PMI RESEARCH & DEVELOPMENT

High Content Screening-Based Analysis to Support Reduced Risk Products Development

Diego Marescotti PhD

High Content Screening Supervisor

PMI R&D

Philip Morris Products S.A.Neuchatel

SLAS Dresden

29th June 2016

Reduced Risk Products

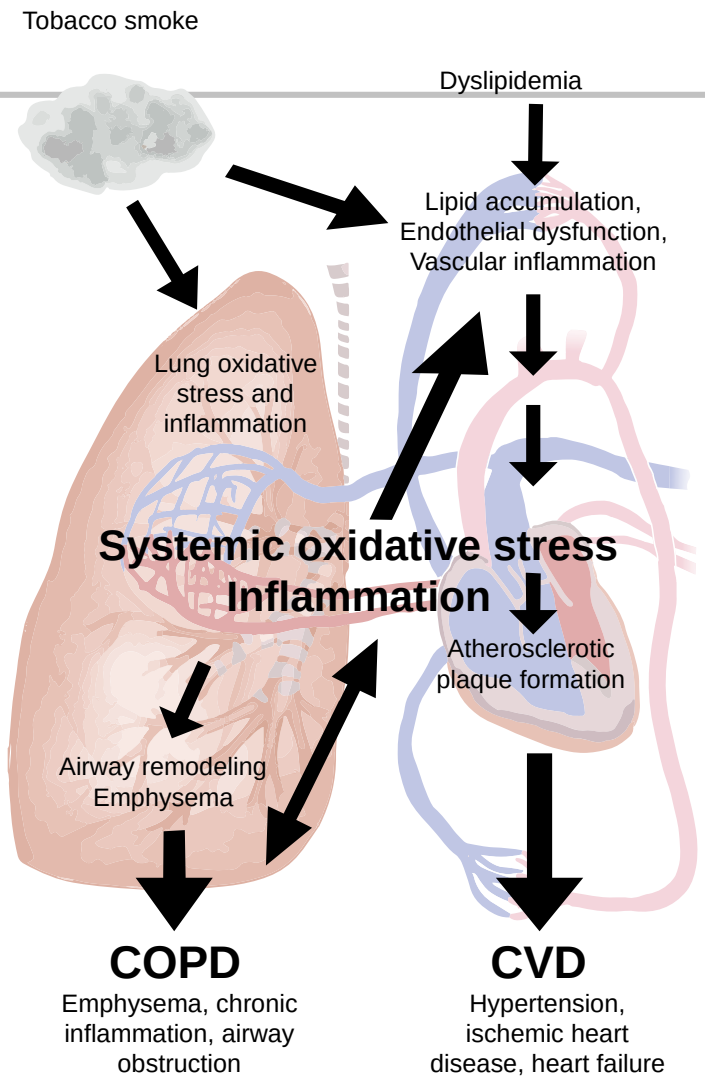
Reduced Risk Products (“RRPs”) is the term we use to refer to products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes. PMI’s RRPs are in various stages of development and commercialization, and we are conducting extensive and rigorous scientific studies to determine whether we can support claims for such products of reduced exposure to harmful and potentially harmful constituents in smoke, and ultimately claims of reduced disease risk, when compared to smoking cigarettes. Before making any such claims, we will rigorously evaluate the full set of data from the relevant scientific studies to determine whether they substantiate reduced exposure or risk. Any such claims may also be subject to government review and authorization, as is the case in the USA today

Agenda

- Smoking-related disease
  - COPD
  - CVD
- HCS assays
  - 2D model
  - 3D model
- HCS for tox testing

Smoking-Related Diseases

- Smoking causes a number of serious diseases including lung cancer, chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD).
- Inflammation and oxidative stress have been recognized as key processes driving the pathogenesis of smoking-related diseases.



Lo Sasso G, J Transl. Med. 2016

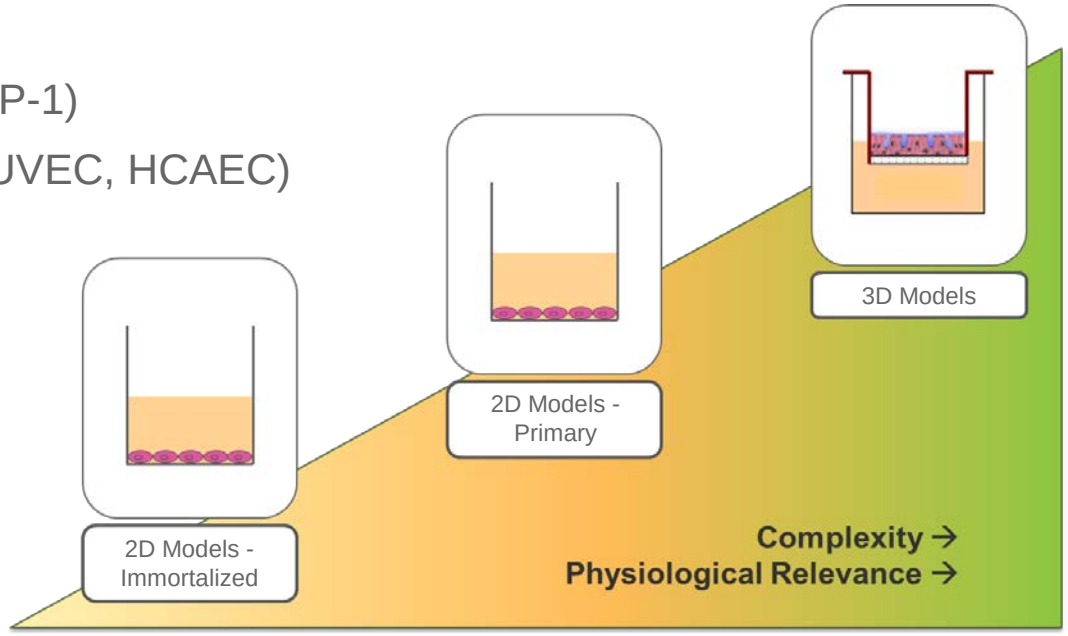
Experimental Model Systems

Lung

- Lung epithelial cell lines (BEAS-2B, BBM, BZR, A549)
- Primary epithelial cells (bronchial epithelial, small airway epithelial) and fibroblasts
- Organotypic models: MucilAir™ (Epithelix SàrL), EpiAirway™ (MatTek Inc.)

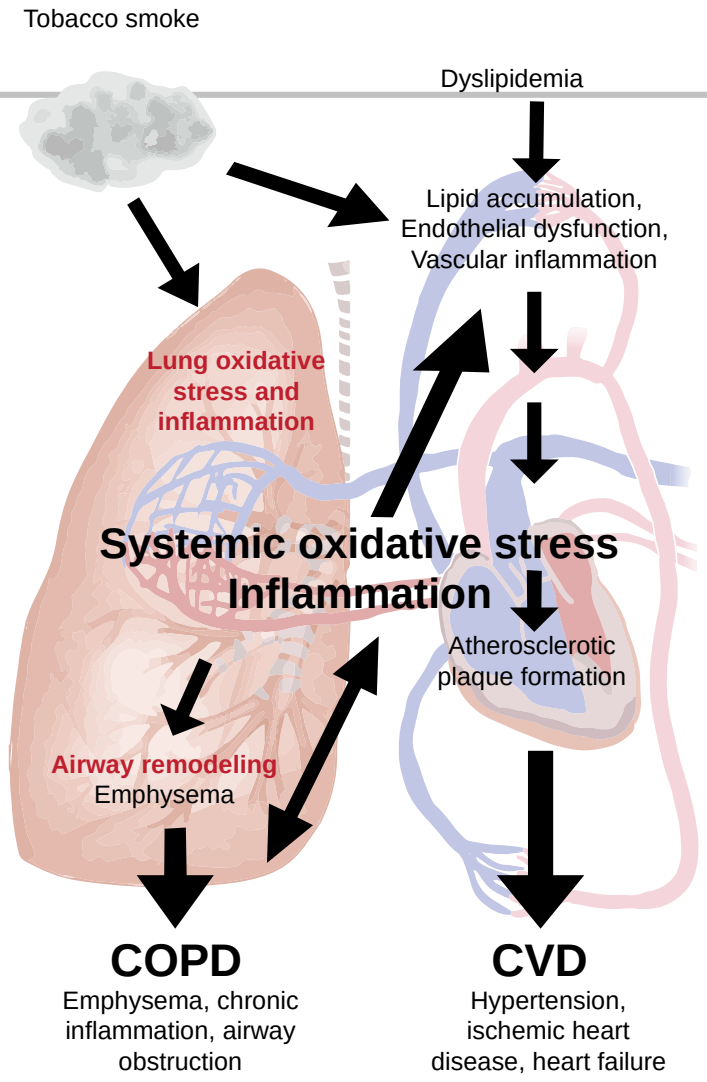
CV System

- Immune cell lines (MM6, THP-1)
- Primary endothelial cells (HUVEC, HCAEC)



Disease-Related HCS Endpoints
COPD

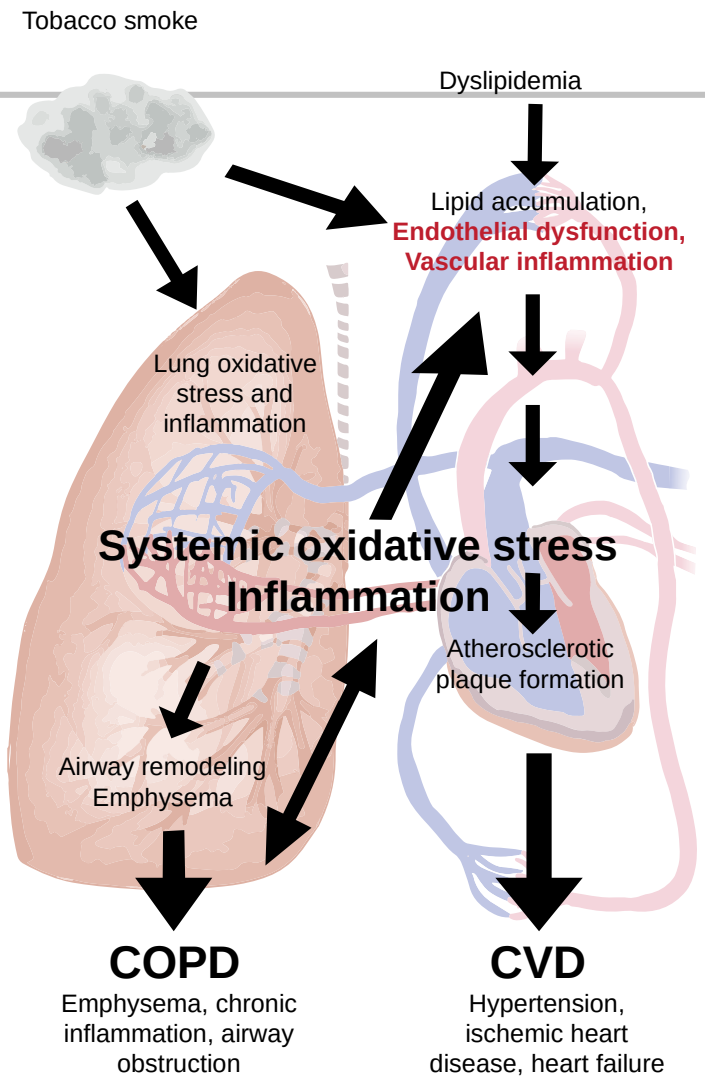
- Viability, cytotoxicity
- Proliferation, cell cycle
- ROS formation
- Intracellular GSH content
- DNA damage
- Cell fate (apoptosis, necrosis)
- NFκB translocation
- Cellular communication
- EMT marker expression
- Barrier integrity (junction protein expression)



Lo Sasso G, J Transl. Med. 2016

Disease-Related HCS Endpoints
CVD

- Viability, cytotoxicity
- Proliferation
- ROS formation
- Intracellular GSH content
- Cell fate (apoptosis, necrosis)
- NFκB translocation
- Monocyte adhesion

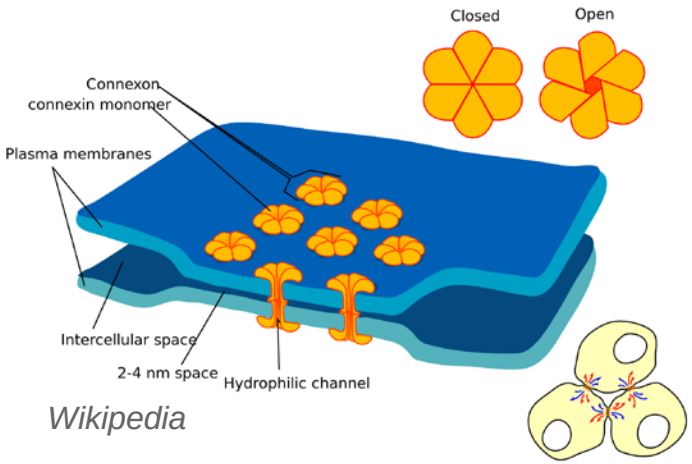


Lo Sasso G, J Transl. Med. 2016

Gap Junction Intercellular Communication (GJIC)

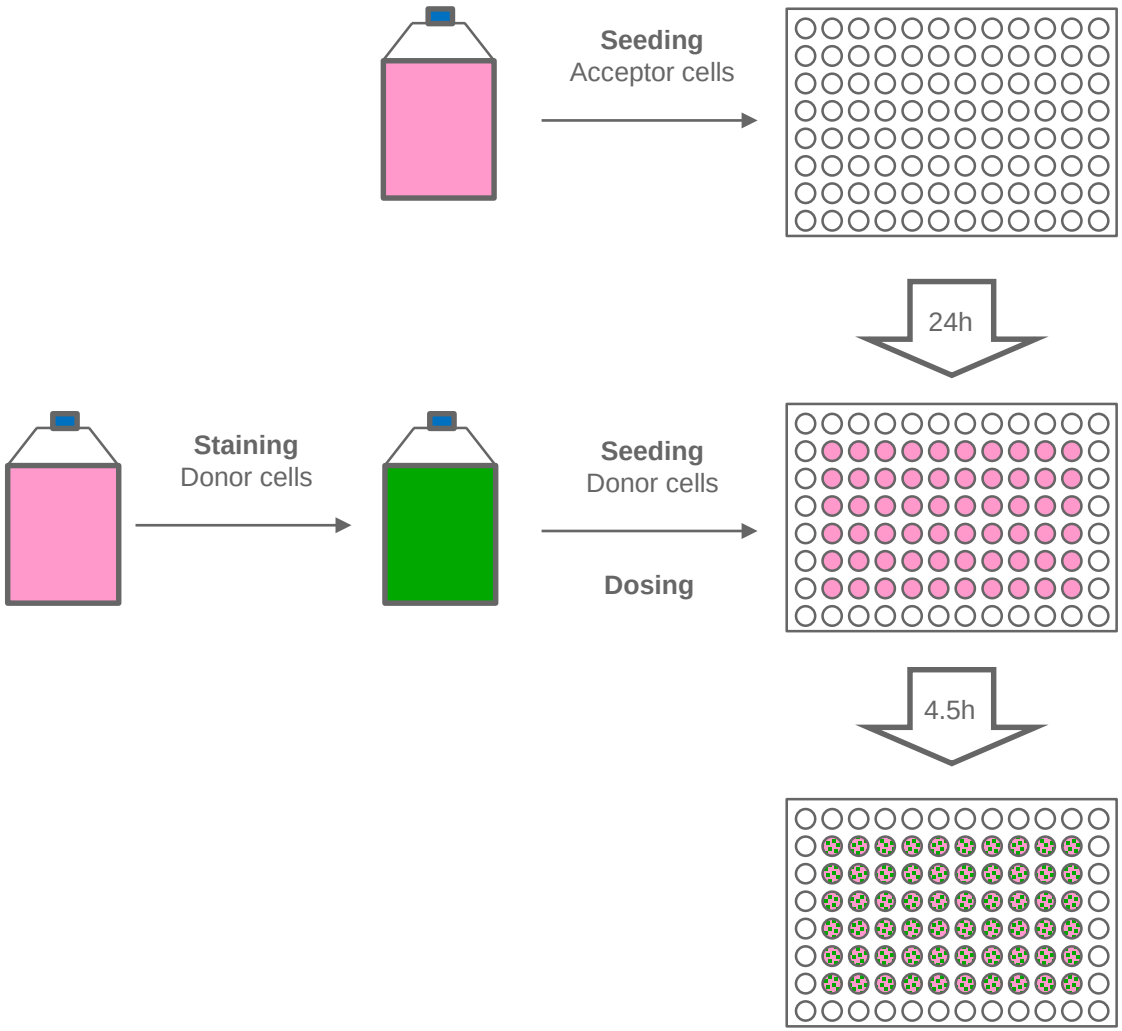
- Gap-junction plays an important role in tissue homeostasis and life-death balance, the impairment of this process during the tumor promotion stage has been linked to the later progression of tumors.
- Breakdown in communication prevents a cell being influenced in terms of growth suppression by its neighboring cells, leading to deregulated cell proliferation and metastatic properties.
- Cigarette smoking is a known risk factor for cancer development, and cigarette smoke is known to induce GJIC inhibition.

Trosko et al. 2004

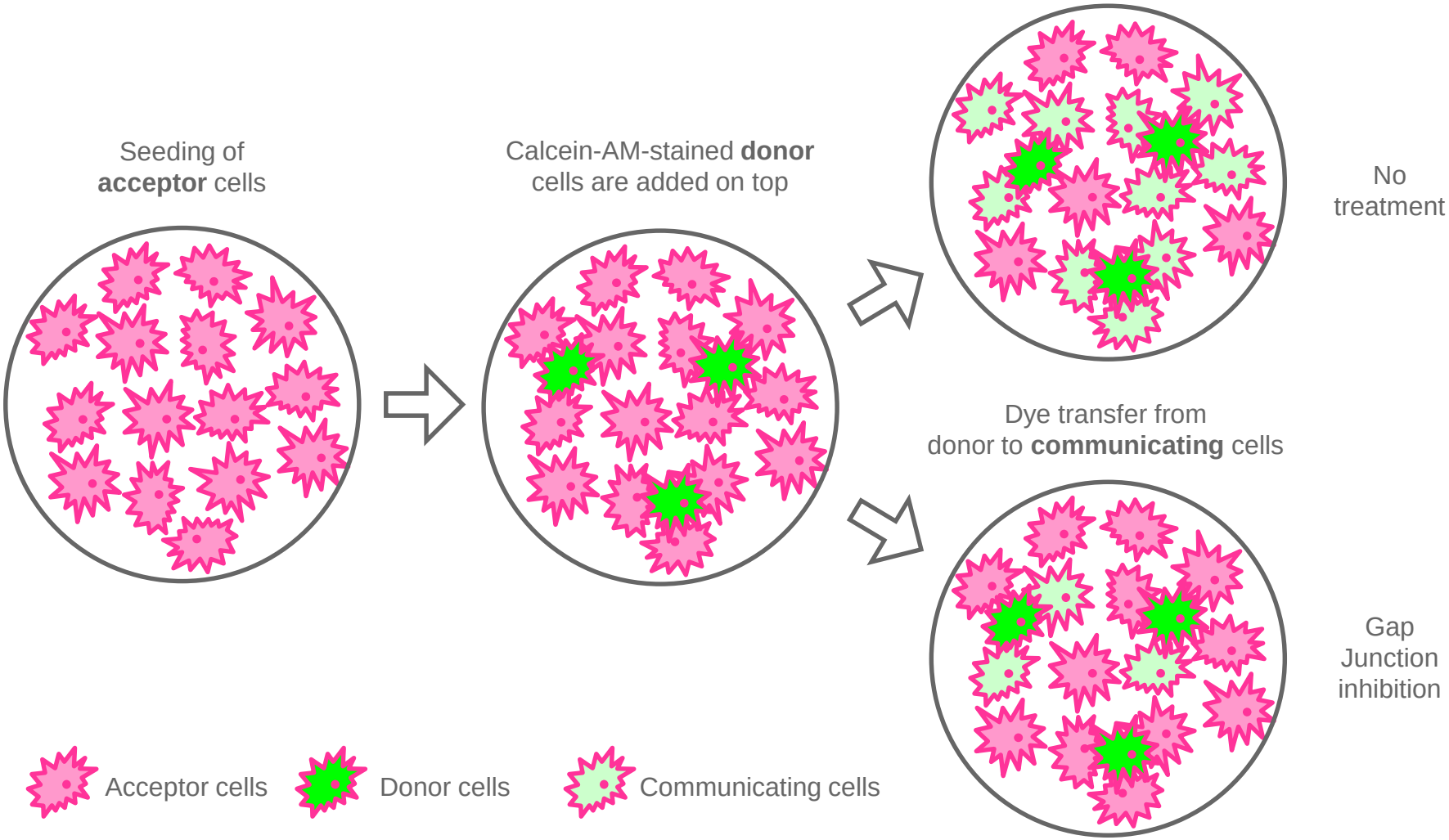


Wikipedia

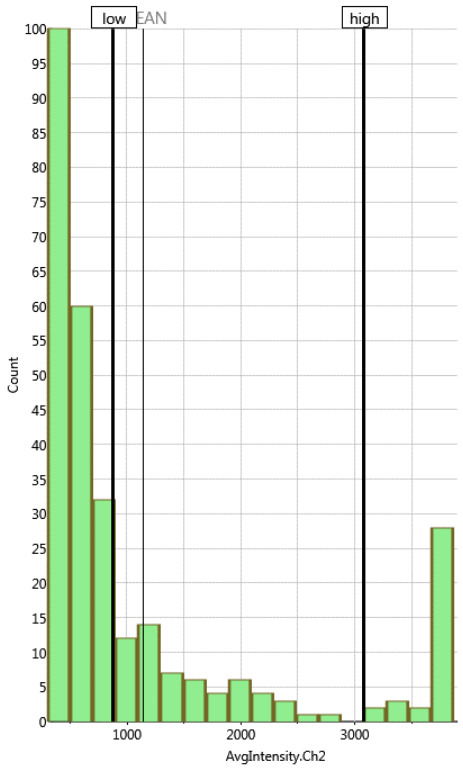
GJIC assay workflow

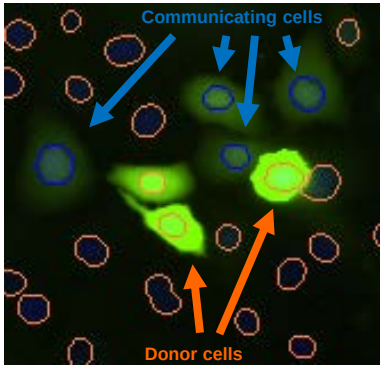


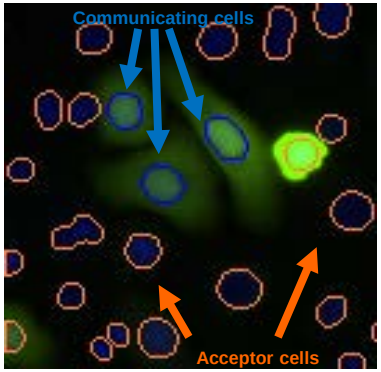
Assay principle

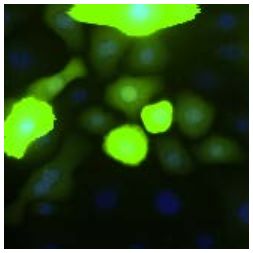


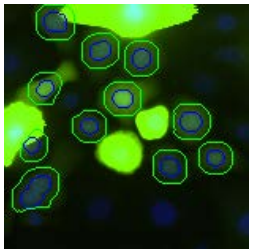
Software analysis

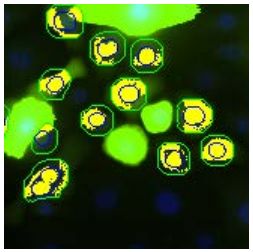












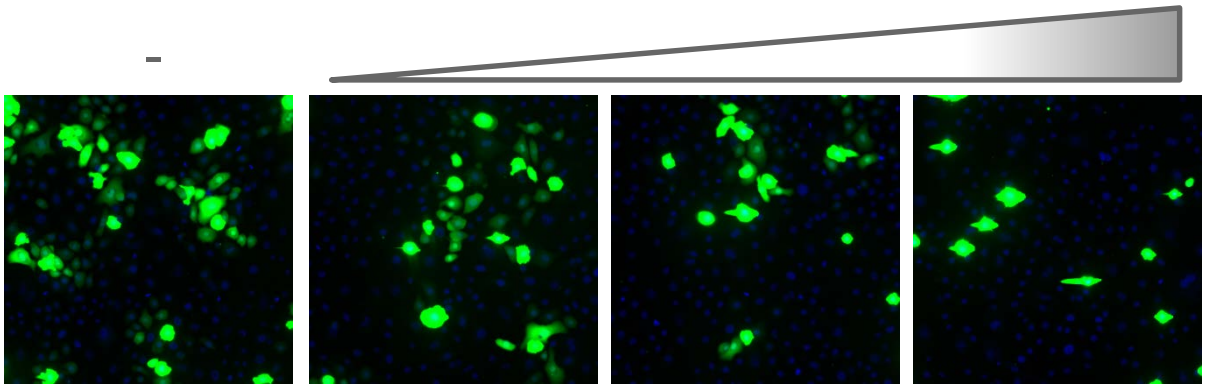
Dye transfer between donor and acceptor cells

Nuclear (**blue** line) and cytoplasmatic (**green** line) area

Detected transferred dye (**yellow**)

Results

18-alpha-glycyrrhetinic acid (AGA)



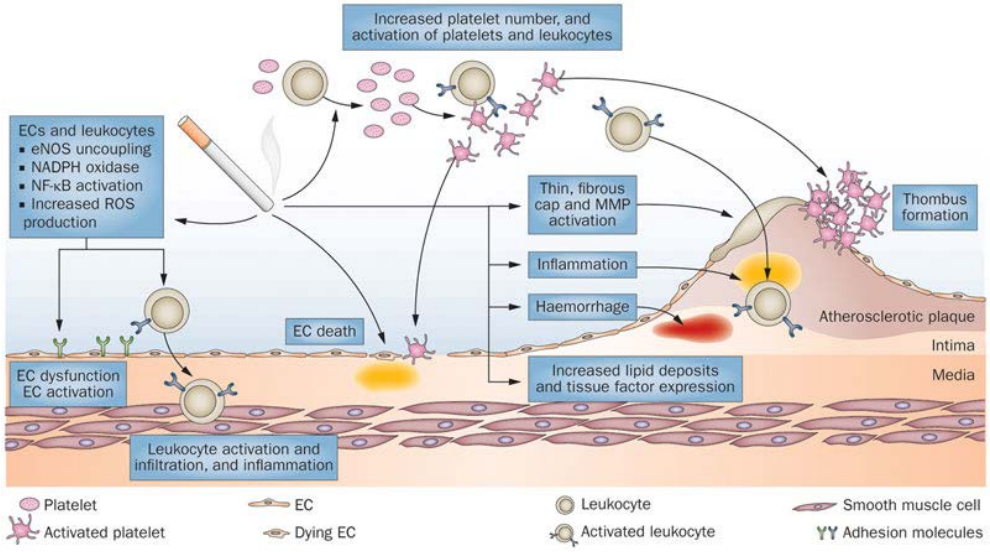
Ratio to vehicle

Ratio to vehicle

Adhesion and atherosclerosis

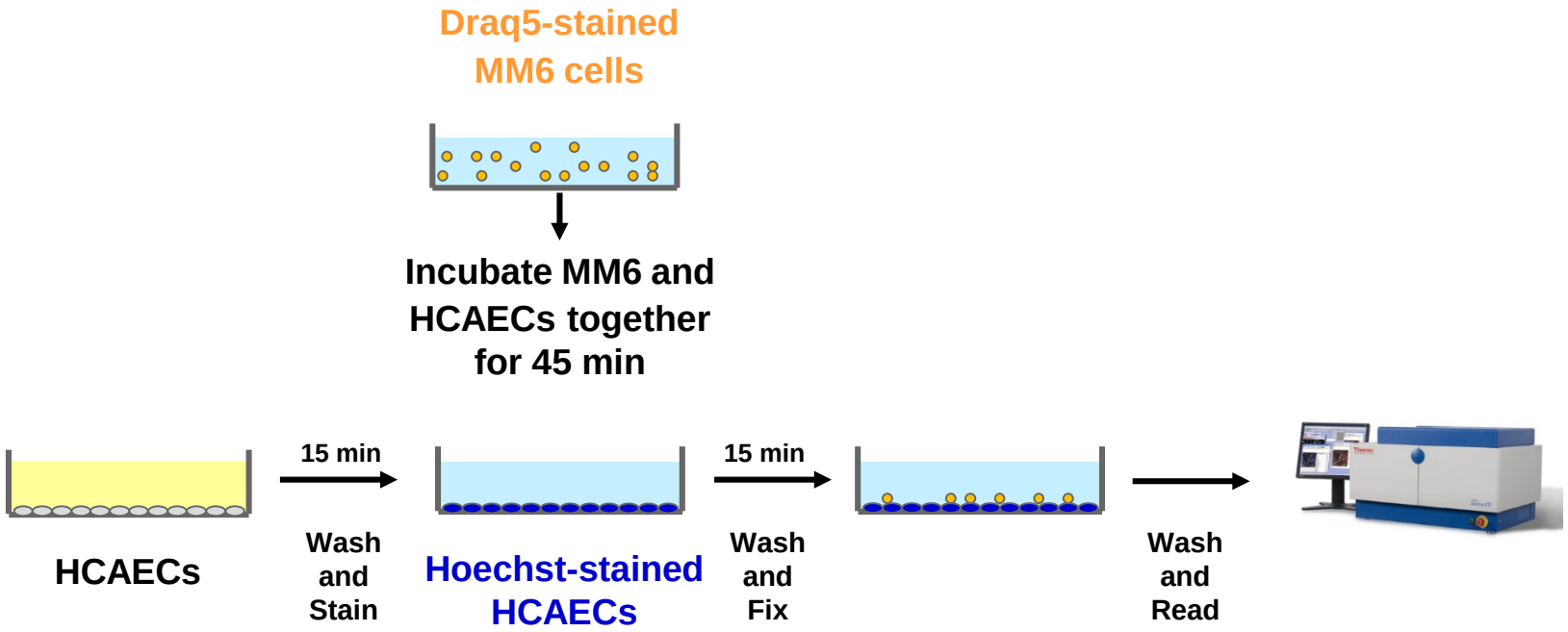
Exposure to cigarette smoke -

- induces multiple pathological effects in the endothelium, several of which are the result of oxidative stress.
- Interferes adversely with the control of all stages of plaque formation and development and pathological thrombus formation via the enhancement of inflammatory processes and the activation of matrix metalloproteases.



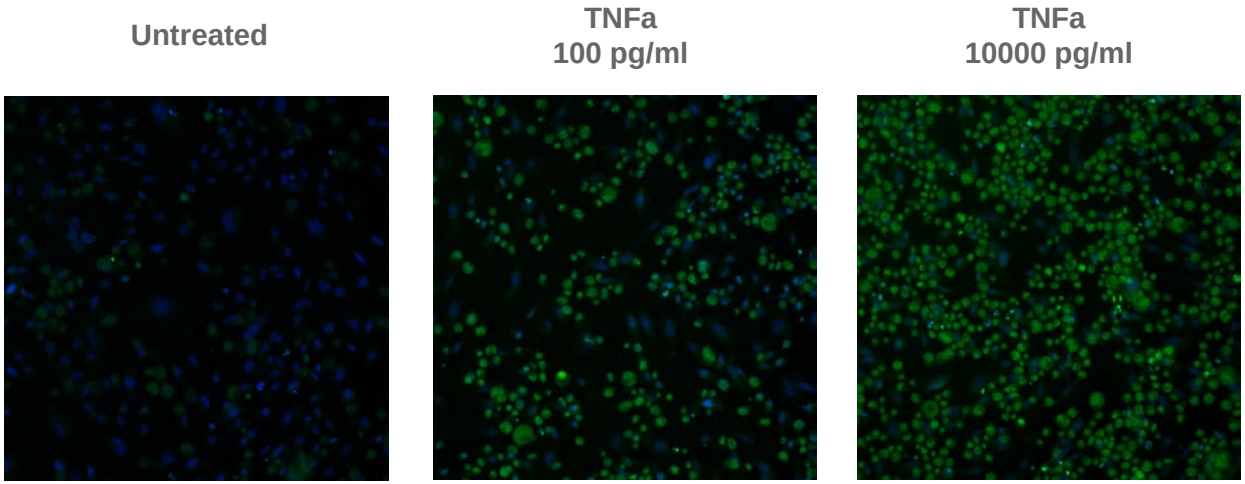
Adam Csordas & David Bernhard
Nature Reviews Cardiology 2013

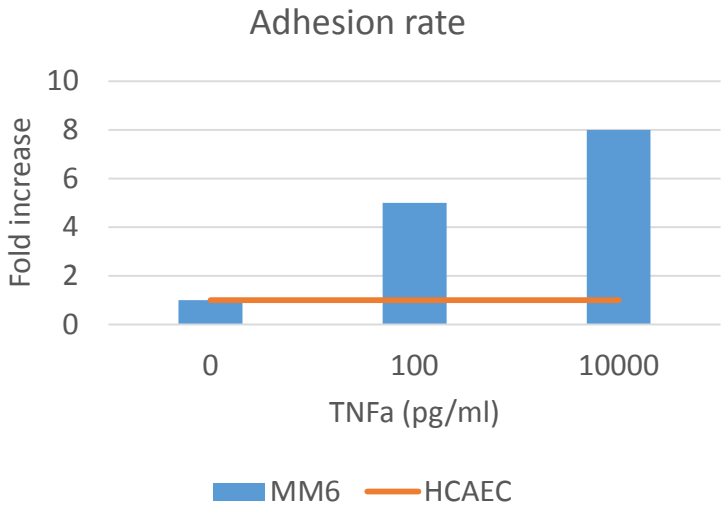
Adhesion assay workflow



Poussin C. Toxicology 2016
Poussin C. Toxicological Science 2015

Results

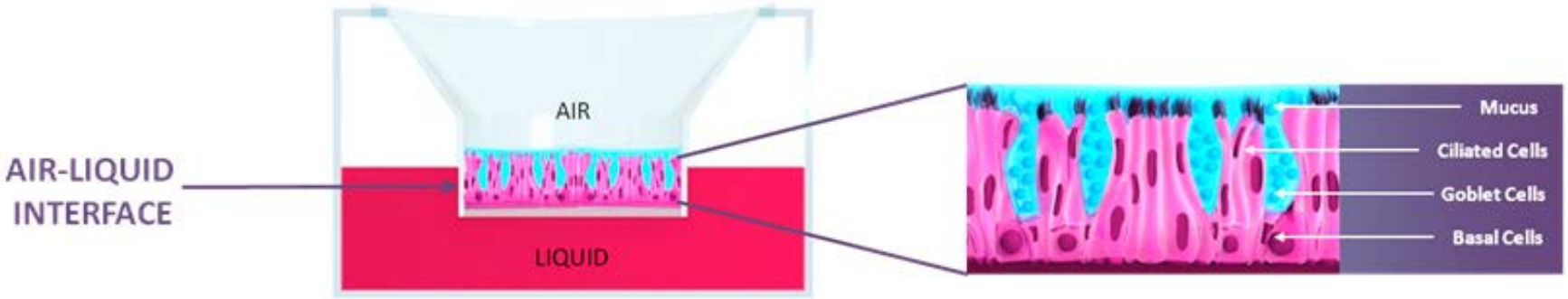

Untreated
TNFa
100 pg/ml
TNFa
10000 pg/ml

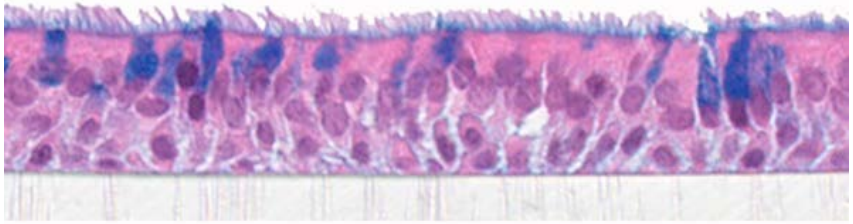

Adhesion rate
Fold increase
10
8
6
4
2
0
0
100
10000
TNFa (pg/ml)
MM6
HCAEC

HCS in 3D cellular bronchial organotypic tissues model

MucilAir – Bronchial

- Reconstituted using human primary cells (single or pool of donor)
- Remains fully differentiated and functional for over one year in culture
- Ready and easy to use





Immuno Histochemistry section

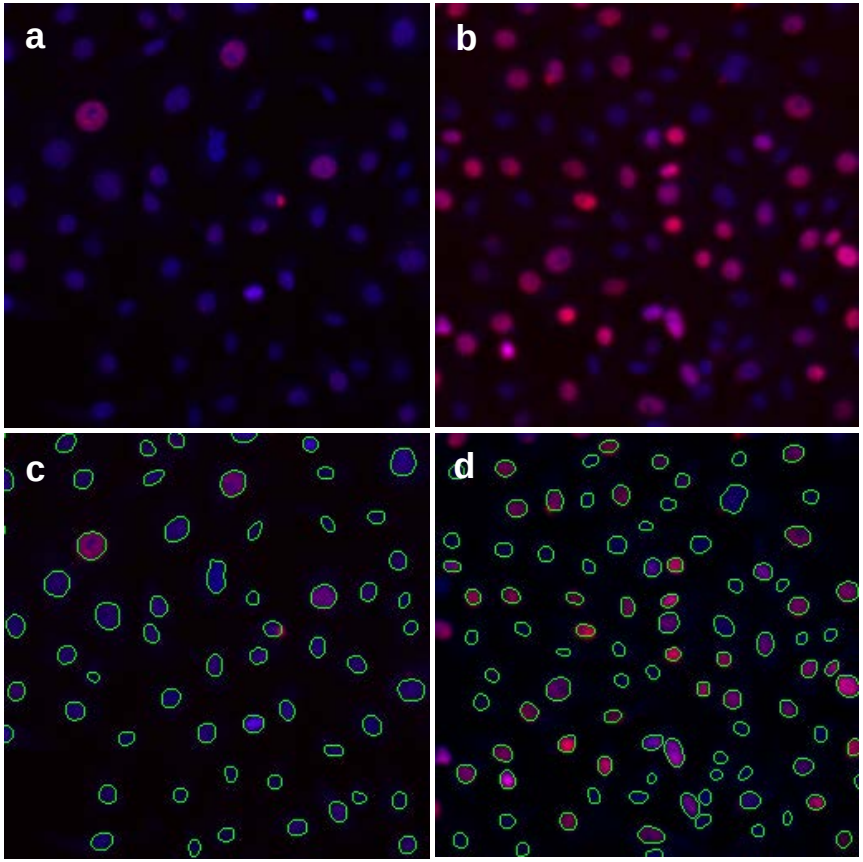
Epithelix Sarl Geneve

DNA Damage in 2D and 3D

NHBE

Vehicle

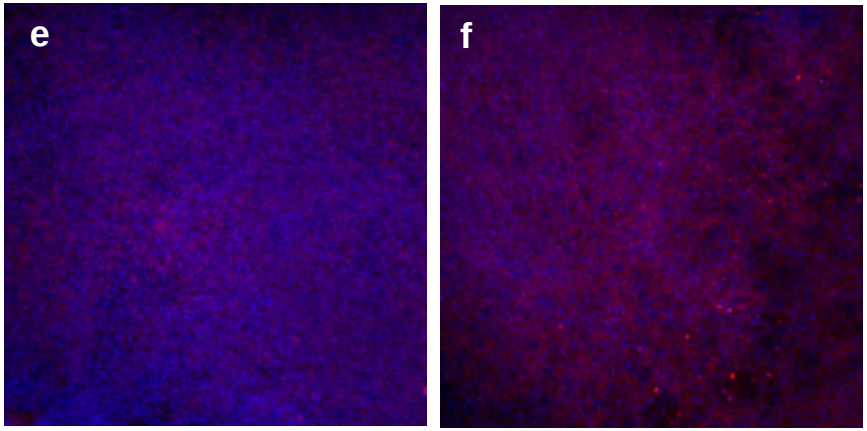
100µM Chlorambucil

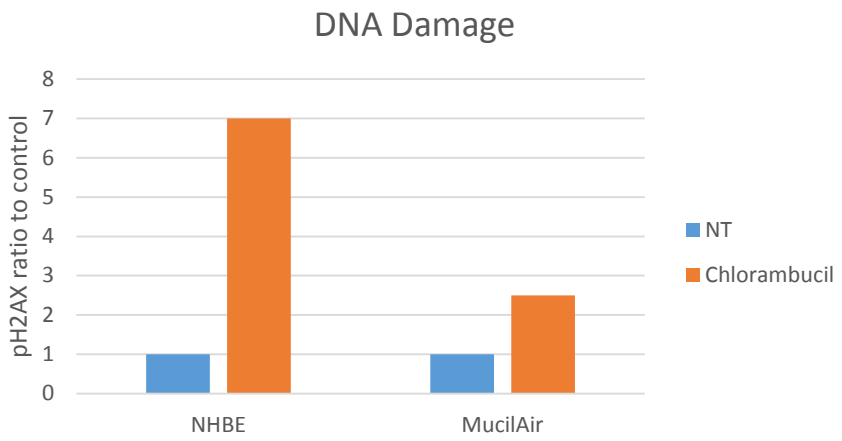


MucilAir

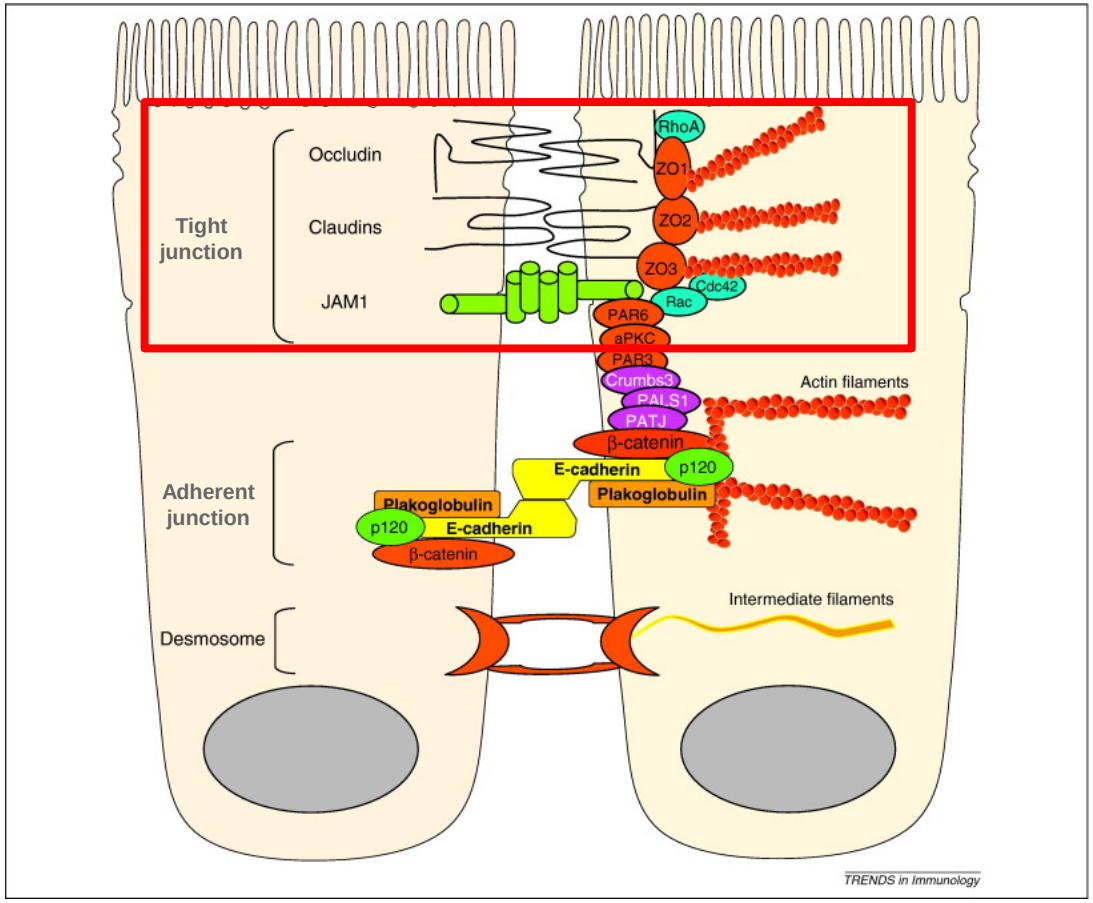
Vehicle

500µM Chlorambucil





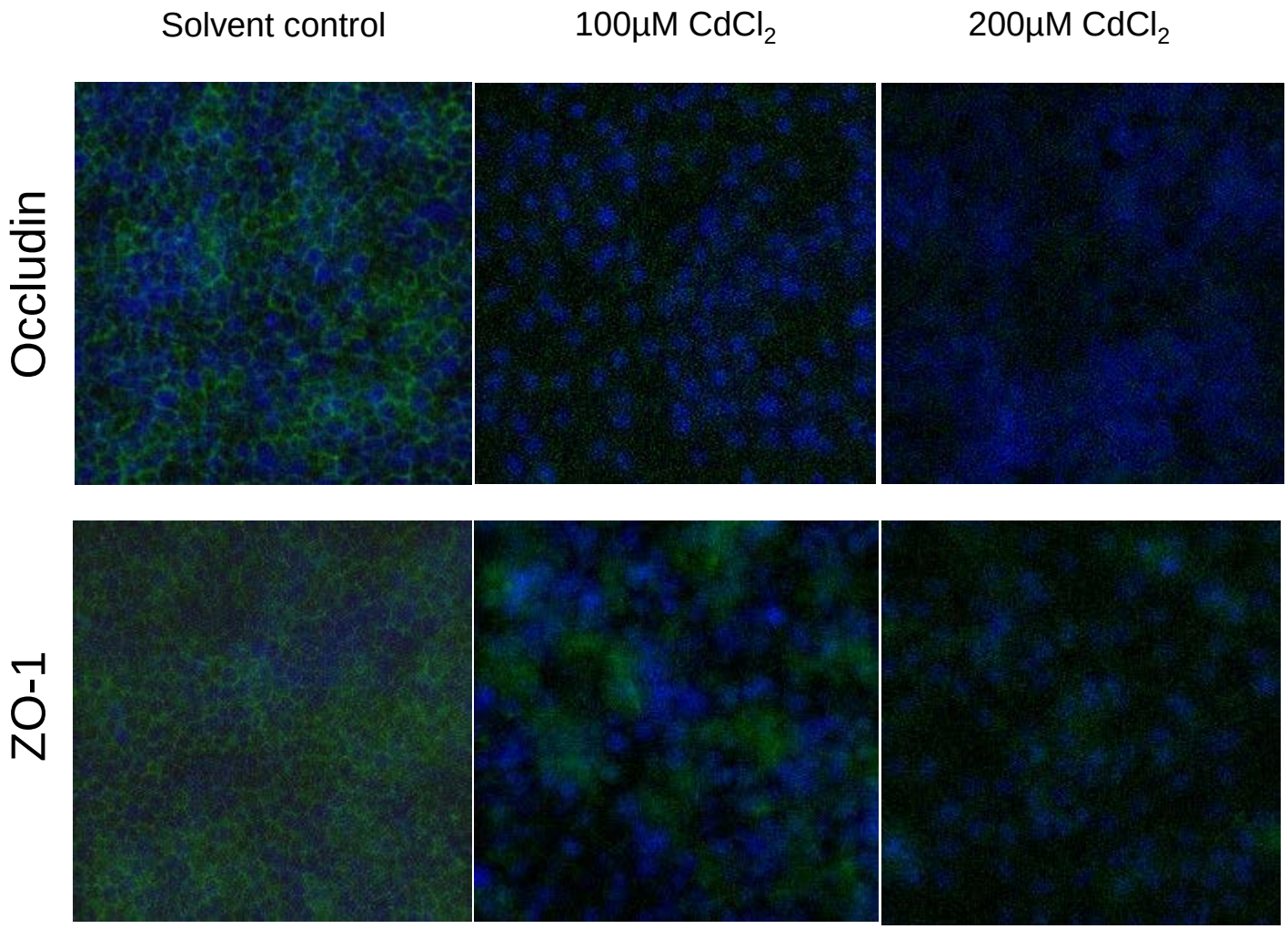
Tight Junction in 3D



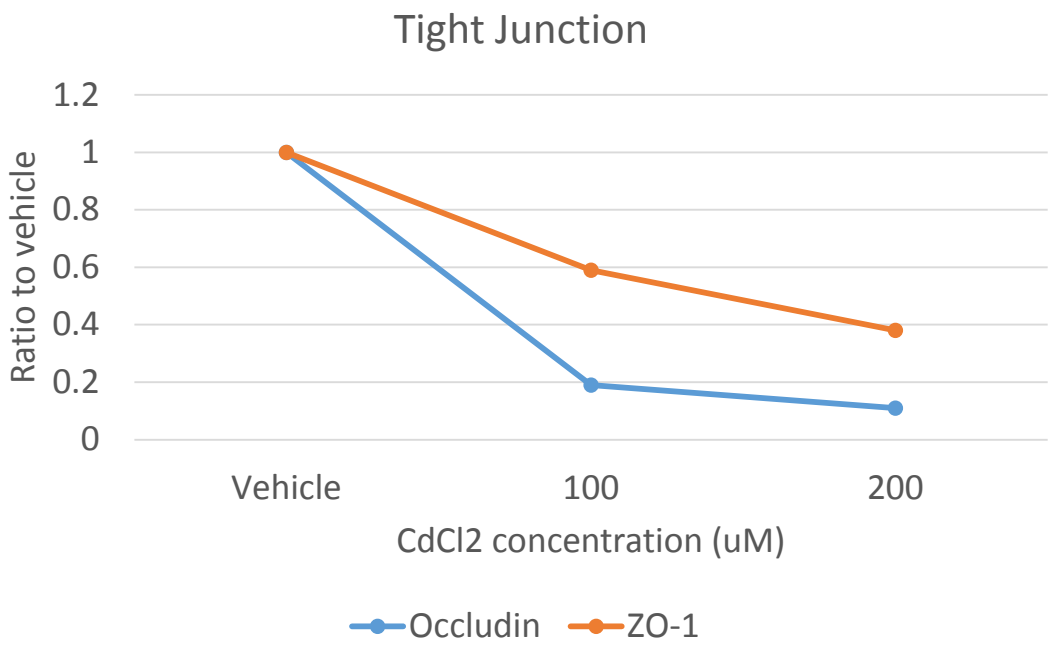
Nawijn MC Trends in Immunology 2011

- The bronchial epithelium is responsible for preserving airway homeostasis in the lung. It possesses innate defense functions and acts as a barrier against inhaled particles or pathogens. Epithelial barrier function is maintained by adherens junctions and, most importantly, by intercellular **tight junctions**.
- Cigarette smoke exposure leads to damage and increased permeability of the airway epithelium.

Tight Junction disruption

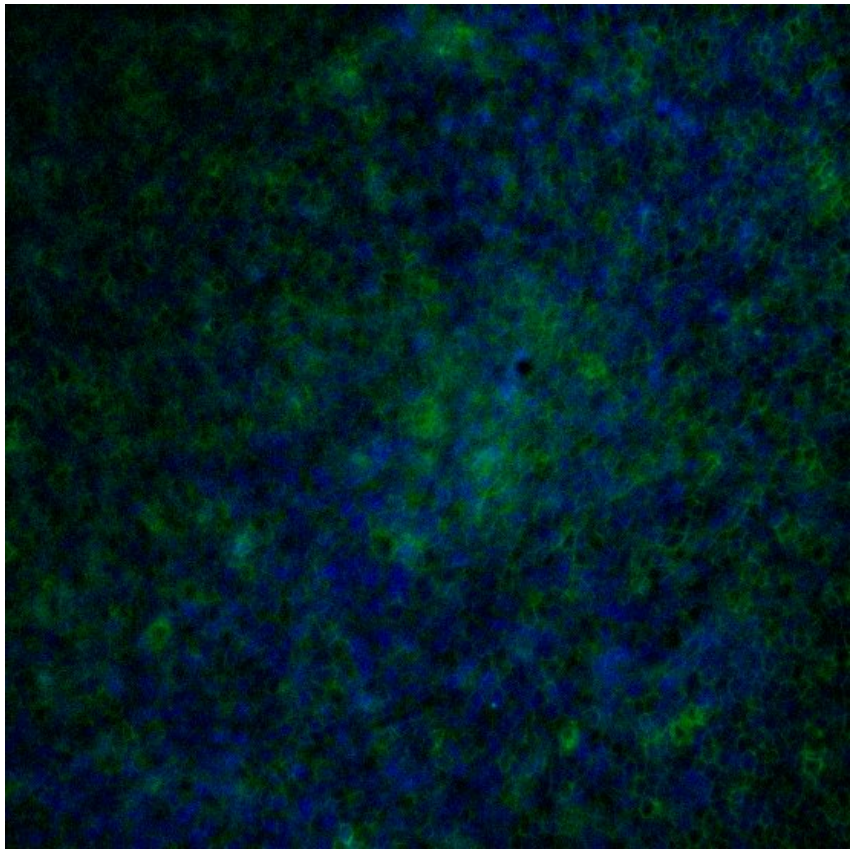


Tight Junction disruption

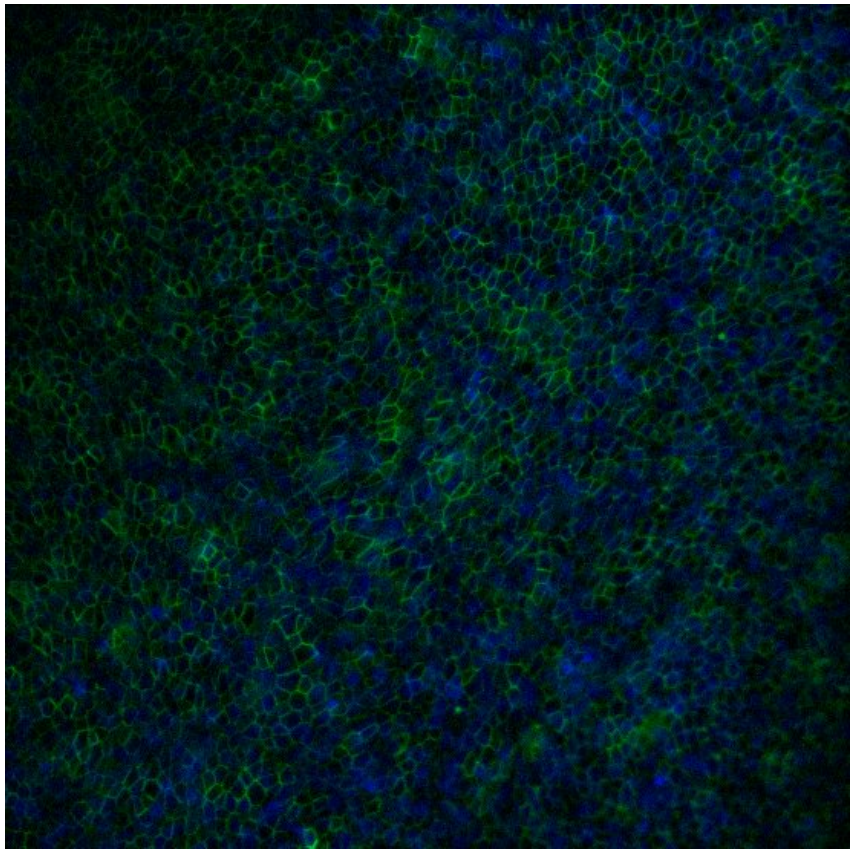


Imaging improvement

Membrane in the insert

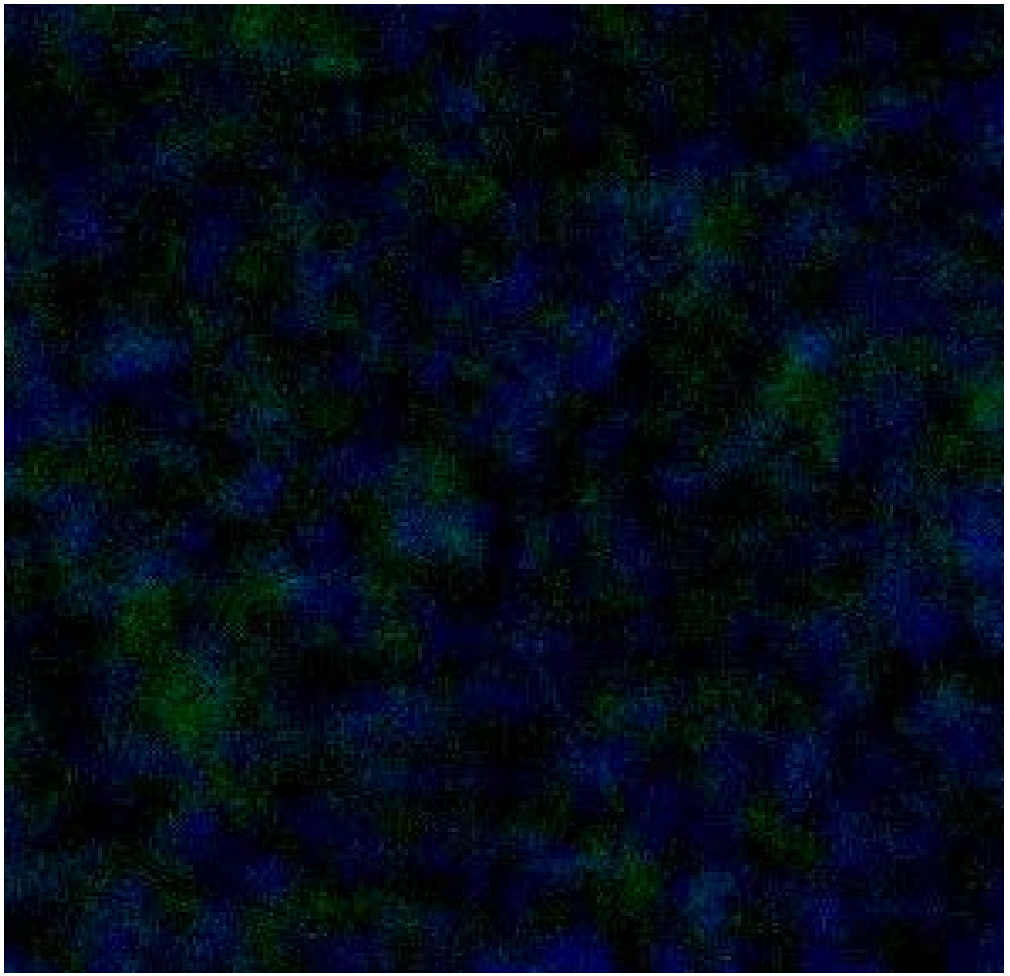


Membrane cut and placed in a well



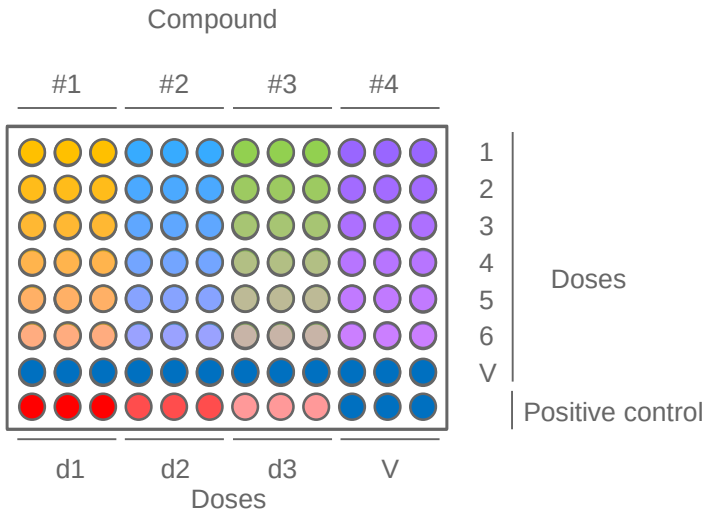
3D reconstruction

ImageJ® video reconstruction of **60** z-stack pictures with **1µm** gap



HCS tox assessment

Assay	#	Biological endpoint
Nuclear parameters (Included in all assays)	1	Cell count
	2	Nuclear area
	3	DNA structure
Cytotoxicity	4	Mitochondrial mass
	5	Mitochondrial membrane potential
	6	Cytochrome C release
DNA damage & Stress kinase	7	phospho-H2AX
	8	phospho-cJun
Proliferation	9	EdU
	10	phospho-H3
NF-kB	11	NF-kB nuclear translocation
Oxidative stress	12	ROS
	13	GSH
Apoptosis & Necrosis	14	Caspase 3/7
	15	Cell membrane permeability



- 1, 4 and 24 hr timepoint for a total of 12 plates/plate set.
- 2 plate sets simultaneously
- 3 experiments/week

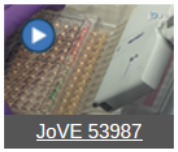
HCS laboratory

- Thermo Arrayscan VTI (2X)
- Thermo CellInsight CX7
- Thermo Orbitor
- Biotek Platewasher
- CyBio Felix

Stefano Acali

Alexandra Laurent

High Content Screening Analysis to Evaluate the Toxicological Effects of Harmful and Potentially Harmful Constituents (HPHC)



Diego Marescotti[1], Ignacio Gonzalez Suarez[1], Stefano Acali[1], Stephanie Johne[1], Alexandra Laurent[1], Stefan Frentzel[1], Julia Hoeng[1], Manuel C. Peitsch[1]

[1]Biological System Research (BSR), **Philip Morris International R&D**

Visit our HCS lab

on Google indoor maps

PMI RESEARCH & DEVELOPMENT

Q&A

Thank you for your attention!!!

Any questions