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Translational biomarker discovery & INTERVALS: a platform facilitating transparent data sharing.

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CORESTA SSTPT Conference

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Outline of the Presentation

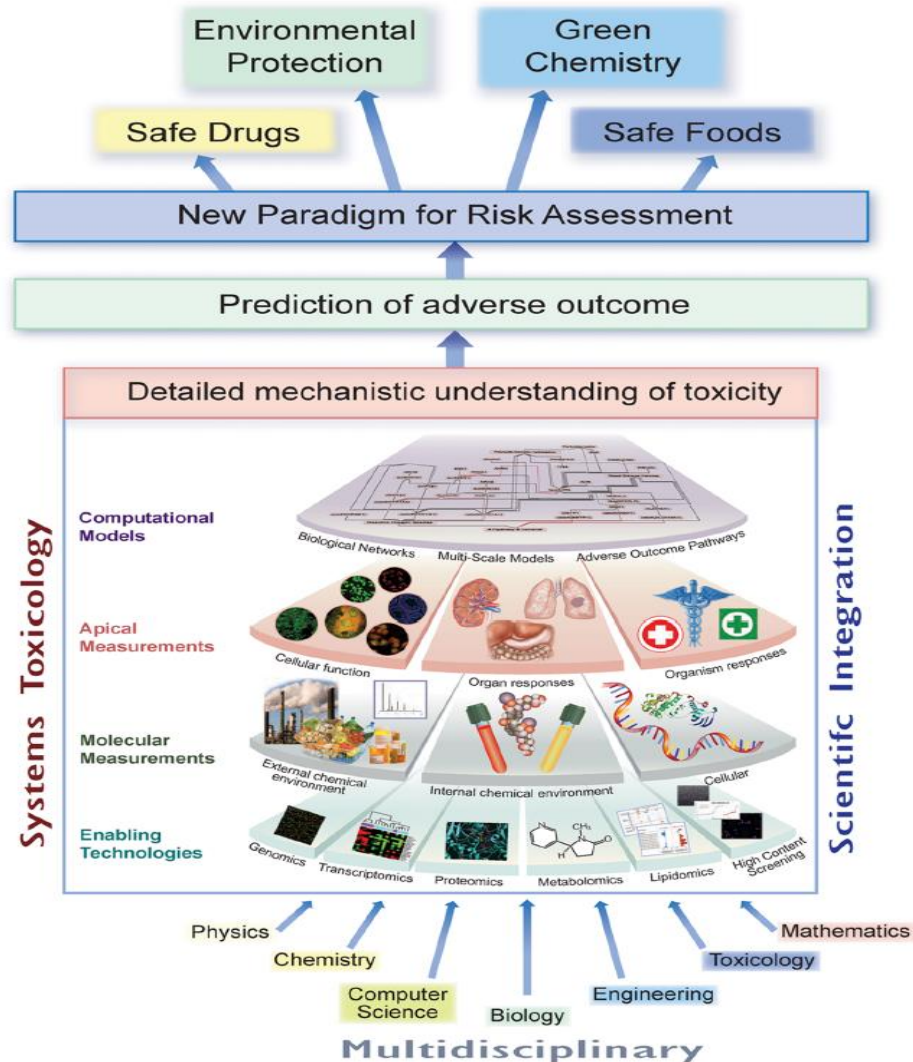
- **PMI R&D**
- **Introduction to our systems toxicology approach**
- **Design of a case study**
- **Proteomics and lipidomics methodologies implemented for identification and quantitation for biomarker discovery**
- **Results**
- **Conclusions and Control of Generated Datasets**
- **INTERVALS: introduction and implementation**

PMI R&D- Background

- Smoking causes serious diseases such as cardiovascular diseases, lung cancer and chronic obstructive pulmonary disease.
- Philip Morris International is developing and commercializing novel products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes.
- To determine whether such potentially reduced-risk products (pRRP), also called modified tobacco risk products (MRTPs) have the potential to reduce individual risk and population harm, we are conducting extensive and rigorous scientific studies comparing their biological impact compared to that of cigarettes



Systems Toxicology Approach For Product Assessment



“Systems Toxicology is the integration of classical toxicology with quantitative analysis of large networks of molecular and functional changes occurring across multiple levels of biological organization.”

- Adds mechanistic insights
- Can support identification of biomarkers for safety assessments
- Toward predictive mathematical models of toxicological processes

Sturla et al. Chemical Research in Toxicology (2014)

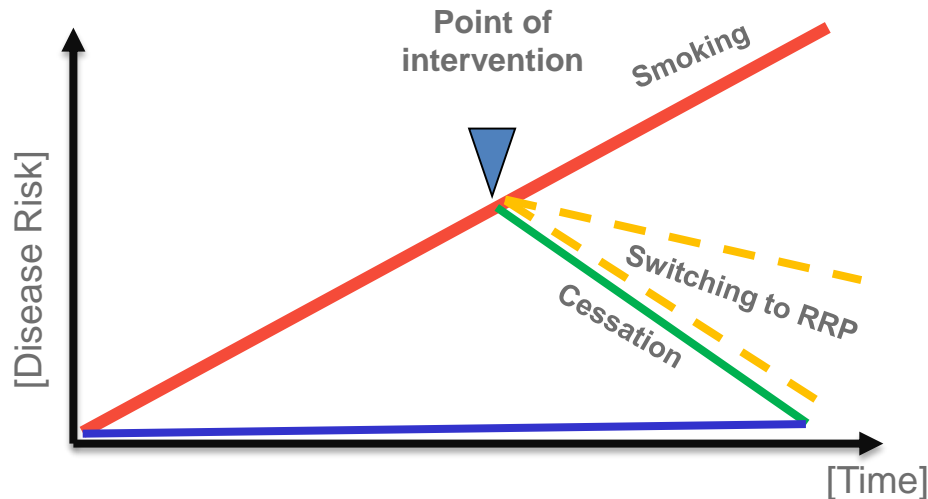


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Design of Case Study

Why Animal Models Switching Studies?

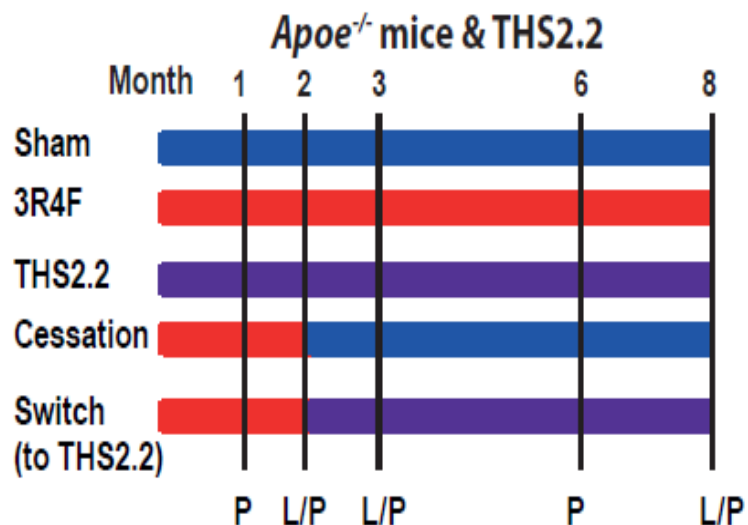
Comparing switching to MRTPs with ongoing smoking and benchmarking against cessation



- Main objective:
Do switching from 3R4F cigarettes to THS2.2, a candidate MRTP (cMRTP) halt or delay the progression of vascular and respiratory pathologies?
If so, what are the cellular and molecular mechanisms affected by switching to cMRTP exposure and how similar are these mechanisms to smoking cessation?

Disclaimer: This graph is for illustrative purposes only

ApoE^{-/-} Study Design



- **ApoE^{-/-} mice model** is an accepted model for the study of cardiovascular diseases. These mice are prone to develop premature atherosclerosis and emphysema.
- **8-month study** on the effects of Sham (control), cigarette smoke (3R4F), cMRTP (THS2.2) aerosol, cessation, and switching to THS2.2 in Apolipoprotein E-deficient (ApoE^{-/-}) mice. All groups n=8 biological replicates analyzed. Months analyzed are: 1, 2, 3, 6 and 8.
- **Nicotine concentration matched between 3R4F and THS2.2** exposure groups (29.9 mg/m³ nicotine)
- **Conducted comprehensive system toxicology study** with special emphasis on respiratory and cardiovascular effects including:
 - In-life observations and biomarkers of exposure
 - Hematology and clinical chemistry
 - Histopathology
 - Aortic arch plaque formation
 - Lung function and BALF analysis
 - Transcriptomics, **proteomics**, and lipidomics

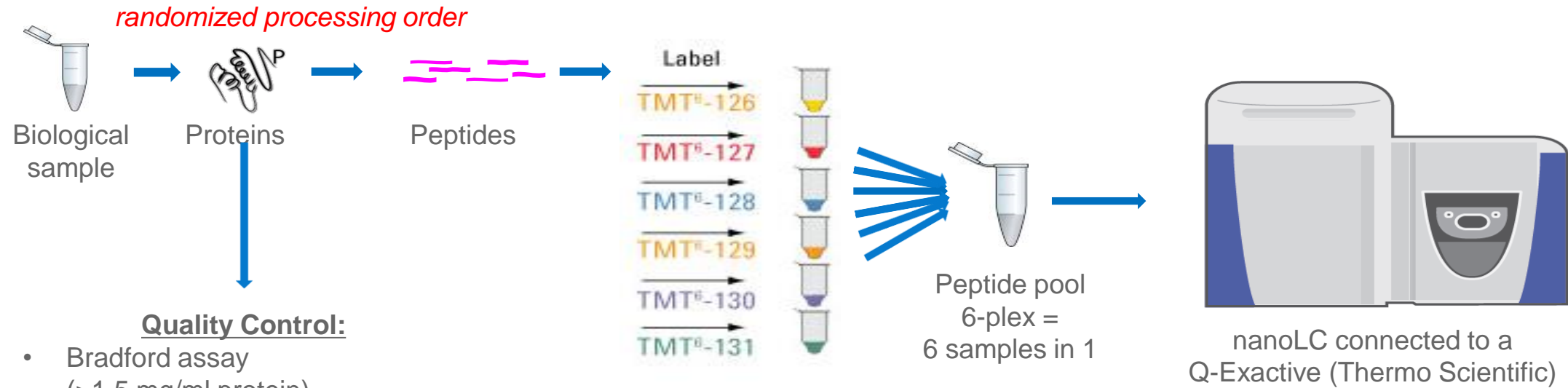
Phillips, B. et al. **An 8-Month Systems Toxicology Inhalation/Cessation Study in ApoE^{-/-} Mice to Investigate Cardiovascular and Respiratory Exposure Effects of a Candidate Modified Risk Tobacco Product, THS 2.2, Compared with Conventional Cigarettes.** Toxicological sciences : doi:10.1093/toxsci/kfv243 (2015).



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Proteomics and Lipidomics Methodologies

Quantitative Proteomics iTRAQ/TMT LC MS/MS Approach

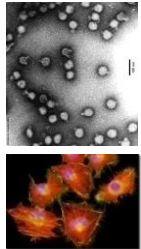
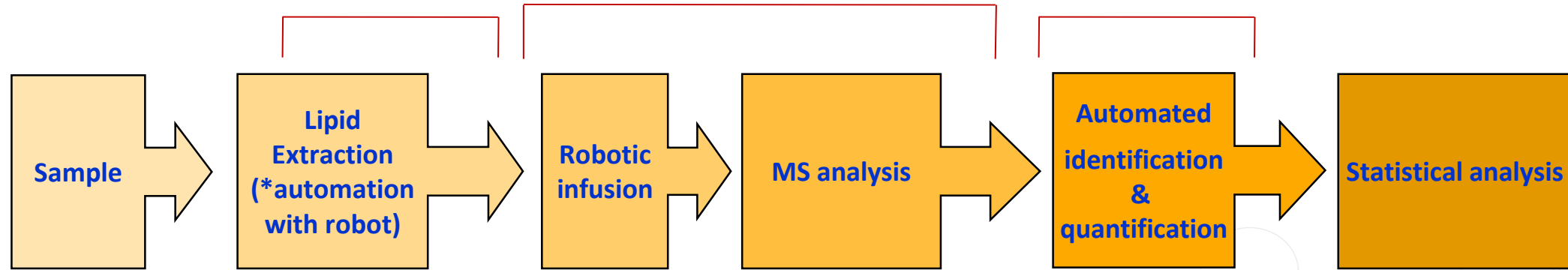


TMT-Labeling:

-Sham
-3R4F
-cMRTP/pMRTP
- Cessation
- Switch
- refmix
→ Pool treatments
→ Months separated

*randomized
channel/pool
assignments*

Shotgun Lipidomics Workflow for Profiling



**Cells
Tissues
Organisms**



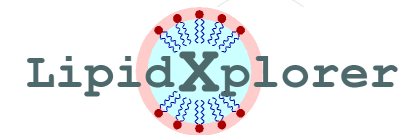
**internal
standards
addition**



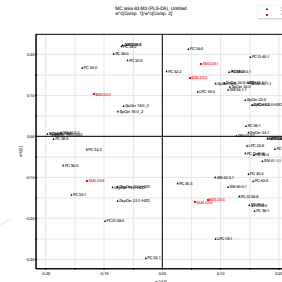
**NanoMate
Robotic interface**



**High resolution
MS-MS/MS
Pos/Neg**



Lipid Xplorer



Ejising *et al* (2009) PNAS 106, 2136-2141

Schwudke *et al* (2006) Anal Chem 78, 585-595

Schwudke *et al* (2007) Anal Chem 79, 4083-4093

Herzog *et al* (2011) Genome Biol 12, R8

Schuhmann *et al* (2011) Anal Chem 83, 5480-5483

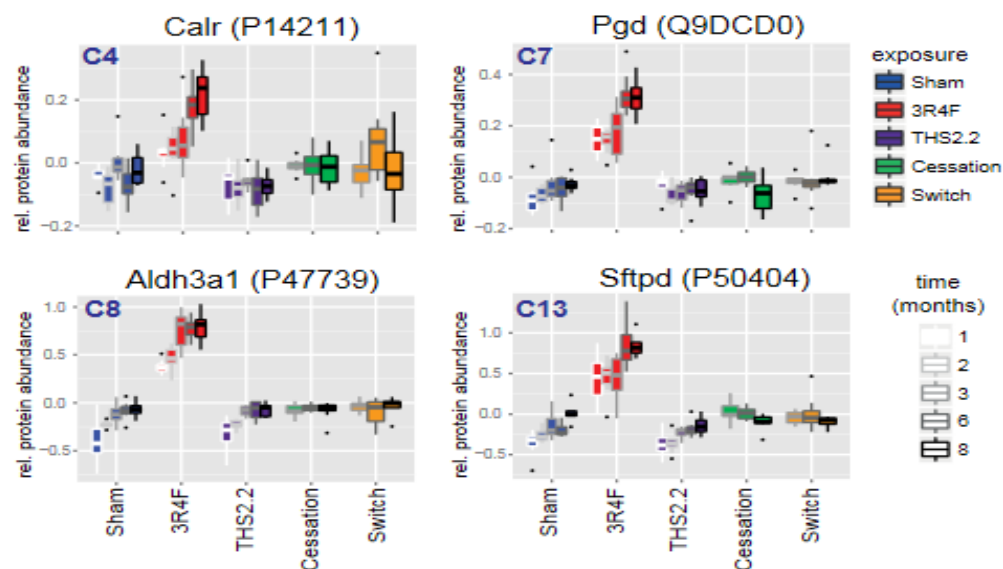
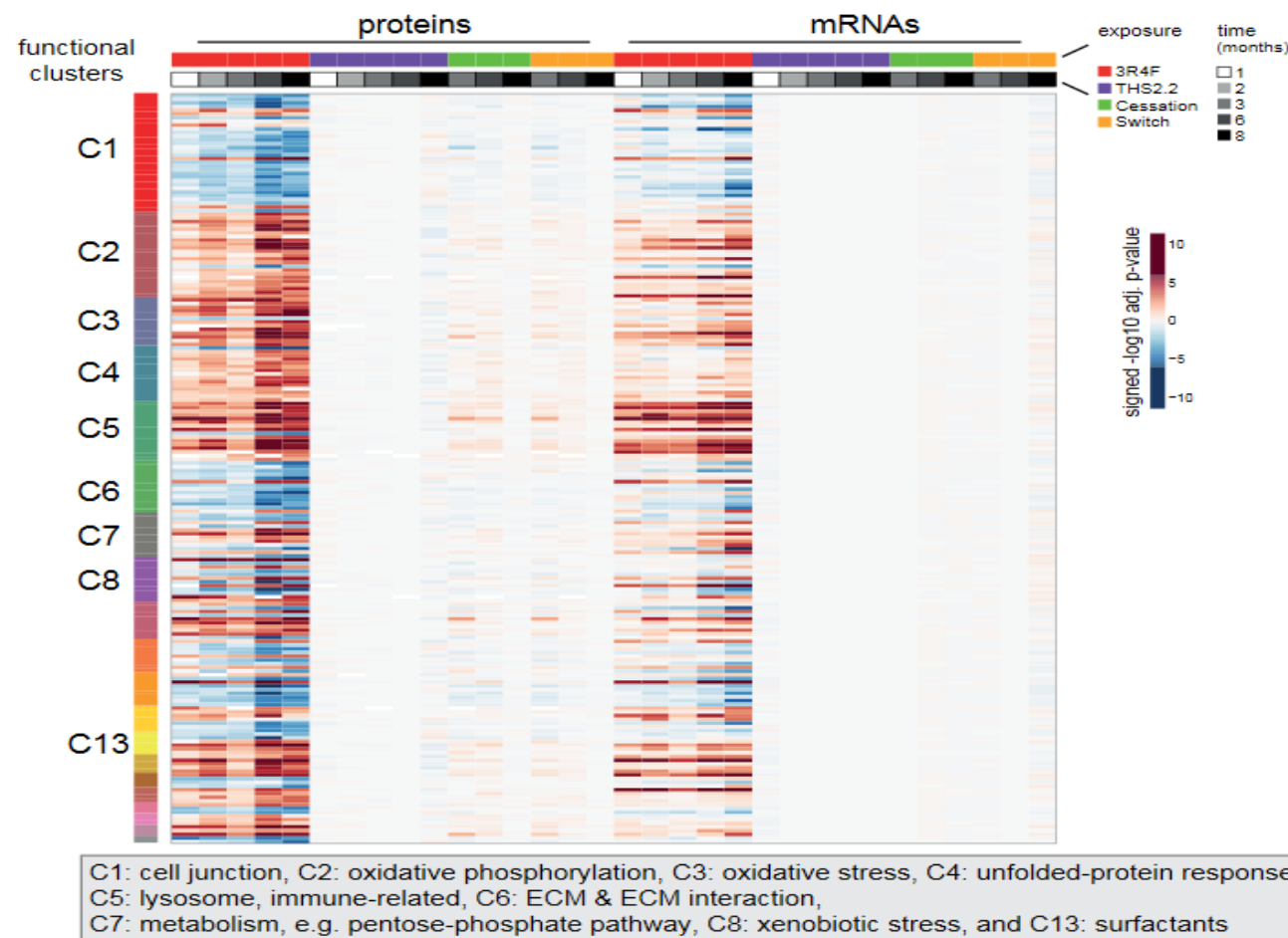
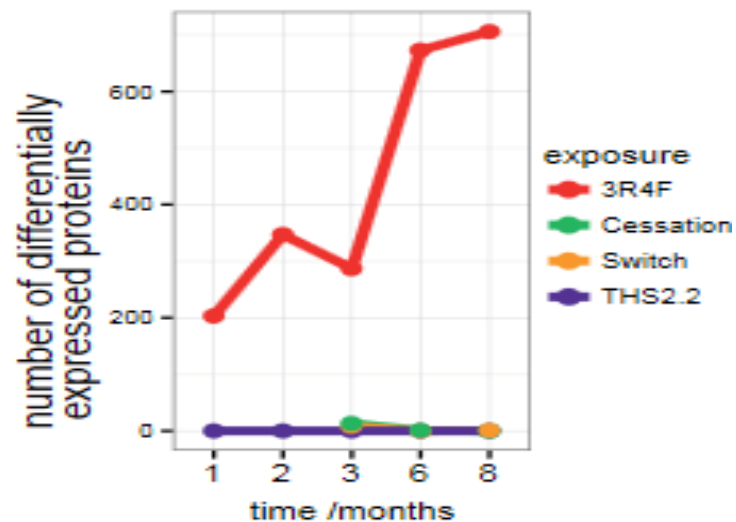
Schuhmann *et al* (2012) J Mass Spectrom 47, 96-104



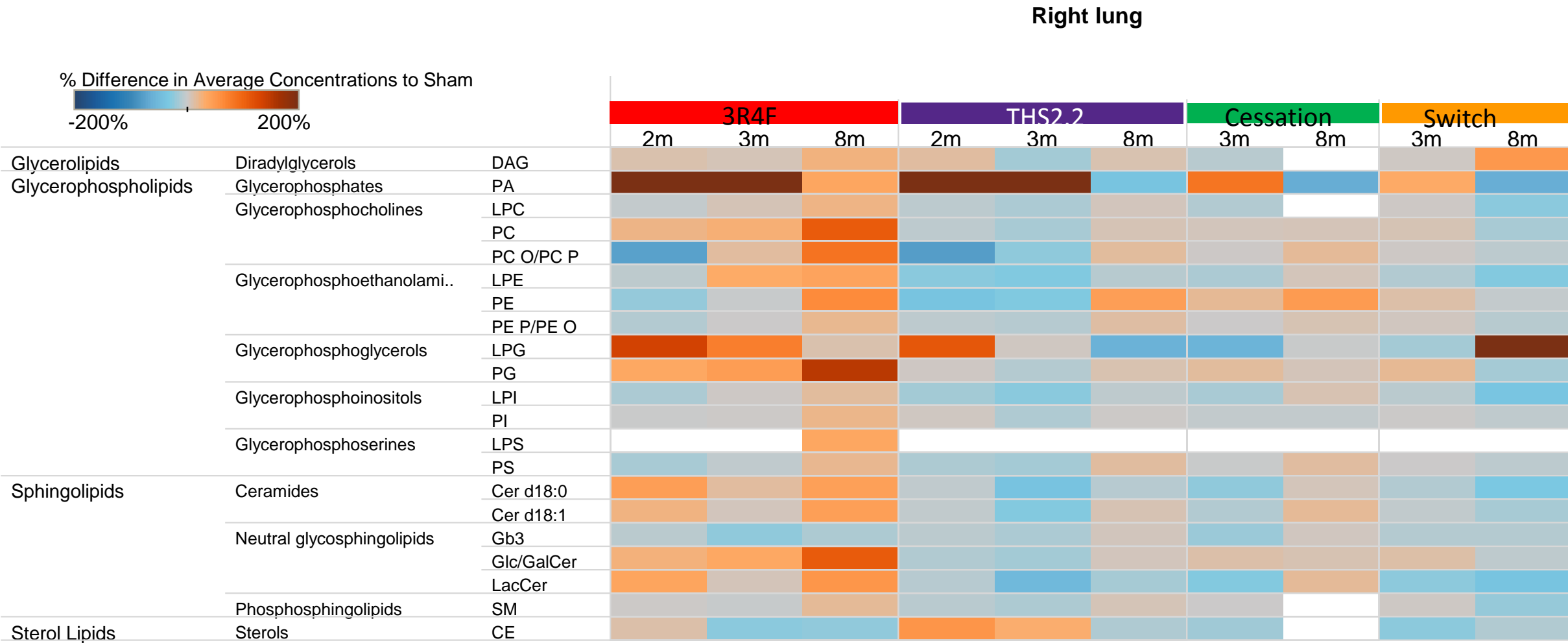
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Results

ApoE-/- Lung Proteome Results



Right Lung Lipidome Results



Titz, B. et al. s. Effects of cigarette smoke, cessation and switching to two heat-not-burn tobacco products on lung lipid metabolism in C57BL/6 and Apoe-/- mice - an integrative systems toxicology analysi Toxicological sciences: doi:10.1093/toxsci/kfv244 (2015).

Conclusions and Control of Generated Datasets

- **iTRAQ proteomics and shotgun lipidomics approaches** were successfully implemented to assess the effects of cigarette smoke (CS), and cMRTP aerosol on lung proteome in an ApoE ^{-/-} mouse study.
- Exposure to mainstream **cigarette smoke (CS)** induced a strong effect on the lung proteome and lipidome. The **other experimental groups showed only a limited number differentially expressed proteins and lipids** as compared to the CS group. **For biomarker verification and confirmation experiments,**
- Targeted LC MS/MS methods will be used on selected protein and lipid biomarkers for verification and confirmation
- **QMS:**
 - Protocols published following QA approved WKIs and SOPs
 - Sample tracking using LIMS and biobanking systems
 - Integrity of raw data managed in validated systems (SDMS)
- **All datasets and protocols are published in INTERVAL** for outside community to be reviewed and accessed



INTERVALS a platform facilitating transparent data sharing & translational biomarker discovery

Workshop - Kitzbühel (Austria) - October 2017

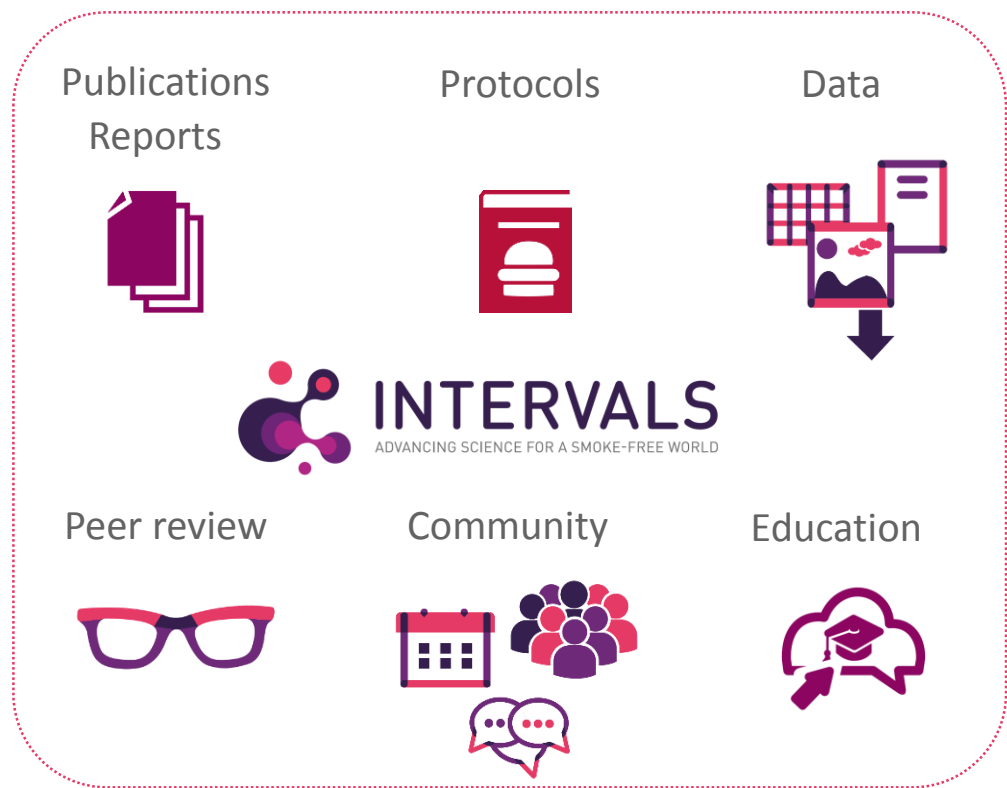
Considerations for the development of INTERVALS

Demonstrate the scientific rigor, thoroughness, validity, precision, conscientiousness, required in Inhalation Toxicology of RRP products

- Many products & flavors to be tested, rapid innovation
- Ensure quality of the data and that the adequate testing strategies are used
- Enable reuse of data sets (3Rs, generation of new hypotheses)
- Novel field of science with many new emerging assay protocols, technologies, and data standards

INTERVALS: Scientific data transparency applied to Industry

Aim: establish a **community** and a **public repository** for 21st-century preclinical and clinical (systems) **inhalation toxicology assessment** data and results that supports open data principles.



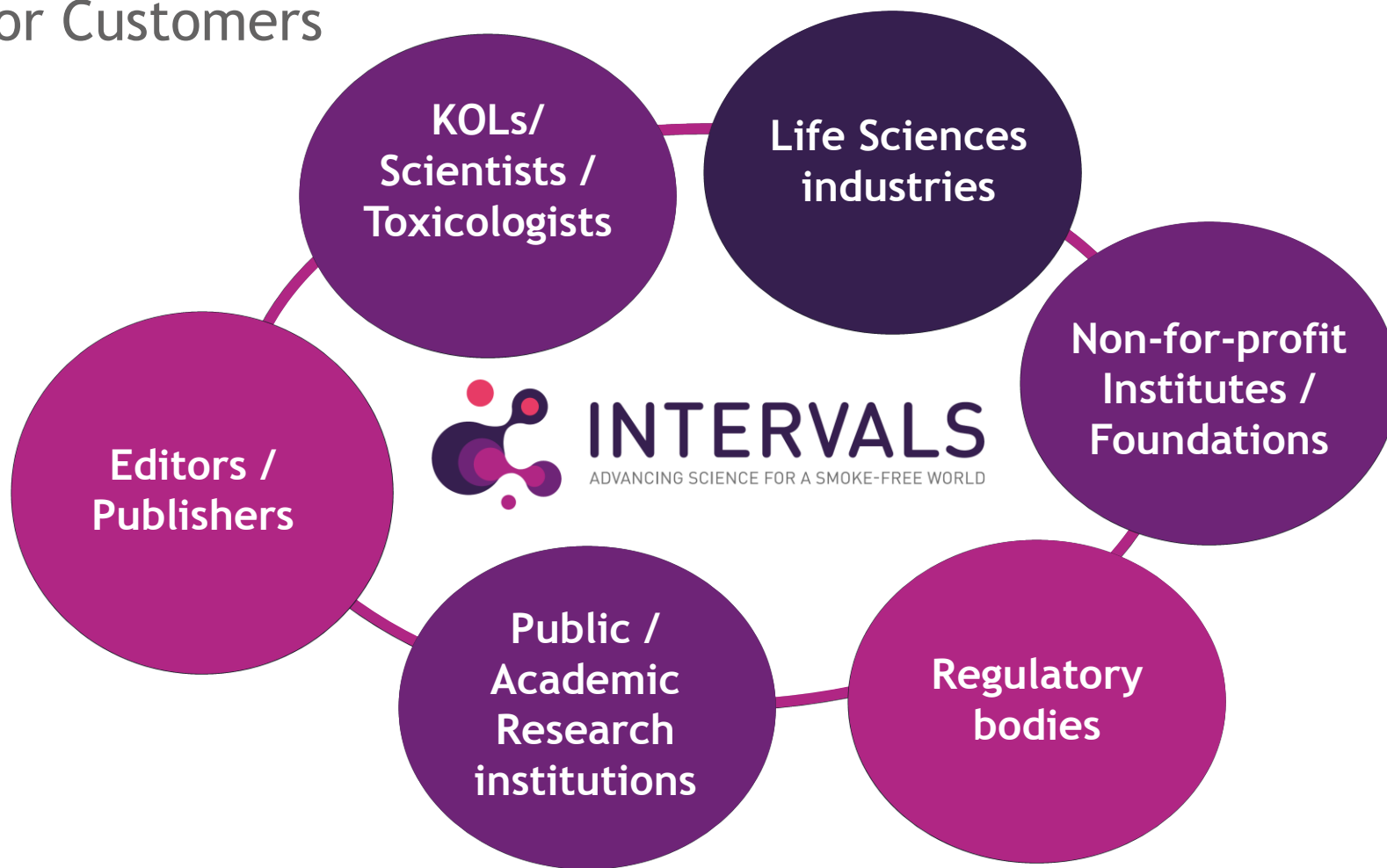
The screenshot shows the INTERVALS website interface. At the top is the **INTERVALS** logo with the tagline "ADVANCING SCIENCE FOR A SMOKE-FREE WORLD" and a "Login" link. Below the logo is a navigation bar with links: **Background**, **Studies**, **Diseases & Pathways**, **Data**, **Tools**, and **Glossary**. The main content area features a large purple banner with the text "Prototype established". To the right of the banner is a graphic with the text "Dive into the Data" and an illustration of a person diving into a pool of data. Below the banner, there is a section titled "Individual risk and population harm associated with cigarettes potentially may be reduced by offering reduced-risk alternatives for smokers who are not willing to quit." followed by a paragraph of text. Below this is a section titled "The quality of the scientific evaluation of MRTPs' associated risk is of the foremost importance." followed by a paragraph of text. At the bottom, there is a section titled "In the first release of INTERVALS, Philip Morris International R&D (for more details, please refer to pmscience.com) shares results obtained during the assessment of a heat-not-burn platform in *in vivo* and *in vitro* studies." followed by a paragraph of text. The footer contains the text "The goal is to grow this initiative and to establish a public repository for 21st century preclinical systems toxicology MRTP assessment data and results, supporting open data principles."

<http://intervals.science>

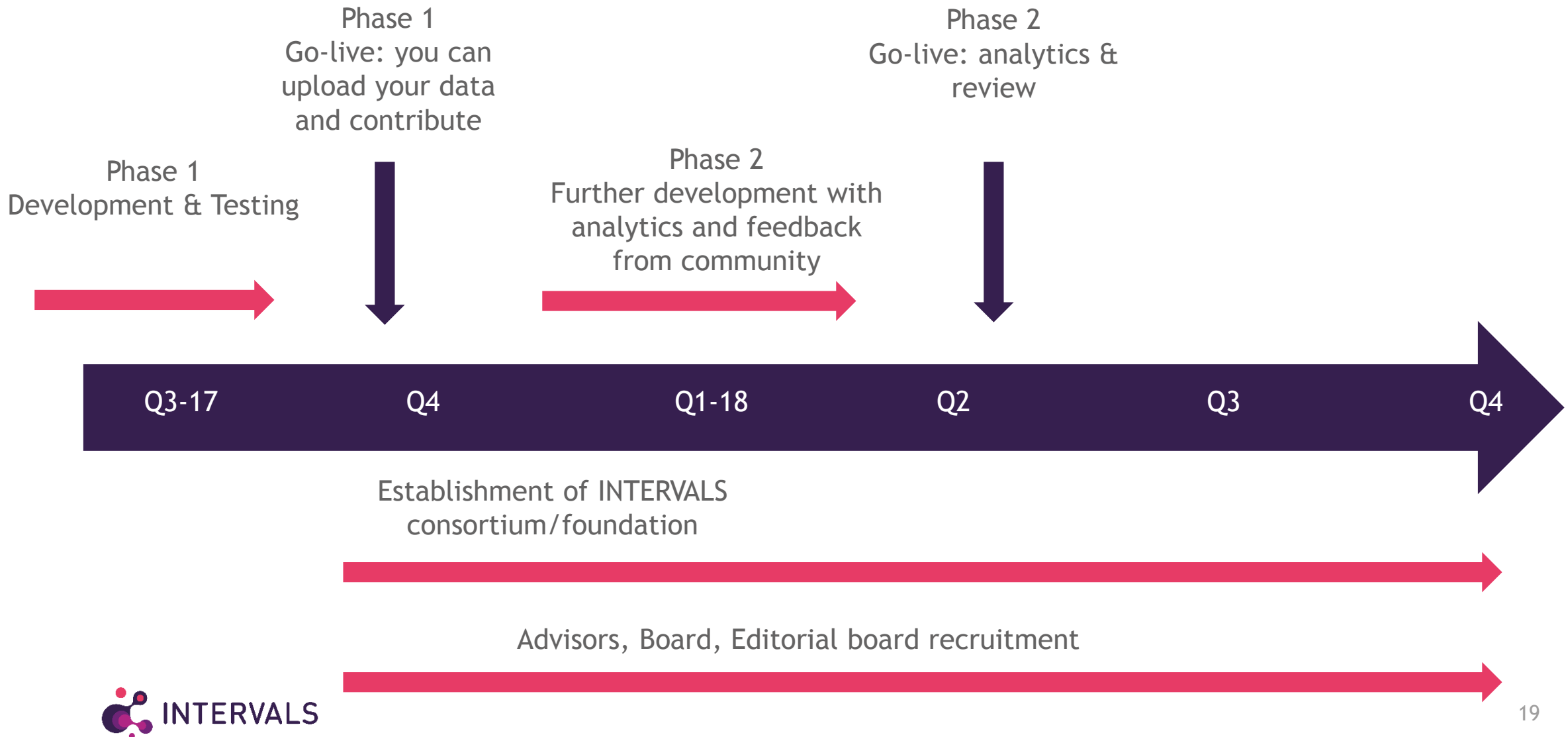
Boué S, Exner T, Ghosh S et al. [Supporting evidence-based analysis for modified risk tobacco products through a toxicology data-sharing infrastructure](#) [version 2; referees: 1 approved, 1 approved with reservations] F1000Research 2017, 6:12 (doi: 10.12688/f1000research.10493.2)

The INTERVALS community / ecosystem

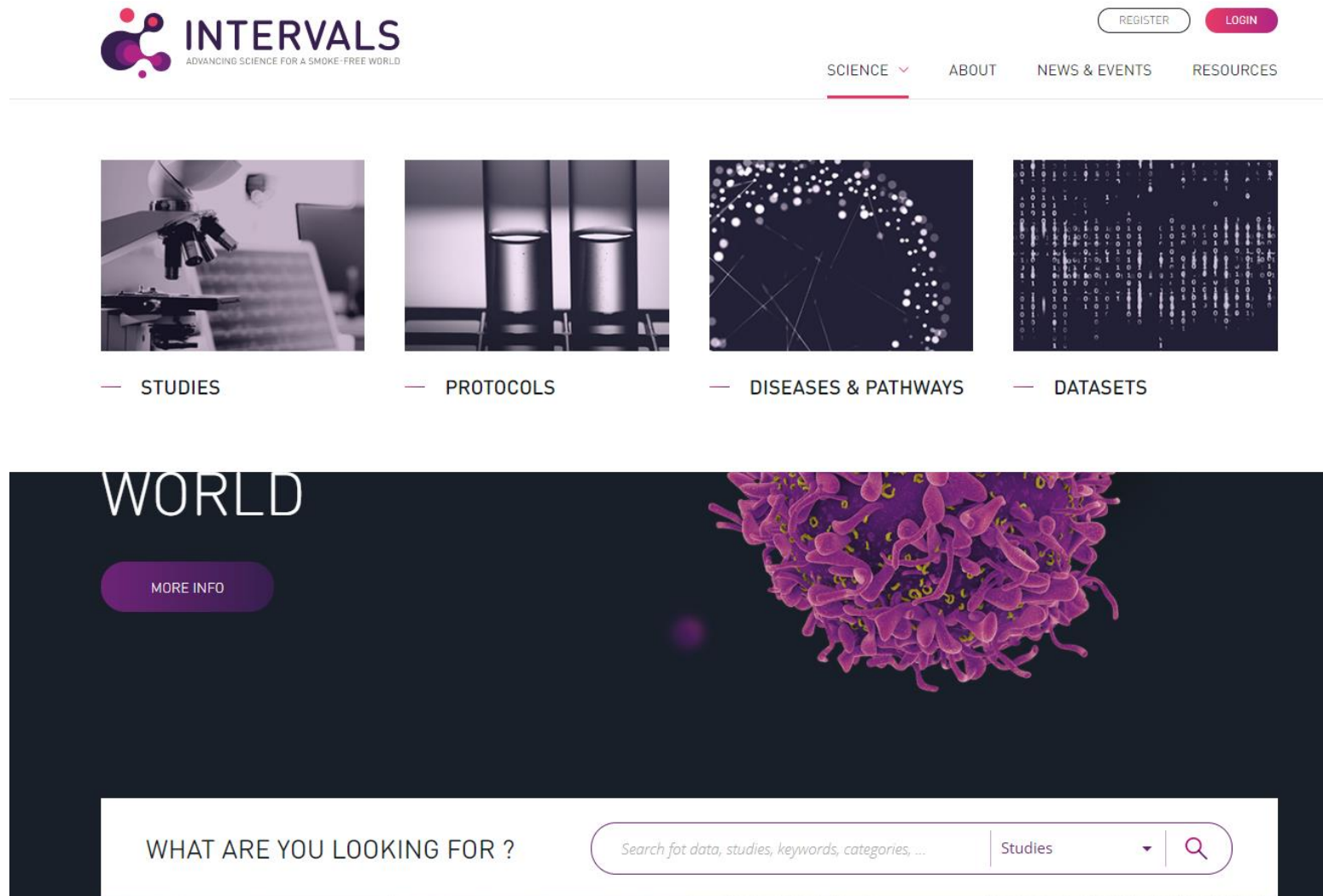
External parties as Ambassadors, Partners / Co-funding members, Sponsors and/or Customers



INTERVALS - Development Roadmap



Overview of the Platform



- science-focused
- 4 entry points:
 - Studies
 - Protocols
 - Diseases & pathways
 - Datasets

Overview of the platform

The screenshot displays the INTERVALS platform interface. At the top left is the logo with the tagline 'ADVANCING SCIENCE FOR A SMOKE-FREE WORLD'. Navigation links include SCIENCE, ABOUT, NEWS & EVENTS, and RESOURCES. User options for REGISTER and LOGIN are at the top right. The main section, titled 'THE STUDIES', contains search filters: EXPERIMENTAL SYSTEM (In vivo), TEST ITEM (THS2.2), and a keyword search bar. An ADVANCED SEARCH section includes filters for ENDPOINT (PK and safety), ORGAN TISSUE (Organotypic gingival), and QUALITY (GCP). Below these filters, a banner indicates '3 results found'. Three study cards are shown, each with a date (09/09/2017), a title, and a 'VIEW ON PORTAL' button.

INTERVALS
ADVANCING SCIENCE FOR A SMOKE-FREE WORLD

REGISTER LOGIN

SCIENCE ▾ ABOUT NEWS & EVENTS RESOURCES

THE STUDIES

EXPERIMENTAL SYSTEM TEST ITEM

In vivo THS2.2

Type keywords

ADVANCED SEARCH

ENDPOINT ORGAN TISSUE QUALITY

PK and safety Organotypic gingival GCP

3 results found

STUDY

09/09/2017

Assessment of acute ths2.2 aerosol exposure in in vitro human nasal epithelial cultures

VIEW ON PORTAL

STUDY

09/09/2017

8-month systems toxicology inhalation / cessation study with THS2.2 in Apoe-/- mice

VIEW ON PORTAL

STUDY

09/09/2017

Nicotine pharmacokinetic profile and safety of the Tobacco Heating System 2.2 (THS2.2) - Japan study

VIEW ON PORTAL

- Faceted search enables quick retrieval of resource of interest
- Detailed protocols
- Clear contact detail
- Community features (news/commenting/events)

How could INTERVALS be useful for Coresta?

- » Share protocols in development
 - » Create a new dashboard
 - » Invite contributors (write access or read-only access, useful for review)
 - » Work on the protocol “privately” until you are ready to make it public
- » Following the same process, share a study or data amongst a restricted number of contributors until you are ready to share with a broader audience
- Contact us if you have questions (contact@intervals.science)

Acknowledgements



Declaration of Interest

All mentioned names in this presentation were employees of Philip Morris Products S.A. (or NAME OF ENTITY IN SINGAPORE) (both part of Philip Morris International group of companies) when they made their contributions to the study. Philip Morris Products S.A. is the sponsor of this project.

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