Application of Systems Pharmacology to Identify Exposure Response Markers in Peripheral Blood After Switching to a Candidate Modified Risk Tobacco Product: the Tobacco Heating System 2.1 (THS 2.1)

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Introduction and Objective

Establishing exposure response markers is necessary for the assessment of candidate modified risk tobacco products (MRTPs) against conventional cigarettes (CC). Biomarkers derived from the primary site, such as the airway, require invasive sampling, whereas blood offers a minimal-invasive alternative for the general population.

Various diseases and exposures, including cigarette smoke, have been shown to alter the molecular profile of the blood. To identify exposure response, we have conducted a whole genome Affymetrix microarray analyses from blood samples of current smokers and never smokers.

The aim of this study was to derive a blood-based current smoker (CS) gene signature with the potential for distinguishing subjects who smoked from those that have quit or have never smoked. Using human biobanking samples, we have developed and applied a "blood signature" which can differentiate between smokers and non-smokers.

Taking advantage of the lessons from the IMPROVER Diagnostic Signature Challenge [3], we have developed a new methodology to derive a "smoking gene signature" that is robust. The key feature of our methodology was to build a prediction model by jointly using high fold-change genes extracted from several publicly available gene expression datasets that profiled blood samples from CS and non-smokers and former smokers (FS). Prior to this study, the development of high fold-change genes from various independent studies has the potential to enforce the robustness of the signature across studies.

The validation was performed with an independent dataset derived from a clinical study initiated by Philip Morris International that aimed to collect whole blood samples for COPD. Finally, from an additional clinical study sponsored by Philip Morris International, we evaluated the blood transcriptome of smokers who switched from CC to Tobacco Heating System 2.1 (THS 2.1) for 5 consecutive days and compared the blood transcriptome of smokers who continued to smoke CC.

Blood samples for BLD-SMk-01 were obtained from a banked repository (Reefactor Biotechnologies Ltd, Belleville, MO. 62221 USA). At the time of sampling, the subjects were between 23 and 63 years of age. Subjects with no disease history and anyone taking prescription medications were excluded. Current smokers had smoked at least 10 cigarettes per day for at least 3 years. Former smokers had ceased smoking at least 2 years prior to sampling and before quitting had smoked at least 10 cigarettes per day for at least 3 years. Current smokers and non-smokers were grouped by age and gender. A total of:

1. 31 blood samples were obtained from current smokers (CS);
2. 30 from never smokers (NS);
3. 30 from former smokers (FS).

For each sample, >20,000 parameters are measured.

Validation on Independent Clinical Samples

The Queen Ann Street Medical Clinical study (referred to as the QASMIC study) was a controlled study conducted at The Heart and Lung Center in London, U.K., according to Good Clinical Practice (GCP) and was registered on ClinicalTrials.gov with the identifier, NCT01780288. It aimed to identify biomarker or a panel of biomarkers that could differentiate the subjects with:

1. 60 COPD (current smokers with a 10 pack year smoking history at GOLD Stage 1 or 2) and three control groups of matched non-smoking subjects:
   a. 60 never smokers (NS);
   b. 40 ex-smokers (FS);
   c. 60 current smokers (CS).

All the COPD patients are Current Smokers. Therefore it enables not only the validation of the smoking signature but also its robustness with respect to the COPD disease status of the patients.

Application to the THS 2.1 Switching Study

The REX-EX-01 study was an open-label, randomized, controlled, two-arm parallel group study (Figure 3) that recruited 42 healthy smokers between 23 and 65 years. It was designed to compare smokers switching CC to smokers switching to a candidate Modified Risk Tobacco Product: the Tobacco Heating System 2.1 (THS 2.1) over 5 consecutive days (Figure 2).

The study was conducted according to Good Clinical Practices (GCP) and was registered on ClinicalTrials.gov, with the identifier NCT0178074. Blood samples were further processed and hybridized to Affymetrix Human Genome U133 Plus 2.0 GeneChips.

The ultimate goal was to apply the signatures obtained to determine whether the impact of switching to THS 2.1 can be readily detected after 5 days of switching in the whole blood transcriptome, thus providing a sensitive and non-invasive tool for assessing the exposure-response in clinical trials. The underlying premise was that the blood transcriptome of smokers who switch to THS 2.1 starts to resemble that of a former smoker. Therefore, instead of characterizing the gene expression profile of a MRTP user that is specific to five days of switching (e.g., by extracting the signature from the REX-EX-01 study data), we set out to find a transcriptomic based exposure response signature that could also serve as an indicator of a longer term switching pattern. This was achieved by establishing the signatures discussed above, that could reliably categorize samples from two current smoker samples.

At the end of each day, a set of 16 biological replicates of smokers who switched from CC to THS 2.1 and 16 of those that remained on CC were collected. The individuals who remained on CC were mainly classified as CS (when classifying current smokers vs. never smokers). This figure 1 shows that for all genes, the expression pattern was very similar.

Conclusions

In conclusion, our systems pharmacology approach enabled the construction of a robust whole blood based smoking gene signature. The ‘signature’ invokes measurement of the expression of 12 different genes which respond to exposure to CC smoke and more importantly, differentiate between current smokers and never smokers. Our systems biology approach was further validated in a large biobank dataset of never and former smokers.

This signature will be used to analyze the whole blood transcriptics in the planned clinical study 2304M-REXa-08-US.

References