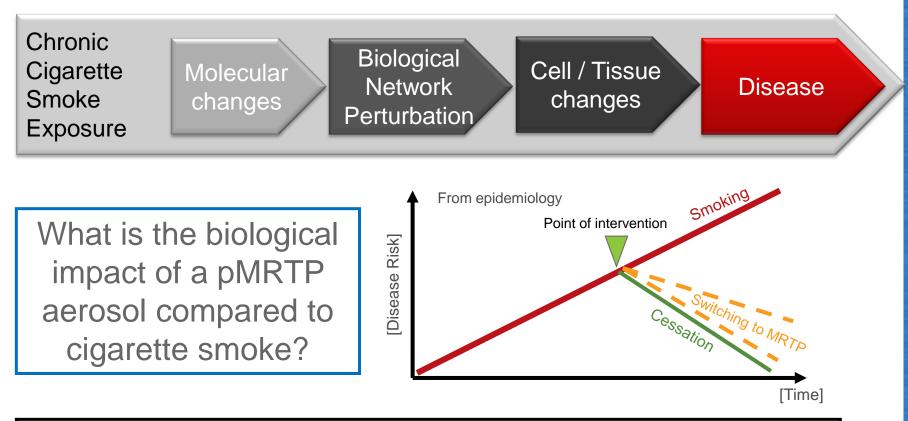


PMI RESEARCH & DEVELOPMENT

Systems Toxicology-Based Comparisons of Smoking Cessation and Switching to a Non-Combustible Tobacco Product in a Murine Model of COPD

Blaine Phillips Philip Morris International Research Laboratories, Pte. Ltd., Singapore July 2, 2013 International Congress of Toxicology, Seoul, 2013

How Does Biology Respond to pMRTP Aerosols?



- Compare switching to MRTP with continued smoking and benchmark against smoking cessation.
- Assess how close switching to pMRTP is to smoking cessation



COPD – Chronic Obstructive Pulmonary Disease

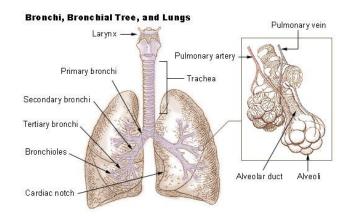
COPD is the co-occurrence of chronic bronchitis and emphysema – characterized by airflow limitations which are not fully reversible

Chronic bronchitis:

- Chronic inflammation of the bronchi
- Increased number and size of goblet cells
- Persistent cough
- Increased sputum and mucus
- Narrowing of airways

Emphysema:

- Progressive destruction of lung tissue (notably around the alveoli)
- Pockets of air and airspace collapse during forced expiration.
- Loss of lung elasticity.
- * Cigarette smoke is the main etiological factor in the pathogenesis of COPD.





Cigarette Smoke-Induced COPD as an *In Vivo* **Model to Assess Smoking Cessation Versus a Switch-to-pMRTP**

Animals: 1224 female C57Bl/6 mice

Exposure groups:

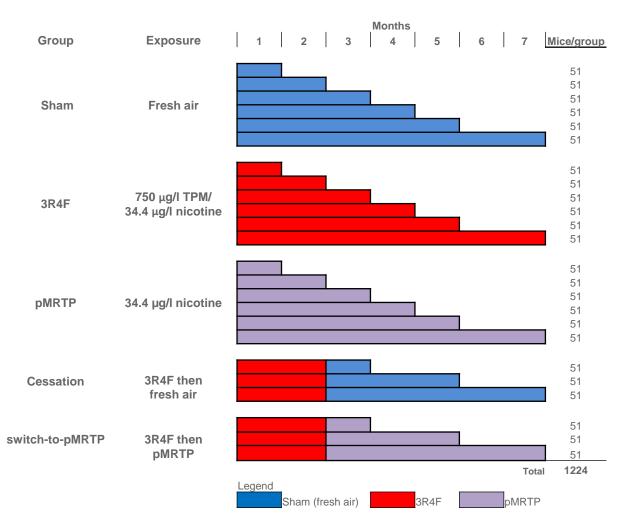
- i) Sham (fresh air)
- ii) 3R4F reference cigarettes (University of Kentucky)
- iii) pMRTP
- iv) 2 months 3R4F, then fresh air up to 5 months (cessation)
- v) 2 months 3R4F, then pMRTP up to 5 months (switch-to-pMRTP)

• Dose:

- 34.4 $\mu g/l$ nicotine in both 3R4F and pMRTP groups
- 4 hours per day, 5 days per week
- 7 months maximum exposure
- Switching/cessation point: after 2 months (when histopathological signs of emphysema begin to emerge)



Experimental Design





Aerosol Generation: Smoke Exposure

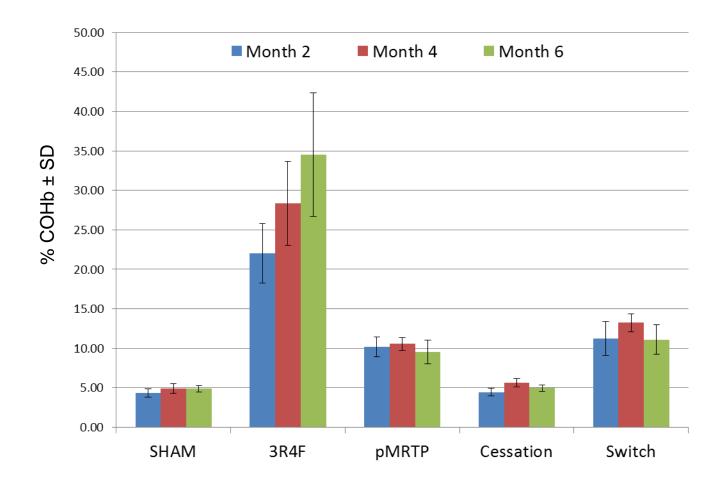




Whole-body exposure chamber (set-up) Conventional cigarette smoking machines

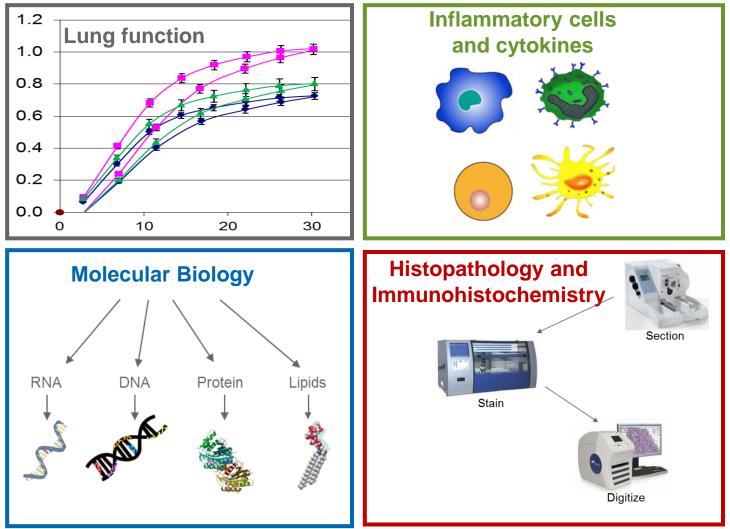


Exposure: Carboxyhemoglobin (COHb) Levels in Blood



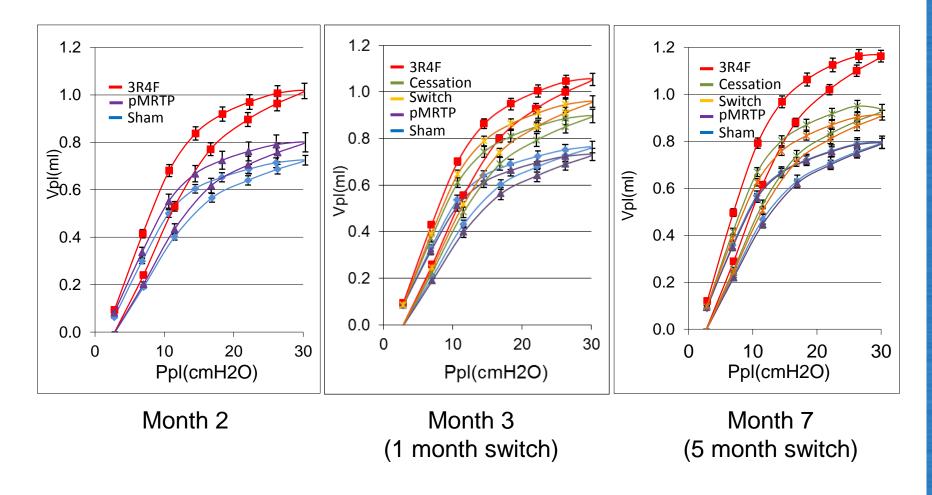


Endpoint analysis: Multi-Parameter Assessment of Emphysema Progression



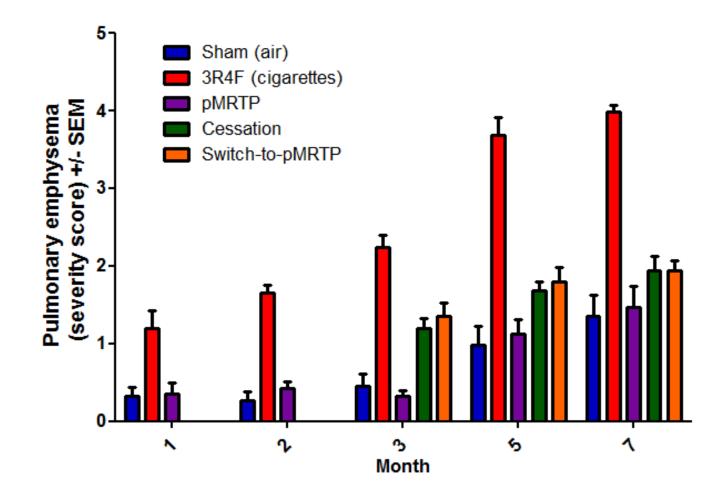


Pulmonary Function: A Physiological Measure of Lung Tissue Compliance and Airflow Resistance



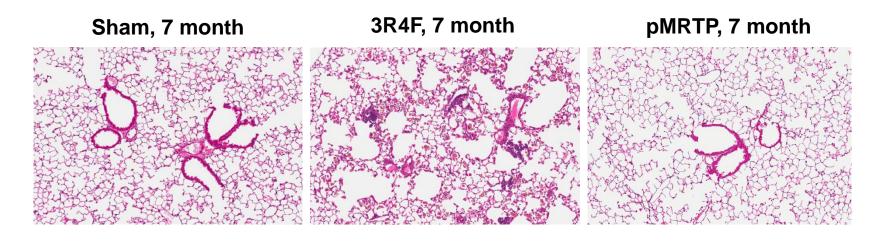


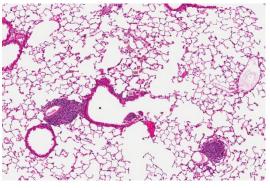
Histopathology: Pulmonary Emphysema Progression



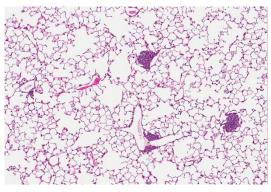


Histopathology: Lung Morphology





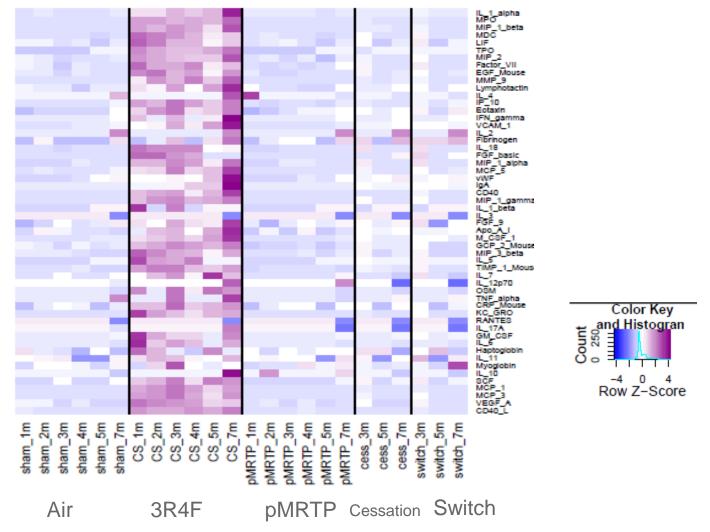
Cessation, 7 month



Switch, 7 month

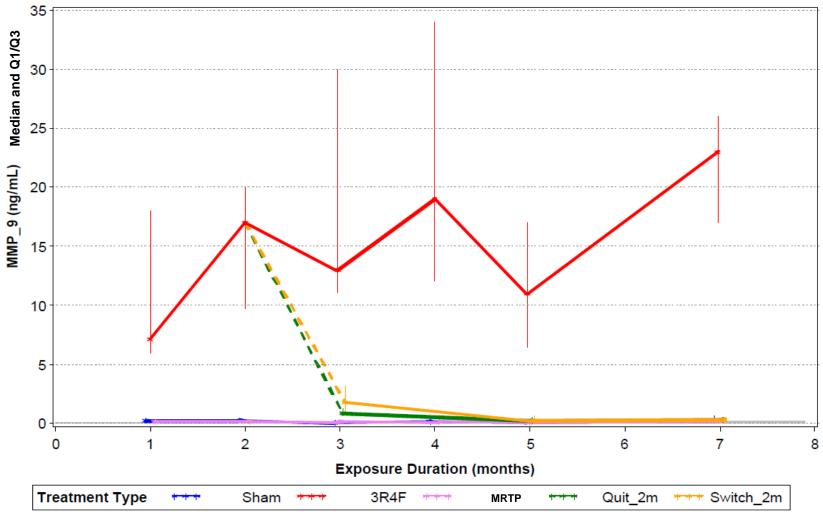


Pulmonary Inflammation: Inflammatory Mediators in the BALF



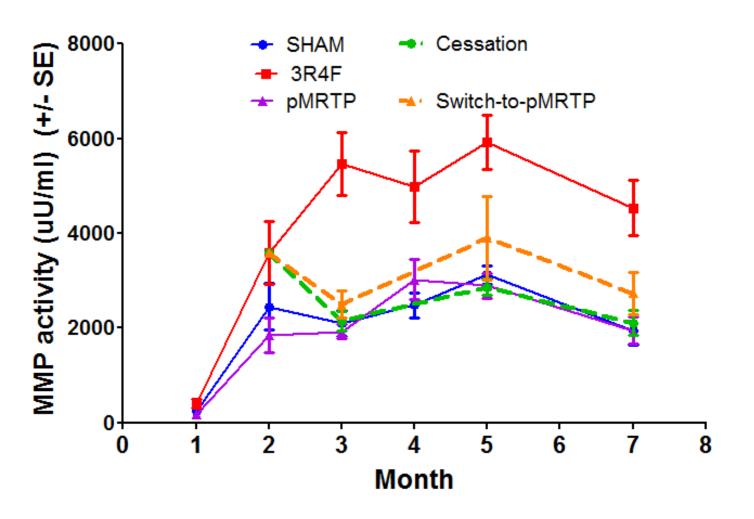


Pulmonary Inflammation: Expression of MMP-9 in the BALF



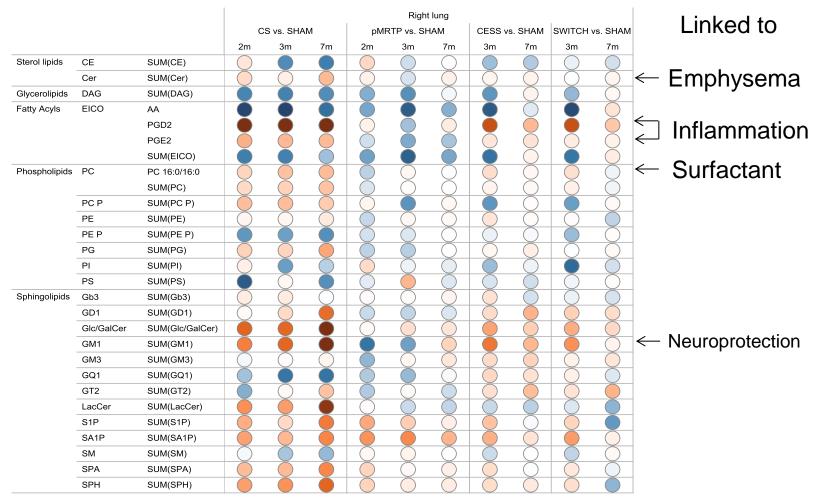


Pulmonary Inflammation: MMP (Gelatinolytic) Activity in the BALF





Lung Lipidomics: Differential Lipid Levels in the Lungs of Smoke or pMRTP-Exposed Mice



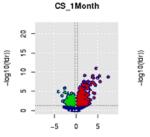
MEDIAN_RELATIVE_CHA..

Relative change (%)

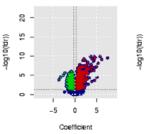
-110%



Transcriptomics: Differential Gene Expression in the Lungs of Smoke or pMRTP-Exposed Mice

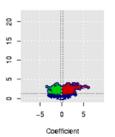


Coefficient

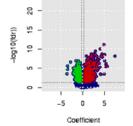


pMRTP 2Month

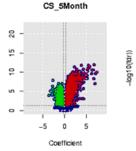
CS 2Month

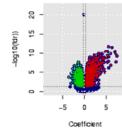


CS 3Month



CS 4Month





CS_7Month

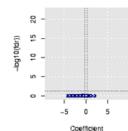
pMRTP 1Month 20 5 log10(ldr)) 2 ŝ

100

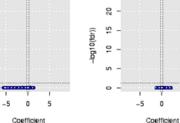
Coefficient

-5 n 5

0



pMRTP 3Month



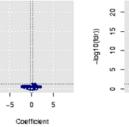
pMRTP 5Month 20

0

log10(ldr)) 5

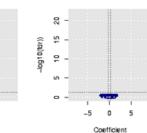
log10(ldr))

pMRTP 7Month



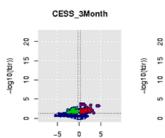
0 -5 5

Coefficient SWITCH_7Month





PMI RESEARCH & DEVELOPMENT



Coefficient



0

Coefficient

5

-5



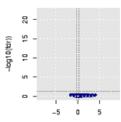
20

5

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log10(ldr))



Coefficient

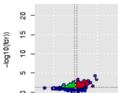
-5



0

5

pMRTP 4Month



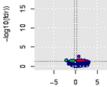
-5

0

Coefficient

5

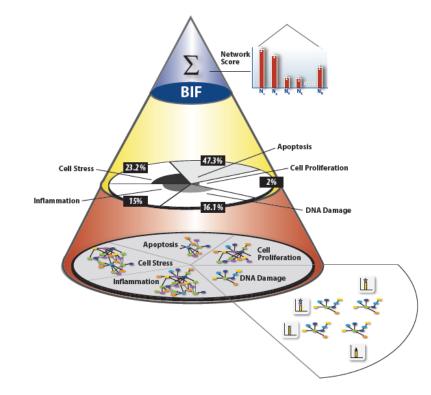




SWITCH_5Month

Coefficient

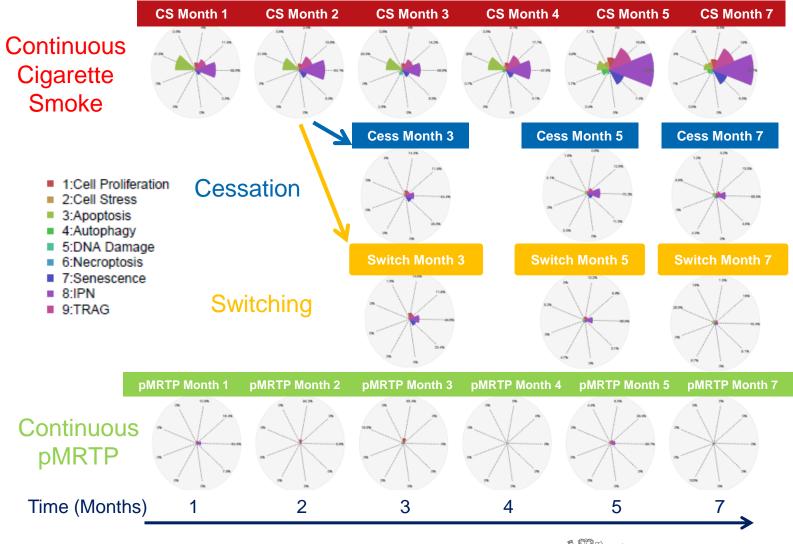
The Biological Impact Factor (BIF)



Relative BIF on lung tissue -0.02 δ= 0.73 ð= 0.87 δ= 0.73 δ= 0.85 δ= 0.94 δ= 0.51 δ= 0.03 ð= 0.69 δ= 0.64 δ= 0.63 δ= 0.27 (REF) δ= 0.07 0.85 0.64 δ= 0.3 0 ģ ð= §= <u>_</u> CS_1Month CS_2Month CS_4Month CS_7Month CS_3Month CS_5Month pMRTP_1Month pMRTP_2Month pMRTP_5Month pMRTP_7Month CESS_3Month CESS_5Month CESS_7Month SWITCH_3Month SWITCH_5Month SWITCH_7Month pMRTP_3Month pMRTP_4Month



Network Perturbations and Biological Impact in the Lung (Gene Expression)





Digging Into the Details: Legend

Cell P lolliferation Cell P lolliferation Formers Hasse Cell P lolliferation Formers Hasse Formers Hasse	m 🚄
N ecroptosis	Ę

NetID

Cell Proliferation/Calcium

Cell Proliferation/Clock

Cell Proliferation/Cell Cycle

Cell Proliferation/Epigenetics

Cell Proliferation/Hedgehog

Cell Proliferation/Jak Stat

Cell Proliferation/Mapk

Cell Proliferation/Notch

Cell Proliferation/PGE2

Cell Stress/Hypoxic Stress

Cell Stress/NFE2L2 Signaling Cell Stress/Osmotic Stress

Cell Stress/Oxidative Stress

Cell Proliferation/Wnt

Cell Proliferation/Growth Factor

Cell Proliferation/Nuclear Receptors

Cell Stress/Drug Metabolism Response

DACS/Apoptosis/-Caspase cascade

DACS/Apoptosis/-TNFR1Fas signaling

DACS/Autophagy/-mTOR signaling

DACS/DNA Damage/-TP53 TS

IPN/Dendritic cell activation

IPN/Macrophage activation

IPN/Mucus hypersecretion

IPN/Neutrophil chemotaxis

IPN/Neutrophil response

IPN/NK cell activation IPN/Tc response

IPN/Th17 differentiation

IPN/Th1 differentiation

IPN/Th2 differentiation

TRAG/Fibrosis and FM1

IPN/Tissue damage

IPN/Treg response

IPN/Macrophage differentiation

IPN/Megakaryocyte differentiation

IPN/Epithelial cell barrier defense IPN/Epithelial proinflammatory signaling

DACS/Necroptosis/-Fas activation

DACS/Necroptosis/-TNFR1 activation

DACS/Senescence/-Replicative senescence

IPN/Dendritic cell migration to lymph node

IPN/Macrophage mediated recruitment IPN/Mast cell activation

DACS/Autophagy/-Protein synthesis

DACS/Autophagy/-Autophagy induction

DACS/Apoptosis/-MAPK signaling DACS/Apoptosis/-NFkB signaling

DACS/Apoptosis/-PKC signaling

Cell Stress/Endoplasmic Reticulum Stress

DACS/Apoptosis/-ER stress-induced apoptosis

DACS/Apoptosis/-Prosurvival mitochondrial signaling

DACS/DNA Damage/-Components affecting TP53 activity

DACS/DNA Damage/-Components affecting TP63 activity

DACS/DNA Damage/-Components affecting TP73 activity

DACS/DNA Damage/-DNA damage to G1S checkpoint

DACS/DNA Damage/-DNA damage to G2M checkpoint

DACS/DNA Damage/-Double-Strand Break Response

DACS/DNA Damage/-Single-Strand Break Response

DACS/Senescence/-Oncogene induced senescence

DACS/Senescence/-Regulation by tumor suppressors

DACS/Senescence/-Regulation of p16INK expression

TRAG/Cell Migration and Adhesion in Wound Healing

TRAG/Growth Factor-Mediated Angiogenesis

TRAG/HIF1A Regulation and Signaling

TRAG/Sprouting and Tubulogenesis

TRAG/VEGF-Mediated Angiogenesis

TRAG/Immune Regulation of Angiogenesis TRAG/Immune Regulation of Tissue Repair

DACS/Senescence/-Stress induced premature senescence

DACS/Senescence/-Transcriptional regulation of the SASP

Cell Proliferation/Cell Interaction

Abbreviation

Calcium

CelCyc

Cellnt

Clock

Epige

GroFac

JakStat

Mapk

Notch

PGE2

HypStr NFE2L2Sig

OsmStr

CasCas

NFkSig

PKCSig

AutInd

ProMitSig

MTORSig

ProSyn

TNFR1FasSi

CoAfTP53Ac

CoAfTP63Ac

CoAfTP73Ac

DNDaG1Ch

DNDaG2Ch

DoStBrRe

SiStBrRe

TNFR1Act

OncIndSen

RegTumSup

RegP16Exp

TraRegSAS

DeCeMiLyNo EpCeBaDe

MacMedRec MasCelAct

DenCelAct

EpiProSig

MacAct

MacDif

MegDif

MucHyp

NeuChe

NeuRes NKCelAct

TcRes

Th1Dif

Th2Dif

TisDam

TregRes

FibEMT

GrEaMeAn

HIF1ARegSi ImmRegAng

ImReTiRe

VEGMedAng

SprTub

CeMiAdWoHe

Th17Dif

RepSen

StInPrSe

TP53TS

FasAct

ERStInAp MAPKSig

OxiStr

Wht

NucRec

DruMetRes

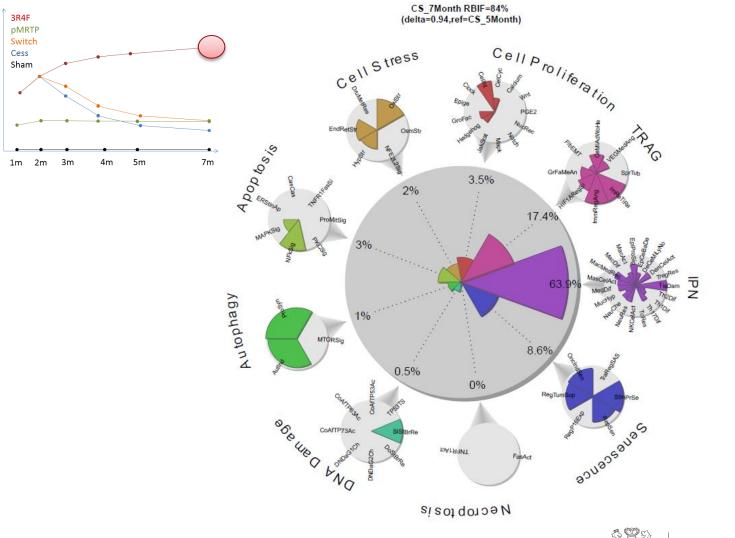
EndRetStr

Hedgehog



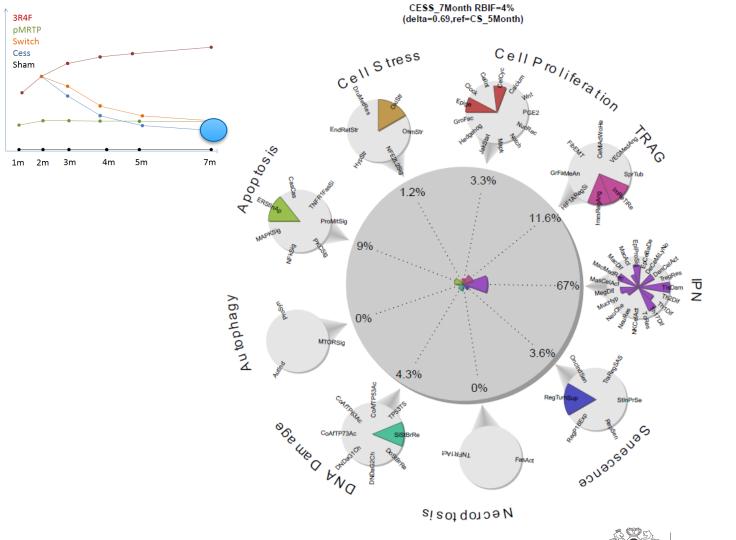


Network Perturbations and Biological Impact in the Lung : Cigarette Smoke, Month 7



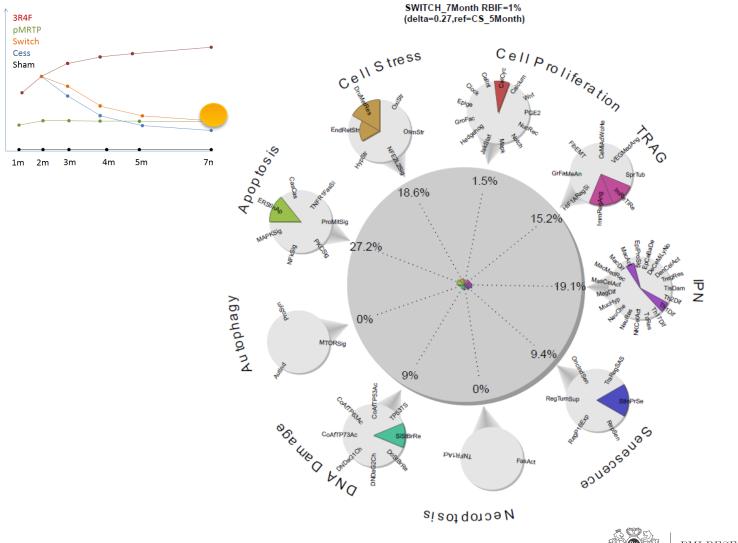


Network Perturbations and Biological Impact in the Lung : Cessation, Month 7

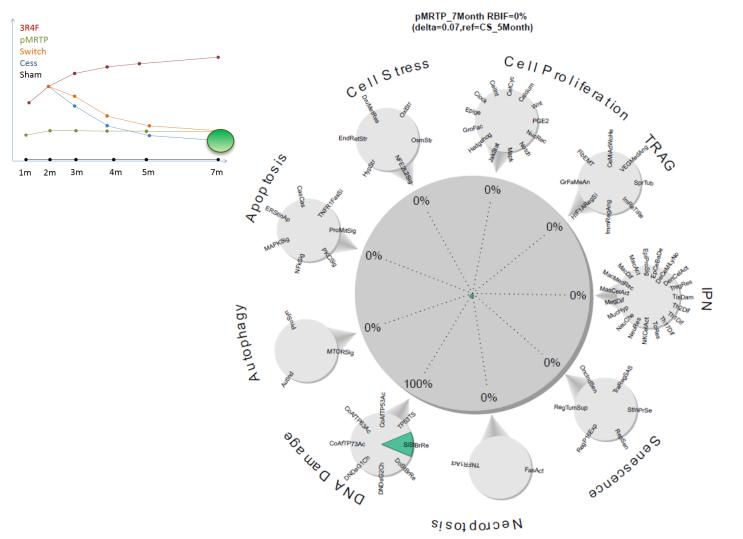




Network Perturbations and Biological Impact in the Lung : Switching to pMRTP, Month 7



Network Perturbations and Biological Impact in the Lung : pMRTP, Month 7





Summary of Results

- Establishment of an *in vivo* model of cigarette smoke-induced COPD with the following characteristics:
 - Pulmonary function was decreased
 - Infiltration of inflammatory cells and mediators in the lungs
 - time-dependent progression of pulmonary emphysema (histopathology)
- The above parameters were ameliorated with cessation or switch-topMRTP
- The perturbations of major biological networks were markedly reduced following switching-to-pMRTP, very similar to cessation.
 - This led to the conclusion that the Biological Impact Factor (BIF) of switching is very similar to that of cessation and markedly different from ongoing cigarette smoke exposure.
- Seven months of exposure to pMRTP had a minimal Biological Impact.

