availability?
- Normal diploid chromosome complements.

VA10







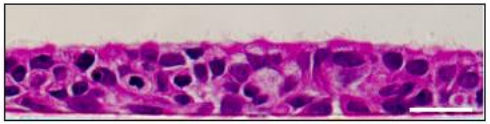
Benediktsdóttir *et al.* (2013) *Pharm Res*

VA10

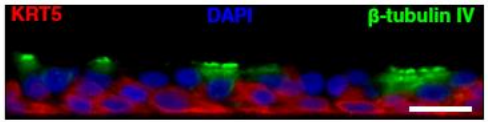
- Basal epithelial cell line established from human bronchial explant by retroviral infection with HPV-16E6/E7. 2007
- Advantages: AIC possible, ciliated
- Disadvantages: BSL2, not commercially available, no information on donor and karyotyping

BCi-NS1.1

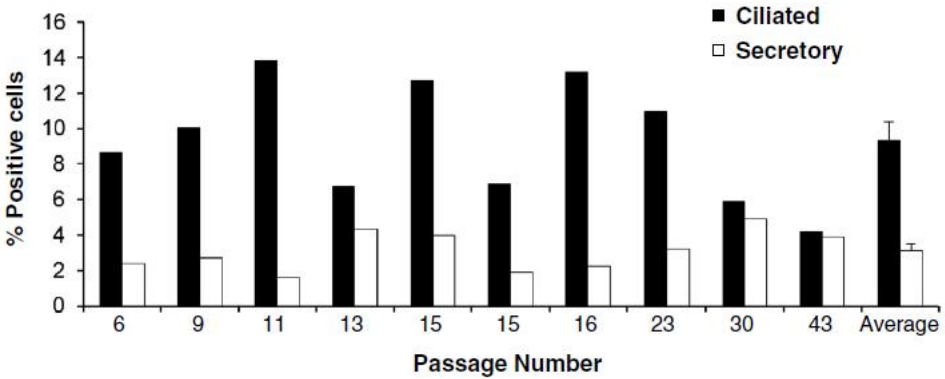
A. H&E, ALI day 28



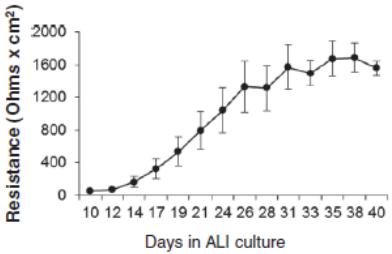
B. Immunofluorescence, ALI day 28



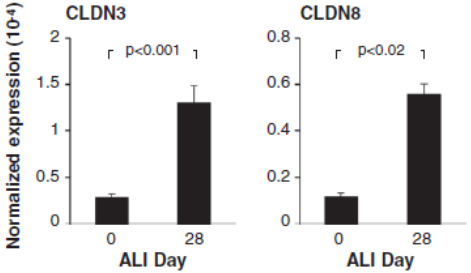
C. Differentiation, ALI day 28



A. Epithelial resistance



B. TaqMan, tight junction genes

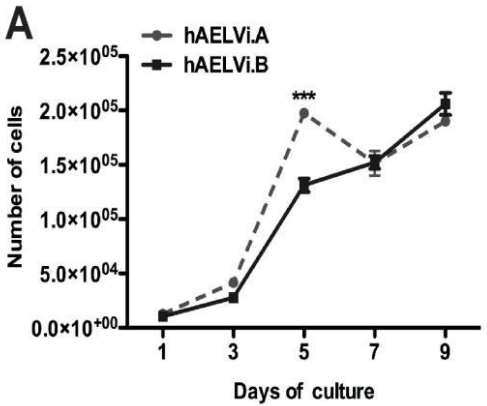


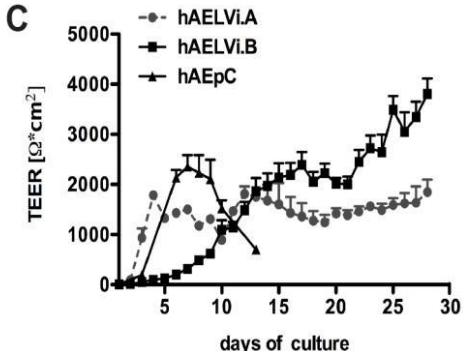
Walters *et al.* (2013) *Respir Res*

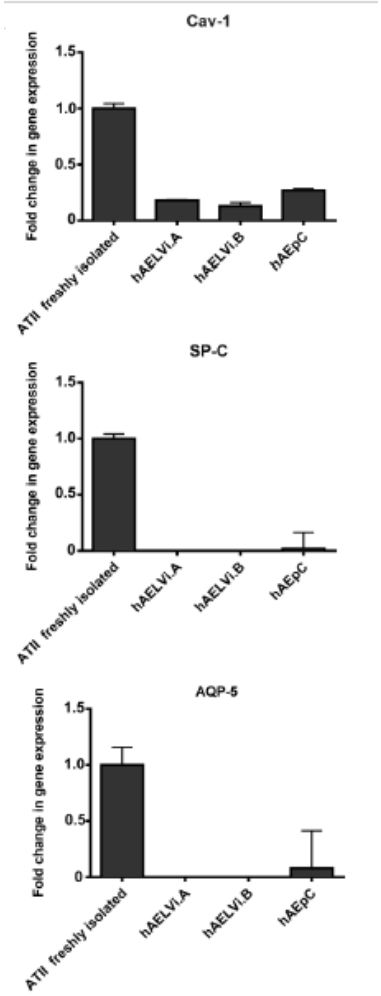
BCi-NS1.1

- Cultured primary airway BC from a healthy 42 year-old Hispanic male non-smoker were immortalised using pBABE-retroviral-mediated expression of hTERT. 2013
- Advantages: AIC possible, ciliated, basal cells that have potential to differentiate
- Disadvantages: BSL2, not characterised for drug absorption studies, availability?
- At passage 9, the majority of the cells had a normal male karyotype (46,XY) with approximately 30% containing trisomy 20 (45% at passage 35).

hAELVi





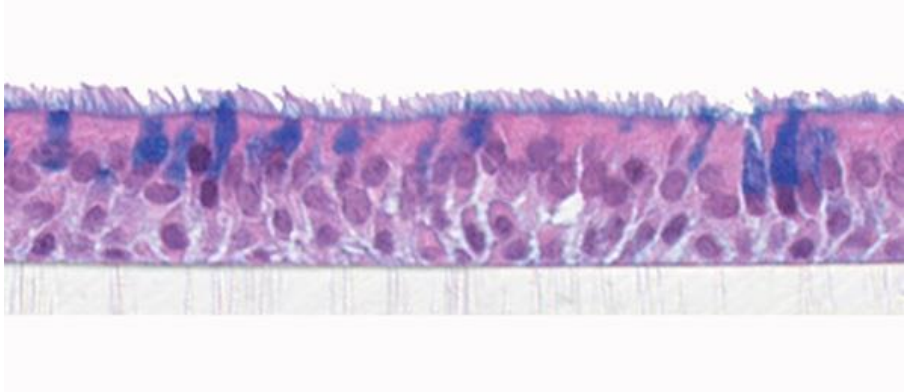


Kuehn *et al.* (2016) *ALTEX*

hAELVi

- Cultured primary alveolar epithelial cells were immortalised using a lentiviral gene library composed of 33 different expansion genes (CI-SCREEN gene library). The expression of recombinant genes was driven by the SV40 promoter. 2016
- Advantages: First human polarised AEC cell line, AIC possible
- Disadvantages: BSL?, not characterised for drug absorption studies, no information on donor (age, sex, disease), availability?
- Thirteen chromosomes revealed no alterations in copy number. The remaining chromosomes revealed several deleted or duplicated chromosome regions.

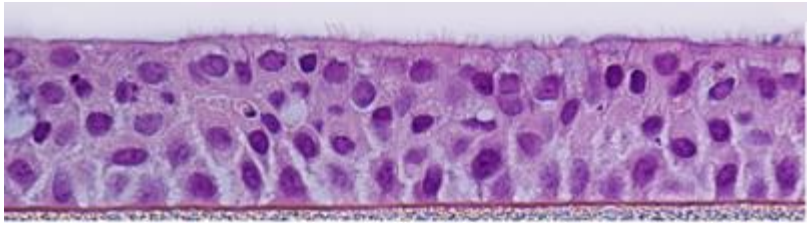
MucilAir™



Proprietary to Epithelix

Characteristics: Air-Liquid interface, Production of mucus, Cilia-beating, Active ions transport, Tight junctions, Metabolic activity, … One year shelf life

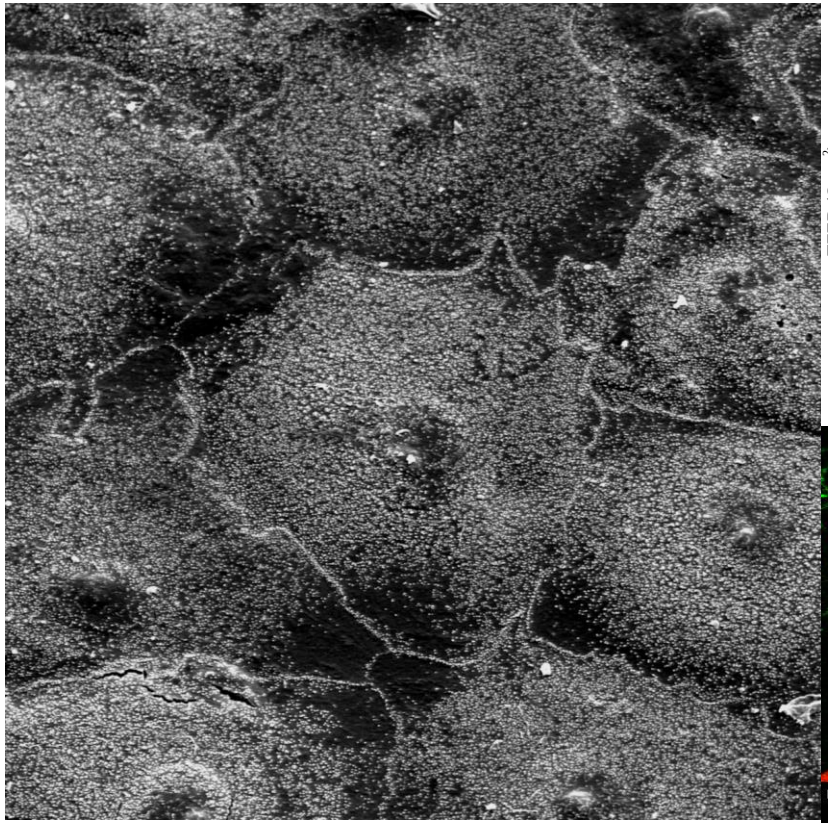
EpiAirway™

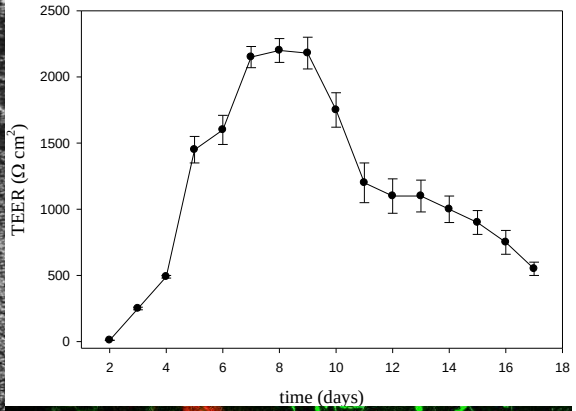


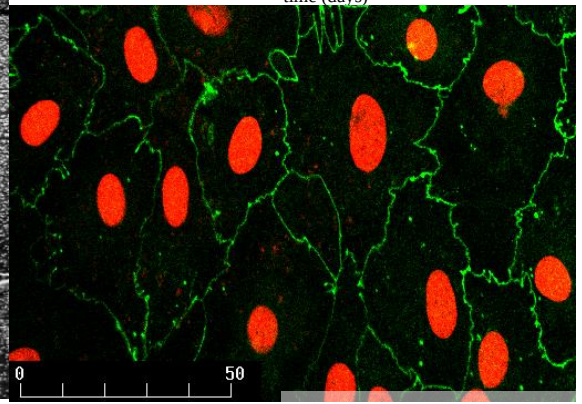
Proprietary to MatTek

MatTek's human 3D *in vitro* respiratory tissue model is redefining preclinical testing in the areas of toxicology, viral research, inflammation & fibrosis and drug development.

Primary culture of human alveolar epithelial cells

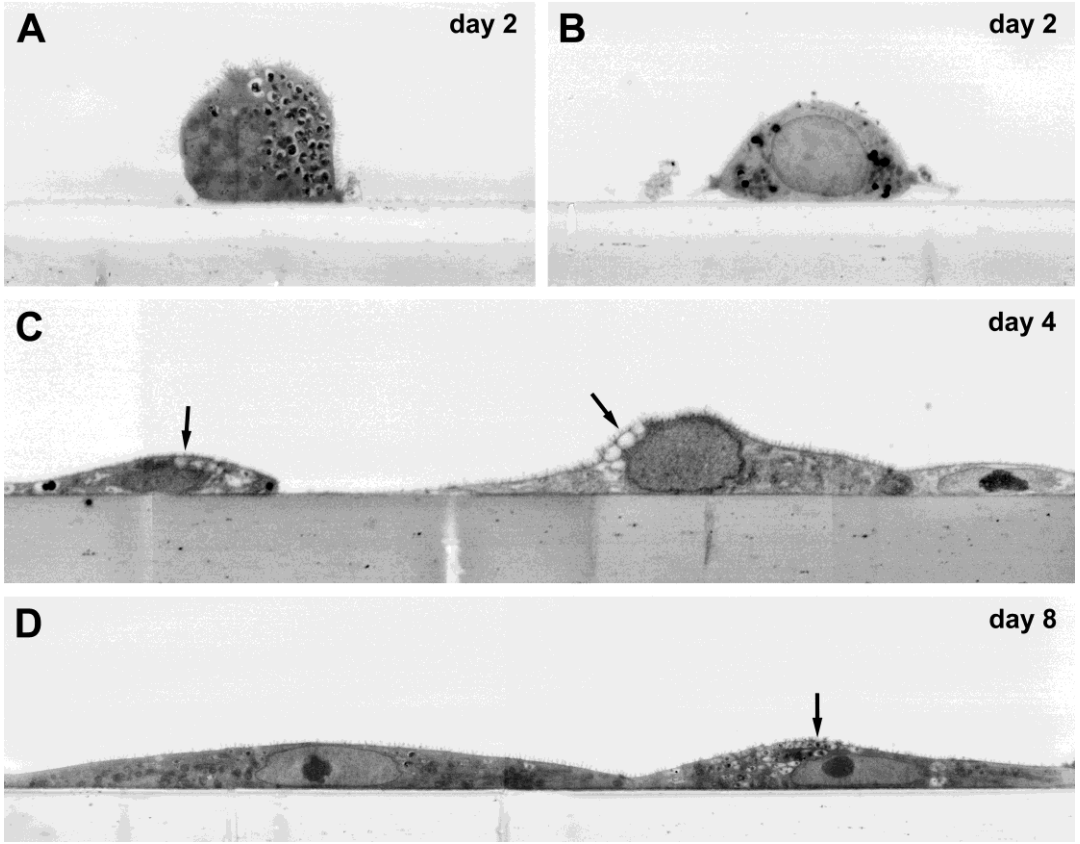






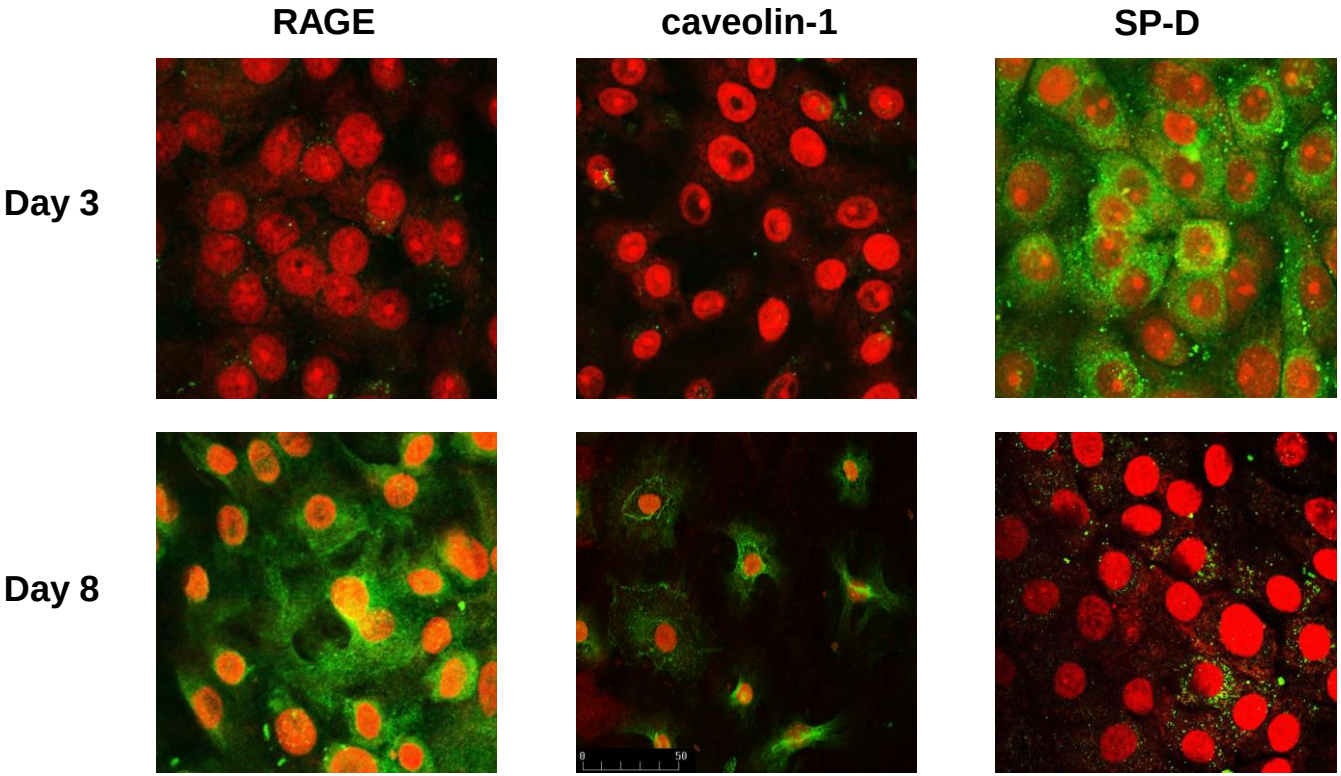
Elbert *et al.* (1999) *Pharm Res*
Ehrhardt *et al.* (2005) *Methods Mol Med*

Transdifferentiation of human alveolar epithelial cells



Fuchs *et al.* (2003) *Cell Tissue Res*

Transdifferentiation of human alveolar epithelial cells



Demling *et al.* (2006) *Cell Tissue Res*

Gene expression analysis in 10 lung cell lines

Number of genes differentially expressed between lung cell models and lung tissues

Relative quantities were calculated based on the comparative threshold cycle method ($RQ = 2^{-\Delta\Delta Ct}$), with BM, PP, AC, or SCC as calibrators. Only the genes that exhibit at least a 4-fold difference in expression ($RQ > 4$ or <0.25) were considered.

Lung Cells and Tissues	16HBE	1HAEO	A549	BEAS-2B	Calu-1	L-132	H292	H358	H460	H727	HBEC
Number of overexpressed genes/BM	92	41	112	27	62	8	24	14	23	20	21
Number of underexpressed genes/BM	128	147	89	132	134	233	138	173	167	220	137
Total	220	188	201	159	196	241	162	187	190	240	158
Number of overexpressed genes/PP	85	44	115	32	58	10	27	18	21	18	33
Number of underexpressed genes/PP	107	123	73	112	105	213	114	148	137	193	119
Total	192	167	188	144	163	223	141	166	158	211	152
Number of overexpressed genes/SCC	107	38	134	40	65	6	35	13	24	19	22
Number of underexpressed genes/SCC	105	111	52	102	101	210	105	129	128	179	87
Total	212	149	186	142	166	216	140	142	152	198	109
Number of overexpressed genes/AC	97	47	131	35	63	6	32	20	18	19	32
Number of underexpressed genes/AC	99	119	55	101	107	209	105	136	132	186	99
Total	196	166	186	136	170	215	137	156	150	205	131

High throughput q-PCT study characterising the expression profiles of 380 genes encoding proteins involved in the metabolism and disposition of xenobiotics in 10 commonly used lung cell lines.

Courcot *et al.* (2012) Drug Metab Dispos

Efflux ratio of rhodamine 123 in different *in vitro* models

Cells	Substrate(s)	Inhibitor(s)	Direction of polarised transport	Efflux ratio
Calu-3[13]	Rhodamine 123,	Ciclosporin,	B–A	11.5 (Hamilton 2001)
Calu-3	Rhodamine 123	Verapamil	B-A	2.4 (Haghi 2010)
16HBE14o-[9]	Rhodamine 123	Verapamil	B–A	2.95
NHBE[16]	Rhodamine 123	Verapamil	B–A	2.95
Alveolar type I-like cells[10]	Rhodamine 123	Verapamil	B–A	3.09
VA10	Rhodamine 123	Verapamil	B-A	1.44
NCI-H441	Rhodamine 123	LY335979	B-A	3.58

Adapted from Madlova *et al.* (2009) *J Pharm Pharmacol*

ASP^+ uptake into different primary epithelial cell cultures

Table 1
Kinetic Parameters of ASP^+ Uptake Into Primary Cultured Human Pulmonary Cells

Cells	V_{max} (nmol/min·mg Protein^{-1})	K_m (mM)
HTEpiC	1.47 ± 0.22	0.741 ± 0.203
NHBE	1.63 ± 0.19	0.543 ± 0.127
HPAEpiC	1.73 ± 0.25	0.592 ± 0.171

Maximum uptake rates (V_{max}) and Michaelis constant (K_m) were calculated using nonlinear least squares regression to fit the uptake rate (v) to the Michaelis–Menten equation (Eadie–Hofstee plot): $v = V_{max}(S)/[K_m + (S)]$, where S is the ASP^+ concentration.

Uptake assay of ASP^+ was performed in triplicate for each donor. Data are represented as the mean ± SD for the averages of a triplicate assay from five different donors.

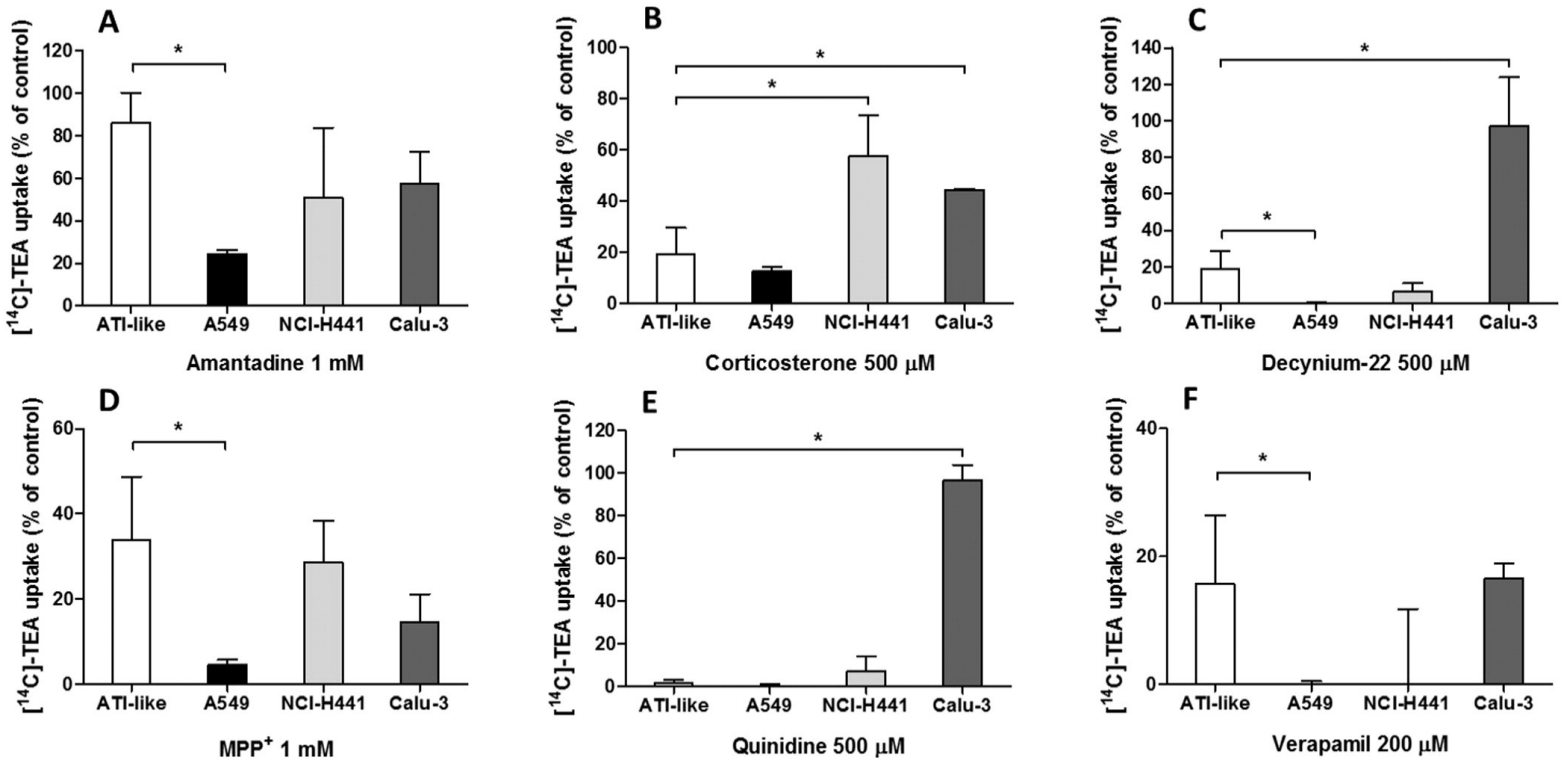
Table 2
Correlation Coefficient Between Protein Expression Levels (fmol/μg Protein) in the Plasma Membrane Fraction and V_{max} of ASP^+ Uptake (Δnmol/min/mg Protein)

Cells	Correlation Coefficient (R^2)				
	OCT1	OCT2	OCT3	OCTN1	OCTN2
HTEpiC	0.0124	0.0270	N.C.	0.965	N.C.
NHBE	0.115	0.0969	N.C.	0.834	N.C.
HPAEpiC	N.C.	0.281	N.C.	0.877	N.C.

Donors were identical to those in a previous study that quantified protein expression of drug transporters, and OCT1, OCT2, OCT3, OCTN1, and OCTN2 protein expression levels in the plasma membrane fraction are taken from the previous data.[8] Correlation coefficient of OCT1 in HPAEpiC, OCT3 and OCTN2 in HTEpiC, NHBE, and HPAEpiC, were not calculated (N.C.) because their protein expressions of plasma membrane fraction were not detected.[8]

Sakamoto *et al.* (2016) *J Pharm Sci*

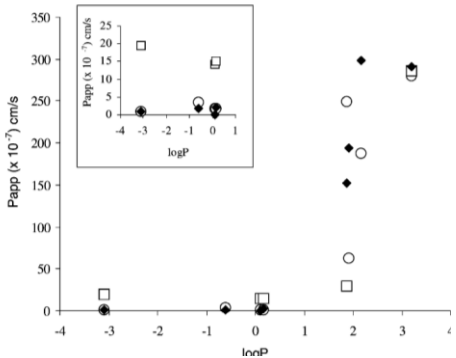
Pharmacological inhibition of TEA uptake



Salomon *et al.* (2015) *Eur J Pharm Sci*

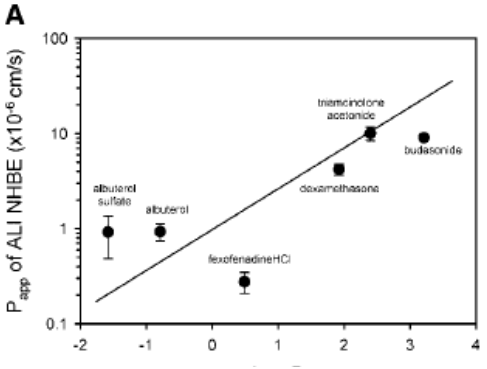
Correlations – physiochemical parameters

16HBE14o-, rAEC, RTEC vs. log P



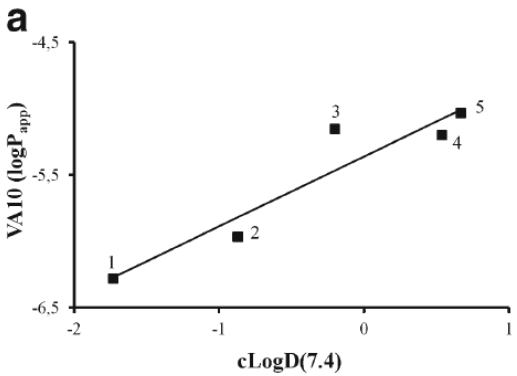
Forbes *et al.* (2003) *Int J Pharm*

NHBE vs. log P

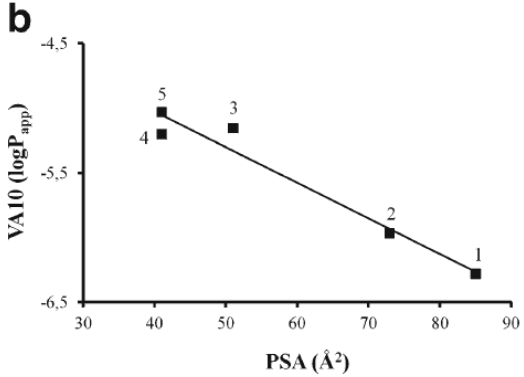


Lin et al. (2007) *J Pharm Sci*

VA10 vs. logP



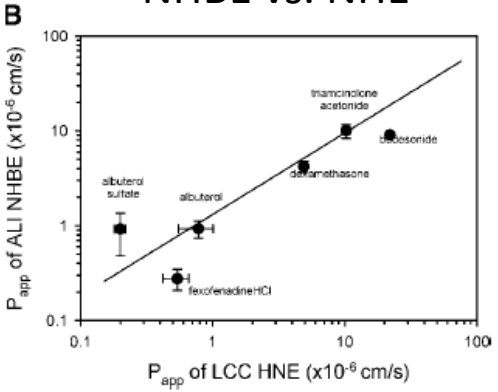
VA10 vs. PSA



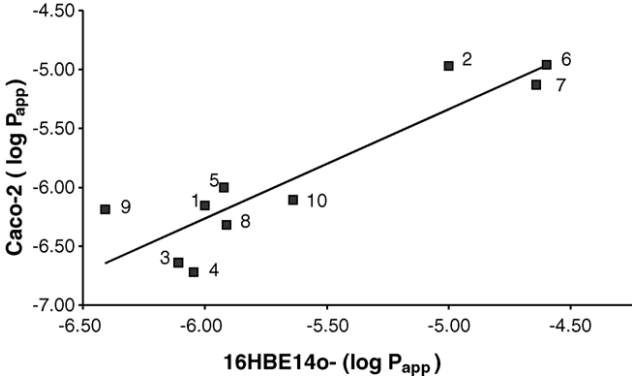
Benediktsdóttir *et al.* (2013) *Pharm Res*

Correlations – with each other

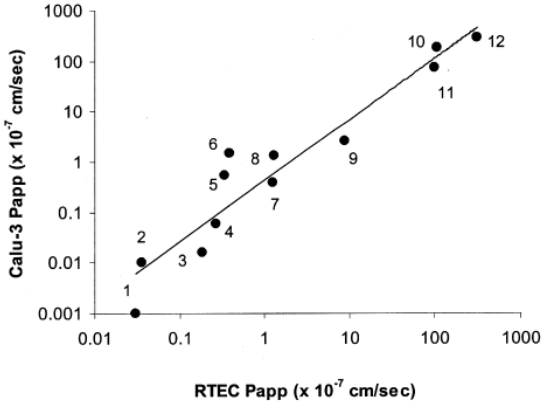
NHBE vs. NHE



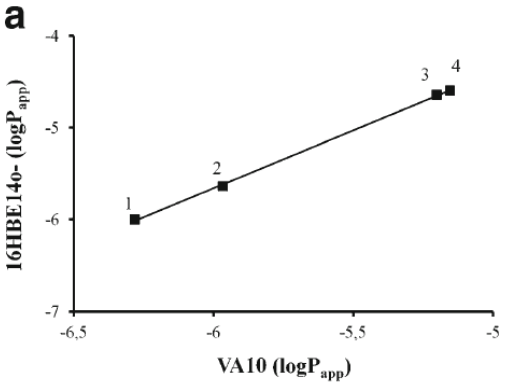
16HBE14o- vs. Caco-2

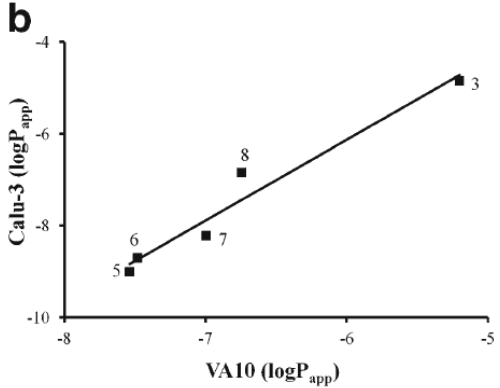


Calu-3 vs. RTEC



VA10 vs. 16HBE14o-



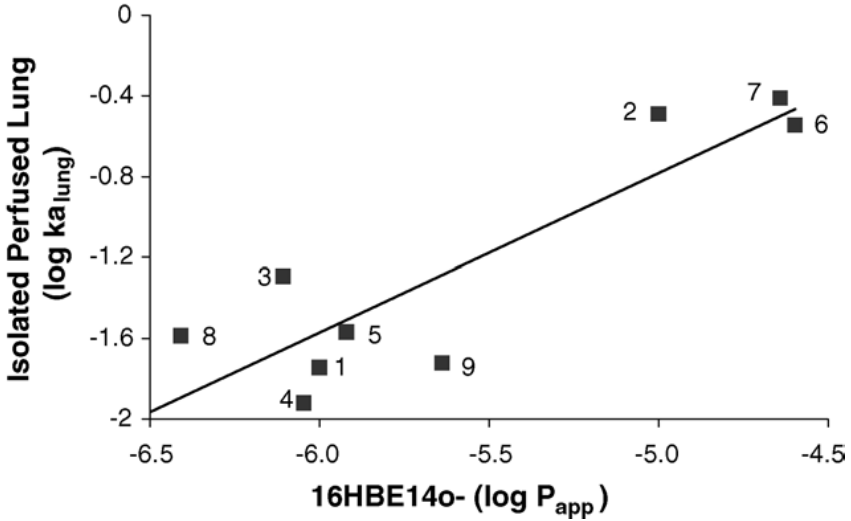


VA10 vs. Calu-3

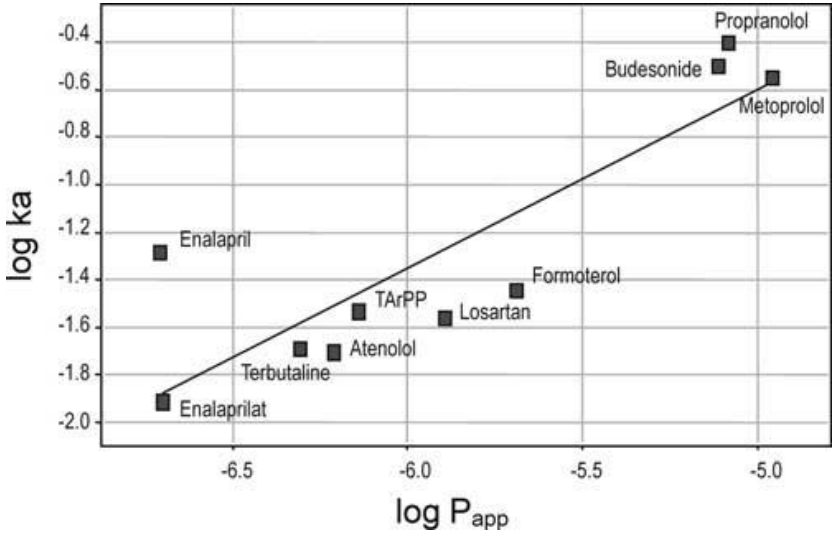
Mathias *et al.* (2002) *J Drug Target* Lin et al. (2007) *J Pharm Sci* Manford et al. (2005) Eur J Pharm Sci Benediktsdóttir *et al.* (2013) *Pharm Res*

Correlations – with isolated and perfused lungs

16HBE14o- vs. IPRL



Caco-2 vs. IPRL

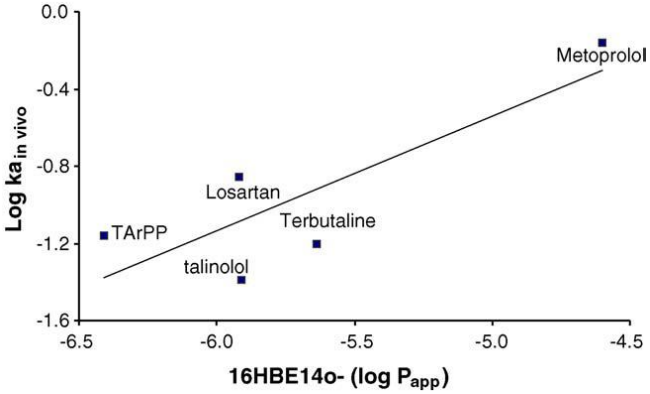


Manford *et al.* (2005) *Eur J Pharm* Sci

Tronde et al. (2003) J Drug Target

Correlations – with *in vivo* instillation/aerosolisation

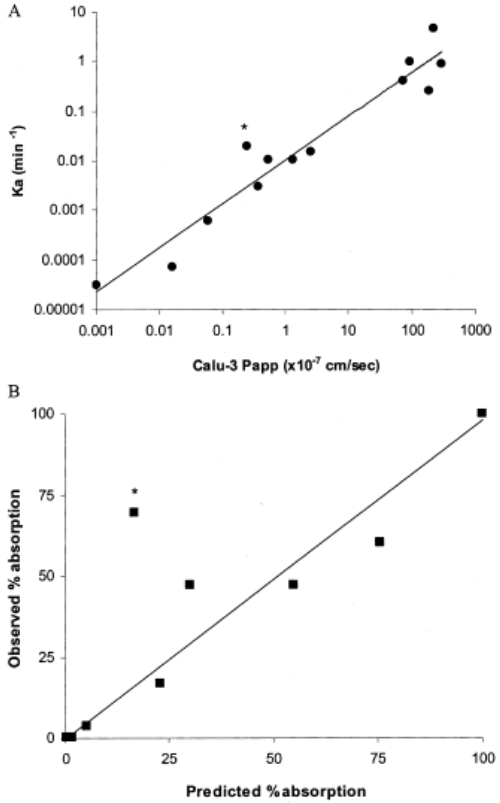
16HBE14o- vs. rat aerolisation



"Permeability in 16HBE14o- cells was less strongly correlated than permeability in Caco-2 cells with the rate of absorption from rat lung *in vivo*"

Manford *et al.* (2005) *Eur J Pharm* Sci

Calu-3 vs. mouse instillation

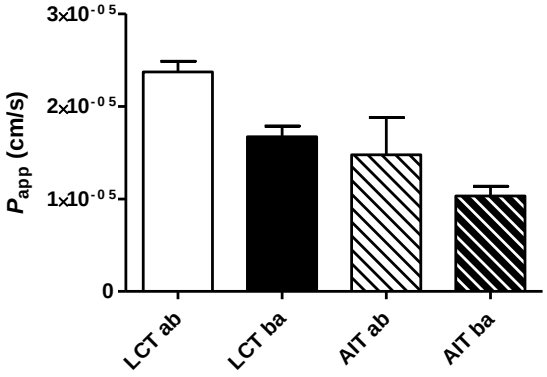


"Based on this relationship, we found that drugs with P_{app} values >5 x 10^{-7} cm/s were completely absorbed, those with P_{app} values <2 x x 10^{-7} cm/s were <50% absorbed, and poorly permeable drugs and/or Macromolecules with P_{app} values <0.1 x 10^{-7} cm/s , were absorbed <10%."

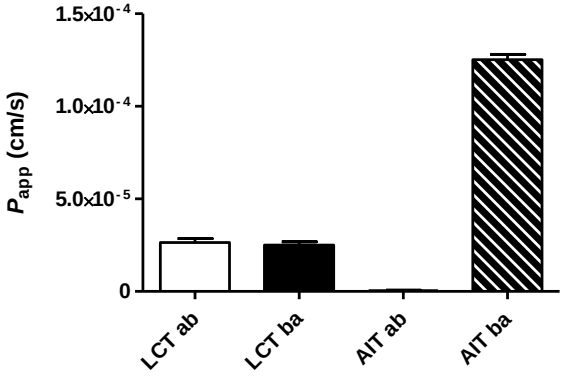
Mathias *et al.* (2002) *J Drug Target*

Air interface transport studies in H441 cell monolayers

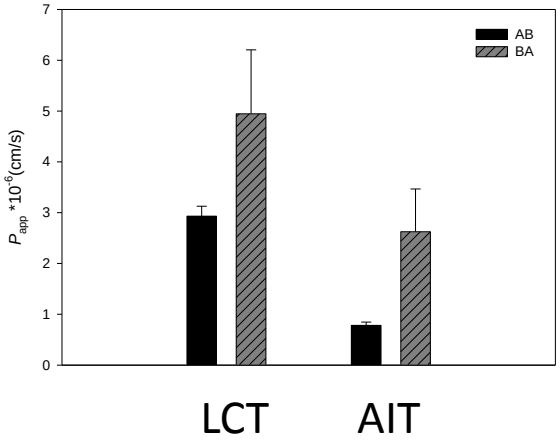
CFDA



[^{3}H]-propranolol



ASP$^+$



Unpublished obersavations

Open questions

- Do we need organotypic cell culture models?
- Do we need different models for different lung sections?
- Do we need complex co-culture models?
- Is the predictive power of the currently available models sufficient to allow IVIVC?
- Do we need a “Caco-2-like” gold standard for the lungs?
- Do transport study conditions matter?