Biomarker (BMK) Sub-Group 2017 Report

Kitzbühel, Austria

October 10, 2017

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Secretary: Kirk Newland
Scientific Commission Liaison: Paul Harp

Biomarker Sub-Group Objectives

- **Objective 1: To review present knowledge of tobacco and smoking-related biomarkers of exposure and effect, and to document these in meeting minutes, CORESTA reports and scientific publications where appropriate**
- **Objective 2: To undertake ring trials / proficiency tests for selected biomarkers as agreed by SC**
- **Objective 3: To source and develop reference materials to support biomarker analysis for those biomarkers selected for ring trials / proficiency tests**

Berlin-Orlando-Kitzbühel

- October 08, 2016 Berlin, Germany
  - 30 delegates attended
- April 19, 2017, Orlando, FL USA
  - 25 delegates attended
- October 08, 2017, Kitzbühel, Austria
  - 38 delegates attended
- The Biomarker SG holds joint meetings with the Product Use Behavior Group
  - BMK SG meets in the afternoon

Biomarker

- Generally refers to a measurable indicator of some biological state or condition (Wikipedia)
- Context-dependent
- Interim measures
- Decision making tools
- Biomarkers typically have two dimensions
  - The biology: What is that we are trying to measure and under what context?
  - Measurement: Analytical component

Two types of biomarkers

- **Broadly two types of Biomarkers exist in the context of Tobacco**
  - **Biomarkers of Exposure: Measures of exposure to tobacco products. Examples include: nicotine, cotinine, TSNAs**
  - **Biomarkers of Effect: Indicate the effect of tobacco use on the consumer.**
    - Could be useful for assessing the health effects of the product and could inform the potential harm
    - Particularly useful in the absence of epidemiology
    - Very diverse in their characteristics: examples; a compound, a protein or its activity, a gene

Scientific Discussions

- **Diverse topics for discussions include, analytical methods to cutting edge science**
- **Methods discussion:**
  - **Flavoromics, profiling for flavors in e-liquids and body fluids (ABF)**
    - Library of flavor compounds, untargeted analysis
- **Biomarkers**
  - **Biomarkers in Clinical Trials (Inflamax)**
    - Focus on respiratory biomarkers, established (FEV1) and emerging (nasal mucociliary clearance)

Biomarkers

- **Methodological considerations for identifying likely users of e-vapor products for ambulatory clinical studies (Altria)**
  - Reduced cigarette use
  - Subject compliance and reporting issues noted in this fairly large clinical study
- **Biomarkers for Cigars - Oral, inhalational, or a combination (Imperial Brands)**
  - Fewer biomarkers/HPHCs in cigar smoke, lower levels in consumers relative to cigarette smokers, and need for additional work

Scientific Discussions

Biomarkers

Biomarkers for tobacco products- proposal for a status review (Inflamax)

- Writing committee formed: Victoria Nelson, Cherrie Small, Piuysh Patel, Patrudu Makena, Michael McEwan, Krishna Prasad, Elizabeth Cerson, Michael Kong, Jeff Edmiston and G. L. Prasad

Biomarkers of Effect review, with a focus on lung biomarkers (NWIP# BMK161)

- Scope may be refined further, and publication costs resolved

Scientific Discussions

Biomarkers

Biomarkers of effect for tobacco products (Altasciences)

- Need for qualified biomarkers for tobacco studies
- Two likely biomarkers-DNA adducts and non-coding RNAs discussed
- Ensuing discussion led to exploration of a meta analysis of existing biomarker data, a potential NWIP

Biomarkers of effect in smokers who switch to electronic cigarettes (ITG)

- Biomarkers of effect in a 2 year product switching study
- Metabolomic data presented

❖ Biomarkers

➢ Proteomic and lipidomic markers from an *in vivo* study involving THS2.2 product (PMI)

- Distinct protein and lipid biomarkers from smoke exposed mice and cessation and product-exposed animals

All about Data

Data

- **Data standards for clinical biomarkers of exposure (Celerion / BAT?)**
  - To standardize and streamline data management and reporting
  - Discussions underway to develop a new project
- **INTERVALS, a platform for transparent data sharing (PMI)**
  - Presentation on data transparency; opportunity for sharing and comparing
  - Role for/ involvement of CORESTA BMK SG?
  - To be discussed further
- **Meta analysis of biomarker data**
  - A likely NWIP (from discussion on biomarkers of effect)
  - More discussion needed

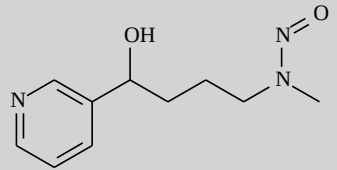
Reference standards

- **Requirements for the Certification of analytical reference standards in tobacco biomarker studies (Objective 3)**
  - **CORESTA Technical Guide No. 20, published – September 2017**
  - **Guideline describes the desired content of Certificate of Analysis for reference standards**
  - **Writing Committee:**
    - Frank Deschamps (Leader), Max Scherer, Mark Bentley, Krishna Prasad, Eckhardt Schmidt and G. L. Prasad

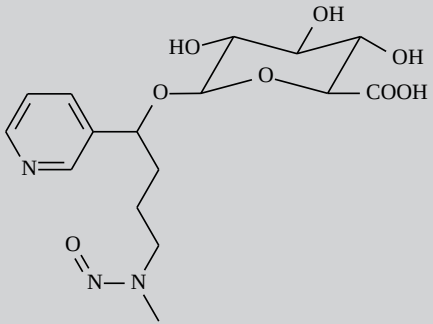
Reference standards

- **Requirements for the Certification of analytical reference standards in tobacco biomarker studies**
  - **To have well characterized analytical standards so that quality data can be generated**
  - **Analytical standards should be well characterized**
    - Prove the identity
    - Purity of the analyte
    - Potency of the analyte
- **Next steps**
  - **Development guidelines for standards for fit-for-purpose bioanalysis, a potential NWIP**

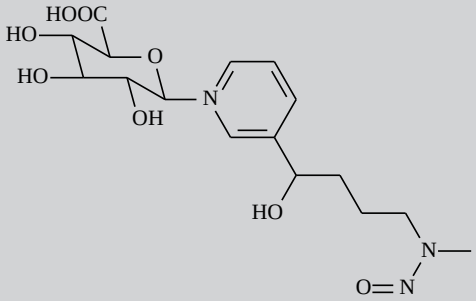
- **Objective 2: Inter-laboratory Comparison : Bioanalytical Assay to measure total NNAL in human urine**
  - **What is NNAL?**
  - **Why NNAL?**



NNAL



NNAL-O-Glucuronide



NNAL-N-Glucuronide

- **NNAL is a major metabolite of the tobacco-specific nitrosamine NNK**
  - **NNK is a known lung carcinogen (IARC Group 1 carcinogen) and is designated as HPHC**
  - **NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol) is also considered as a carcinogen.**
  - **NNAL is enzymatically conjugated as a glucuronide and excreted in urine**
  - **Urinary levels of total NNAL (free + glucuronidated NNAL) are widely used as a biomarker of exposure to tobacco products**

Potential labs

- **Several laboratories showed interest**
  - ABF GmbH
  - SEITA – Imperial Tobacco
  - China National Tobacco Quality
  - Shanghei Tobacco Group
  - Zhengzhou Tobacco Research Institute of CNTC
  - KT&G Research Institute
  - University of Minnesota
  - Covance Laboratories
  - Celerion, Inc., **Organizing lab**

Sponsors

- Altria Client Services
- British American Tobacco
- Imperial Tobacco Ltd
- Japan Tobacco
- Philip Morris International
- RAI Services Company

The cost associated with the study were shared the by the sponsors

Fewer labs

Study Results

- **Final data was received from 4 participating laboratories**
  - ABF, SIETA, U Minnesota and Celerion

- **Final data was not available from 5 of the 9 laboratories due to issues of:**
  - Labs unable to receive biological samples from Celerion
  - Bioanalytical assay not available
  - Testing not completed/ Results were not provided

Study design

- Shared Quality Controls at 3 concentrations
  - 15.0 pg/mL (n=6)
  - 70.0 pg/mL (n=6)
  - 750 pg/mL (n=6)
- Individual Lots of Smoker Urine from 9 Volunteers (n=3)
- NIST Smoker Urine (n=3)
- Control Blank Matrix (n=3)

- 1 Set of Imbedded Standards
  - 9 Standard Concentrations
  - Range: 5.00 to 1000 pg/mL
- Original Participating Labs: 9
- Labs Completing Analysis: 4

Preliminary Results

- When each laboratory used their own source of reference material, the bioanalytical results were not comparable within the standard bioanalytical acceptances (>30% R.E).
- When the same reference material and standard set was used for quantitation, all 4 labs produced data within 15% of the expected concentrations

Preliminary conclusions

- With the 4 laboratories evaluated no analytical method issues were noted. The same variability was observed for aglycone and total NNAL samples.
- The use of a single set of calibration standards resolved the bias observed for both aglycone and total NNAL samples.
- Final Report anticipated Jan 2018

Publications

- **The inter-laboratory comparison study for the urninary acrolein biomarker, 3-HPMA was published.**

Beiträge zur Tabakforschung International
Contributions to Tobacco Research
Volume 27 @ No. 5 @ January 2017
DOI: 10.1515/cttr-2016-0006

An Inter-Laboratory Comparison for the Urinary Acrolein Biomarker 3-Hydroxypropyl-Mercapturic Acid (3-HPMA) *

by
Gerhard Scherer [1], Wolf-Dieter Heller [2], Michael McEwan [3], Thomas Göen [4], Peter Joza [5], Nan Liu [6], Kirk Newland [7], Thomas Schettgen [8], Sheng Wang [9], Hyung-Ok Sohn [10], Valerie Troude [11], Dai Yuki [12], Saijing Zheng [13], Guojun Zhou [14]
[1]

Thank You