Identification of smokers that are responsive to cessation by global gene expression analysis
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INTRODUCTION
Approximately 5600 constituents have been identified in cigarette smoke (Perfetti & Rodgman 2011). A number of these compounds are known to have specific toxicological properties. In order to demonstrate any reduction in the impact of these tobacco smoke toxicants on smokers, a number of research tools are employed. We describe here the results of a global gene expression study as part of a clinical study designed to assess reversibility of biomarkers in a cohort of smokers participating in a smoking cessation program. The aim of this study was to examine gene expression profiles before and after smoking cessation and identify candidate biomarkers responsive to reduction in cigarette smoke exposure.

METHODS
A longitudinal clinical study was conducted at the Centre of Research and Information on Smoke (CeRIF), Messina, Italy, with approval of the local ethical committee and according to principles of Good Clinical Practice. 43 smokers successfully completed the cessation program.

Clinical assessment at the time of screening and at 28, 90 and 180 days following smoking cessation included analysis of salivary cotinine, exhaled carbon monoxide, clinical blood and urinalysis. In addition, whole blood samples were collected into CPT vacutainers for global gene expression analysis.

Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation within four hours of collection, and cells were lysed in TriZol Reagent. Following extraction of total RNA, biotinylated cRNA was prepared and hybridized to Affymetrix HG U133 Plus 2.0 arrays. Microarray data were analyzed using GeneSpring v11.5. Statistical analysis was carried out using SASv9.2, SAS Enterprise Guide v4.2 and JMP 9.0 (SAS Institute, Carey, NC).

RESULTS
Repeated measures ANOVA showed that 109 genes were differentially expressed in quitters over time (p<0.001). Few differences were seen between time 0 (baseline) and day 28. However, expression of this 109-gene signature markedly changed at day 90 with increased median intensity levels, remaining at similar levels at day 180.

Cluster analysis revealed a clear difference between individual quitters: some quitters appear to respond clearly after entering the cessation program, whereas others show no or little detectable change following smoking cessation. Hierarchical clustering of 109 differentially expressed genes, identified using a repeated measures ANOVA (corrected p-value P <= 0.001), demonstrates the relationships between gene expression and quitters (Figure 1).

SUMMARY
The most significant relationships were associated with total nicotine, total cotinine, COHb and MCHC.

Subjects with a different trend have been shown to be among the cohort with the highest levels of nicotine equivalents at baseline. These subjects may need more time to obtain the benefit from smoking cessation, although this is a hypothesis given that the data were not conceived for studying subgroup differences. Another possibility is that these subjects are not sensitive to changes observed in the subset of genes in PC1.

Other variables were studied such as age, gender, time smoking, pack years and BMI. No statistical significance was observed for these subgroups.

CONCLUSION
The study indicates there is not a predictable biological response (for the biomarkers used) to smoking cessation, rather the response(s) is individual-based and possibly confounded by a number of other factors, for example, blood consists of a complex, heterogeneous mix of several cell types at various developmental stages varying from time to time and subject to subject.

Further analyses of these data is being conducted to better understand these complex responses.

References