MUT/2022/12

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

Horizon Scan Item

Update on a recent meeting and workshop of interest to COM.

Introduction

1. This paper presents some of the current issues being discussed at a recent meeting and workshop covering issues that may be of interest to COM.

2. Attached at Annex A is a brief overview of topics discussed at the IGG Next Generation Sequencing Workshop, held in May 2022 in London.

3. Attached at Annex B is a summary of some sessions of the UKEMS Annual Meeting, held in July 2022 in Harrogate.

Questions for the Committee

4. Members are asked to consider the list of topics discussed in Annex A and Annex B and discuss whether these should be considered by COM for future horizon scanning?

IEH-C under contract supporting the UKHSA Secretariat
October 2022

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IGG Next Generation Sequencing Workshop, held in May 2022 in London.

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Session 1. ECNGS: Current and Emerging Technologies

Introductory presentations were given by Jesse Salk (Twinstrand Biosciences, USA) who presented an '**Introduction to DuplexSeq, BotSeq, etc**..' and Inigo Martincorena (Sanger Inst, UK) who provided an '**Introduction to NanoSeq'**.

Bob Young (GTTC-Error Corrected Sequencing Workgroup, USA) presented '**Duplex Sequencing™ and its potential to transform cancer safety assessment'**. This presentation explored whether DS can be used to identify early biomarkers of cancer risk.

Session 2. ECNGS Concepts and Mutational Signatures

Jill Kucab (Kings College London, UK) provided an overview of the unique mutational signatures that have been identified for carcinogens, including environmental agents in the presentation '**Investigating mutational signatures of carcinogens and chemotherapeutics using human tissue-derived organoids'.**

Clint Valentine (TwinStrand Biosciences, USA) presented '**Fundamental concepts with Duplex Sequencing mutagenesis data and trinucleotide signatures'**.

Session 3. Mutagenesis, carcinogenesis and regulatory testing

Carole Yaulk (University of Ottawa, Canada) presented **'Exploring the utility of error-corrected Duplex Sequencing in regulatory toxicology: proof of concept and validation studies in vivo and in vitro'.** The importance of such studies when translating research NGS assays into regulatory assays was explored.

Patricia Escobar (Merck, USA) presented **'Genotoxic v nongenotoxic carcinogens'.**

Kritine Witt (NIEHS, USA, retired) presented '**Duplex Sequencing: a game-changer in genotoxicity testing and cancer risk assessment?** The advantages of DS in genetic toxicology were outlined.

Roland Frötschl (BFarm, Germany) presented **'Regulatory Challenges and Opportunities'.** Challenges included the implementation of new methods into standard tests, data interpretation, defining the context of use, preparation of International Guidelines. Opportunities were seen to be the higher accuracy of DS assays which was not linked to specific cells, tissue or animal model.

Session 4: Skills for ECNGS

Simon Reed (Cardiff University, UK) presented **'Induce Seq technology',** which detects breaks in the genome.

Anne Ashford (AstraZeneca, UK) presented ' **Advanced assessment of mutations in an in vitro Pig-a assay through Duplex Sequencing'.**

MUT/2022/12 Annex B

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UKEMS Annual Meeting, held in July 2022 in Harrogate.

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UKEMS Annual Meeting

July 2022, Harrogate

IGG Session 1 – Nitrosamines and Industry Topics

Jo Elloway (AstraZeneca, UK) presented 'N-Nitrosamine impurities: Introduction to Risks and Challenges'. N-Nitrosamines are found in trace amounts in food, water and air. The mutagenicity and carcinogenicity of N-Nitrosamines is dependent on their capacity to form DNA reactive intermediates through metabolism and is structure and size dependant, e.g. smaller alkyl N-nitrosamines NDMA and NDEA are potent mutagenic carcinogens. The discovery of NDMA in the pharmaceutical Valsartan in the USA led to current regulatory guidance including the requirement for an assessment of the risk of N-Nitrosamine impurity formation, including from large complex 'drug-like' nitrosamines which may have lower capacity to form mutagenic intermediates. N-Nitrosamine impurities are currently controlled to acceptable levels based on carcinogenicity data or a justified similar structure – this is generally available for small nitrosamines and it is difficult to achieve for large complex N-Nitrosamine impurities. In addition, interim limits of 18 ng/day set for N-Nitrosamine impurities, where read across is not possible, are analytically challenging. Ames testing is included in ICHM7 but there are questions as to optimisation of the assay for the detection of mutagenic N-Nitrosamines. Additional mutagenicity tests are also being evaluated.

David Ponting (Lhasa) presented 'Structure-activity relationship for nitrosamine mutagenicity and carcinogenicity'. The available dataset for nitrosamine mutagenicity and carcinogenicity is relatively small but sufficient for the development of structure-activity-relationships (SAR) for nitrosamine subclasses. Several different categorisation methods have been developed which can be used separately or in combination to assess potency of nitrosamines, which vary over a large range. For those considered to be of concern, SAR classes can be used to determine higher (nitrosodiethylamine) and lower (nitrosopiperazine) levels of concern. This can help in prioritising risk assessment, supporting in silico assessments of mutagenicity and Ames test results and the assignment of acceptable intake limits by supporting read-across or utilisation of a subclass-based limit.

Rachel Tennant (Lhasa) presented 'Can the Ames test adequately predict the carcinogenic potential of N-nitrosamines? Mutagenicity data is a core component of the safety assessment data required by regulatory agencies for the assessment of drug compounds and impurities. OECD-471 bacterial reverse mutation (Ames) assay is most widely used as a primary screen for mutagenic risk. It may not be sensitive enough to detect the mutagenic potential of N-nitrosamines to accurately predict a risk of carcinogenicity. Evaluation was carried out using public Ames and rodent carcinogenicity data relating to the N-nitrosamine class of compounds. The predictive performance of the OECD 471-compliant Ames test was impacted by the introduction of variations in the assay including type of metabolic activation, solvent type and pre-incubation/plate incorporation methods. When the guideline is followed the Ames test is highly sensitive.

George Johnson (Swansea University) presented 'Quantitative analysis of in vivo mutagenicity dose-response data for risk assessment and regulatory decision-making: A case-study of Nitrosamines'. There is a need for the refinement of specific critical effect sizes for genetic toxicity data along with guidance on the use of uncertainty factors to calculate PDE from these data, in addition to the justification for using the PDE approach.

Discussion

Q: Do we need to worry about the levels of nitrosamines added in vivo as a consequence of taking a drug when there are larger sources of nitrosamines in the diet.

A: It is a requirement to keep levels of nitrosamines/impurities in drugs to a minimum to make sure the drugs are as safe as possible, even though endogenous levels will be higher – the endogenous levels and level of exposure are taken into account when setting the guidance value for drugs.

Q: The induction process in S9 and its route of administration seem to have an effect?

A: This is something industry is looking into currently.

Q: Can we believe a negative Ames test? Why not look at the whole test battery ?

A: SAR also being used for identifying risk but is a work in progress. The length of time taken to do the whole test battery may be an issue and also there may be questions around the solubility of the test chemicals that need to be answered.

Q: How are nitrosamines transported into the cell and can that effect testing – is it the same for in vitro and in vivo?

A: It is possible to visualise using drug uptake receptors.

Karen Philip (Gentronix) presented 'Establishing lab proficiency of OECD 488 big blue transgenic rodent somatic and germ cell mutation assay'. Assessment of new drugs and chemicals for their mutagenic potential and risk to human health and the environment is a regulatory requirement. The OECD 488 transgenic rodent mutation (TGR) assays play a key role in determining in vivo mutagenicity risk of substances that have a positive in vitro mutagenicity data. A proficiency exercise was carried out for detection of mutation frequency at the cll locus in liver, duodenum and male germ cells of Big Blue rats. Frozen tissues from previously conducted Big Blue rat studies where a 28 day exposure was followed by either 3 or 28 day (somatic tissues) or 28 day (germ cells) fixation period for untreated and N-Ethyl-N-nitrosourea (ENU) treated as a positive control. ENU treatment produced a statistically significant increase in mutation frequencies over control for all tissues tested; fold increases were 7, 8 and 16 in liver, germ cell and duodenum respectively. The data generated by Gentronix was within 95% control limits of historical data showing proficiency with the methodology. Noted that there is a need to build up historical control data for different rat tissues, e.g., bone marrow. Throughput is an issue with 10-12 drugs per year.

James Whitwell (Labcorp) presented 'Comparison of lowest positive concentrations for inducers of MN in vivo with in vitro positive concentrations (IVIVC). There is a paucity of data to demonstrate whether genotoxicity substances are detected at lower concentrations in cell culture in vitro than can be reached in the blood of animals treated in vivo. The lowest concentration required for induction of chromosomal damage in vitro (lowest observed effective concentration, or LOEC) was compared with the lowest dose required for biologically relevant induction of micronuclei in vivo \lowest observed effective dose, or LOED) for 83 substances. Of these, 39.8% were positive in vivo at blood concentrations lower than the LOEC in vitro, 22.9% were positive at similar concentrations and 37.3% of substances were positive in vivo at higher concentrations. The scatter of clastogens and aneugens across the 3 categories was similar. The speaker concluded that the ability to detect induction of micronuclei in bone marrow in vivo is not soley dependent on the concentration of test substance that induced chromosomal damage in vitro. Noted that there is concern over the direct application of IVIVC models to in vitro concentration response for the lowest hazard.

Carol Beavers (XXXX) presented 'A weight of evidence review of the genotoxicity of Titanium Dioxide'. TiO2 is a ubiquitous white colourant found in a wide range of products that has been considered no longer safe for use in foods (nano and microparticles of E171) by the European Foods Safety Authority (EFSA) due to concerns over genotoxicity. An independent panel of experts have developed a weight of evidence assessment of the genotoxicity of titanium dioxide- based o the available data (identified from many sources). Expertise included genetic toxicology, general toxicology, bioavailability, carcinogenicity and nanoparticle characterisation. A total of 192 datasets for endpoints and test systems considered the most relevant for identifying mutagenic and carcinogenic potential were reviewed and discussed for both reliability and relevance (by weight of evidence) and in the context of whether the physicochemical properties of the particles had been properly characterised. Only 34 met the criteria, of which 10 were positive and all from studies of DNA strand breakage (comet assay) or chromosome damage (micronucleus or chromosome aberration assays). All positive findings were connected with high cytotoxicity, oxidative stress, inflammation, apoptosis, necrosis or combinations of these and the speaker considered that as the DNA and chromosome breakage can be secondary to physiological stress it is highly likely that the observed genotoxic effects of titanium dioxide, including those with nanoparticles, are secondary to physiological stress. Consistent with this finding there were no positive results from a limited number of gene mutation studies in vitro and in vivo although it was concluded that data from more robust in vivo gene mutation studies would be useful in reaching firmer conclusions. It was noted that 10% of papers meeting the relevance and reliability criteria is a low number but that highlights the importance of having the correct expert reviewers for genotoxicity data in journal papers which isn't always being achieved. Reasons for papers 'failing' were varied but included a lack of positive control or consideration of historical data etc. Publishing of poorly conducted studies which show positive findings may have led to regulatory decisions being based on data that don't apply the correct regulatory guidelines, e.g. nano characterisation. Negative data is hard to publish.

Session 3 – New Technologies

Jill Kucab (King's College, London) presented 'Using NanoSeq to examine mutational signatures of chemotherapeutics and carcinogens in human tissue-derived organoids'. Distinct mutational signatures associated with exposure to specific environmental carcinogens (e.g. tobacco smoke) and chemotherapeutics (e.g. temozolomide, TMZ) can be detected in the DNA of human tumours and normal tissues using next-generation sequencing. In order to better understand the mutations observed in people the speaker aims to characterise signatures induced by mutagenic exposures experimentally. Currently this is done using normal human tissue-derived organoids along with a genome-wide duplex sequencing technology (NanoSeq) to examine mutations caused by a panel of environmental and chemotherapeutic agents. NanoSeq enables highly sensitive error-free detection of subclonal mutations, and using organoids derived from stomach, colon, kidney and pancreas all treated with several carcinogens (aristolochic acid, benzo(a)pyrene, aflatoxin B1 and 2-amino-1-methyl-6-phenyl7imadazo[4-5-b]pyridine) the speaker found that all models accumulated mutations as detected by NanoSeq. The speaker re[ported carcinogen-specific mutational signatures consistent with those previously identified by conventional whole-genome sequencing. In gastrin organoids treated with 30 chemotherapeutics the speaker identified a single base substitution signature for TMZ that matches a signature observed in human tumours (COSMICSBS11) as well as SBS signatures for mitomycin C and nitrogen mustard alkylating agents (e.g. chlorambucil). Methodological questions only following the presentation.

Marc Audebert (INRAE, France) presented 'Use of the H2AX/pH3 genotoxicity assay in combination with high throughput toxicokinetics models to provide human dose context in chemical risk assessment. The development of in vitro genotoxicity assays as an alternative to animal experimentation is of growing interest however the extrapolation of toxicity data between in vitro and in vivo systems is hampered by differences in the biotransformation of chemicals. The speaker described a newly developed and validated genotoxicity assay based on histones H2AX and H3 quantification. The novel methos is considered to low effective discrimination between aneugens, clastogens and cytotoxic compounds in all cellular models. The assay together with the use of human cell lines with different bioactivation capacities permit differentiation between direct genotoxins and bioactivated ones. Human dose context was provided by applying data from the H2AX/pH3 genotoxicity assay to high throughput toxicokinetics (HTTK) models. It was noted that the test could be improved using 3D tissue models but H2AX/pH3 is considered the most sensitive genotoxicity biomarker to date. An OECD guideline application will be made as regulators won't accept the data without guideline compliance, but it was acknowledged that this is likely to be a lengthy process.

Fiona Chapman (Imperial Brands) presented 'The in vitro ToxTracker and Aneugen Clastogen extension assay as a tool in the assessment of relative genotoxic potential of e-liquids and their aerosols'. *In vitro* (geno)toxicity assessment of electronic vapour products (EVPs), relative to conventional cigarette, currently uses assays, including the micronucleus and Ames tests. Whilst informative on induction of a finite endpoint and relative risk posed by test articles, such assays

could benefit from mechanistic supplementation. The ToxTracker and Aneugen Clastogen Evaluation analysis can indicate the activation of reporters associated with (geno)toxicity, including DNA damage, oxidative stress, the p53-related stress response and protein damage. Here, we tested for the different effects of a selection of neat e-liquids, EVP aerosols and Kentucky reference 1R6F cigarette smoke samples in the ToxTracker assay. The assay was initially validated to assess whether a mixture of e-liquid base components, propylene glycol (PG) and vegetable glycerine (VG) had interfering effects within the system. This was achieved by spiking three positive controls into the system with neat PG/VG or phosphate-buffered saline bubbled (bPBS) PG/VG aerosol (nicotine and flavour free). PG/VG did not greatly affect responses induced by the compounds. Next, when compared to cigarette smoke samples, neat e-liquids and bPBS aerosols (tobacco flavour; 1.6% freebase nicotine, 1.6% nicotine salt or 0% nicotine) exhibited reduced and less complex responses. Tested up to a 10% concentration, EVP aerosol bPBS did not induce any ToxTracker reporters. Neat e-liquids, tested up to 1%, induced oxidative stress reporters, thought to be due to their effects on osmolarity *in vitro*. E-liquid nicotine content did not affect responses induced. Additionally, spiking nicotine alone only induced an oxidative stress response at a supraphysiological level. In conclusion, the ToxTracker assay is a quick, informative screen for genotoxic potential and mechanisms of a variety of (compositionally complex) samples, derived from cigarettes and EVPs. This assay has the potential for future application in the assessment battery for next-generation (smoking alternative) products, including EVPs. Methodological questions only following the presentation.

Amy Wilson (AstraZeneca) presented 'High Content, High Throughput, Image Based Genotoxicity Screen: Micronucleus Assessment and Beyond. The speaker described the development and validation of an automated high content, high throughput, multiparametric image assay with machine learning, based on the in vitro Micronucleus assay. The assay detects micronuclei, cytotoxicity and cell-cycle profiles from Hoechst staining and the mechanism of action information is determined by kinetochore labelling in micronuclei (aneugenicity) and gH2AX foci analysis (DNA damage). Applying computational approaches in R and implementing machine learning models alongside Bayesian classifiers allows the identification of, with 95% accuracy, aneugenic, clastogenic and negative compounds, reducing analysis time by 80% whilst minimising human bias. Over 1000 compounds have been assessed in the screen, which shows good concordance with the regulatory assay, significantly reducing the number of unexpected IVM positive responses during regulatory testing. Additional mechanistic understanding of genotoxic responses can be determined by the inclusion of additional endpoints, e.g. p53 and 53BP1, and markers targeted to phosphorylated Histone-H3, β-Tubulin and Aurora-B, as well as by the inclusion of metabolic activation for the assessment of reactive metabolites. The screen has identified chemical clusters associated with genotoxicity and combining screening data with hierarchical clustering of compound biding affinities allows the identification of specific sub-classes of epigenetic modulators that contribute to genotoxicity. The endpoints utilised in the screen have also been applied to multiple novel assays; including CRISPR screens, to identify novel genes

associated with micronucleus induction. Methodological questions only following the presentation.

Gillian Conway (Swansea University) presented 'Nanomaterial Exposure Induces Expressional Alternations in 3D HepG2 Liver Spheroids'. Exposure to engineered nanomaterials (ENM) is considered inevitable, therefore the development of robust, predictive *in vitro* hazard testing systems is essential. This study aimed to develop a panel of biomarkers to detect key events (Kes) that are indicative of liver carcinogenesis to better support predictive toxicology. The speaker reported that their data demonstrates that short-term exposure to Ag, TiO_2 and CB results in a large number of transcriptional alterations that may be important in driving hepatocellular carcinoma. Interestingly, the genes highlighted at 6hrs (*CDKN1A, RXRA,* and *IGFBP3*) have all previously been linked to both liver fibrosis and inflammation which can be precursors to liver carcinogenesis. In addition, changes in expression observed at longer-term exposures (*RXRA, TGFBR2, GJB1, CDKN1A*) could be more relevant to liver carcinogenesis. The study enabled the development of the understanding of biological alterations induced by ENM exposures while also demonstrating the potential for the identification of new biomarkers that could predict pathological outcomes. Methodological questions only following the presentation.

Katherine Chapman (Swansea University) presented 'Investigating the impact of high glucose and carcinogen co-exposure on mitochondrial genotype and phenotype in human lymphoblastoid cell line'. People with diabetes are at increased risk of developing cancer compared to the general population. A common symptom of untreated diabetes and prediabetes is high blood glucose levels, or hyperglycaemia which is associated with increased oxidative stress, possibly contributing directly to mitochondrial damage and genetic mutation. The speaker outlined a study which aimed to determine whether mitochondrial toxicity endpoints could distinguish hyperglycaemia and carcinogen (acetaldehyde, bis(2-ethylhexyl) phthalate, hydrogen peroxide and 1-nitropyrene) co-exposure from carcinogen-only exposure in human lymphoblastoid cell lines TK6 and MCL5. When combined with high glucose, all carcinogens elicited statistically significant ($p<0.05$) effects on mitochondrial activity. Fluorescence microscopy indicated statistically significant reductions in mitochondrial activity for 1-nitropyrene and high glucose (30 mM). For genotypic changes, mitochondrial variant allele frequency was significantly altered. The speaker concluded that the results suggest that hyperglycaemia combined with carcinogens can increase mitochondrial damage when compared to elevated glucose or carcinogen treatments individually. Methodological questions only following the presentation.

Awadhesh Jha (University of Plymouth) presented 'Evolving concepts in genetic ecotoxicology or eco-genotoxicology: a stride through linking human and environmental health' (Jim Parry Award Lecture). Quality of human life is dependent on the 'health' of the environment, which also includes natural species with whom we share this planet. These species also serve as sentinels or surrogates to assess toxic potentials of environmental stressors, including increasing range and amount of anthropogenic chemicals discharged or present in the environment with

diverse mode of actions. In this context, historically, a large number of studies using aquatic organisms have provided a wealth of information for fundamental understanding of life processes and for basic biomedical research bearing in mind that we share highly conserved genetic information and, qualitative induction of genetic damage in humans and natural biota are analogous. On the other hand, it is also being recognised that compared to human health arena only limited progress has been made in defining the significance of exposure to genotoxins and other stressors on natural species with fundamental gaps in our knowledge on the long-term implications of exposures of these toxic agents on the natural species. To address these broader issues, over the years, attempts were made to develop a range of sub-lethal biological or biomarkers responses at different levels of biological organisation in ecologically relevant aquatic species. These required adoption of interdisciplinary approaches linking 'toxicokinetics' with 'toxicodynamics' processes to determine the bioavailability, body burden of chemicals and using appropriate statistical and modelling approaches in order to elucidate relative sensitivity of different biological responses following exposure to a range of priority and emerging contaminants. The synthesized information provided indication of the health status of the organisms. The speaker suggested that adoption of such an approach could go some way towards embracing preventive measures for the protection of human health and environmental sustainability. No questions following the presentation.

Session 4 – Cancer / disease prevention

Phil Quirke (Yorkshire Cancer Research) presented 'The microbiome and colorectal cancer'. Humans are holobionts, i.e. they are composed of their own cells and also host a complex collection of bacteria, yeasts and viruses that live on and within them forming a very close and intricate relationship. These provide mucosal protection, generate chemicals beneficial to the host and in return are afforded nutrients and shelter. These ecosystems are unique to an individual and are affected by breast feeding, diet, exercise, geography, ageing, disease and drugs. It varies from mouth to anus and also whether it is adjacent to the mucosa and is applied to the mucus layer or in the lumen of the gut. The microbiome has been shown to be associated with many diseases, including as a major contributor to CRC. It can be studied in many ways, but currently next generation sequencing methods lie at the centre of analyses either through 16S studies or metagenomics. Geographical variation, stool weight, faecal bacterial composition have all been associated with CRC but now we have evidence of associated oncomicrobes, the importance of bacterial toxins in carcinogenesis and inflammation and direct evidence of mutagenesis by colibactin a protein produced by the *pks* operon found in some E. *coli*. This has been shown to cause mutations in an area of the adenomatous polyposis coli gene. Around the world there is a difference in levels of pks on E.coli and so it is possible that there may be a role of initiation of pks on E.coli in the Western diet in CRC. Promotional events are likely to also be critical as the same mutation number may or may not lead to CRC development. This could be a key intervention target. PKS on E.coli is common in the population and there is an interest in a possible link with early onset CRC which is becoming more common. The most likely place for an environmental mutagen to cause damage is at the mucosa.

Joseph Rothwell (Inserm) presented 'Association of circulating amino acids with colorectal cancer risk in the EPIC and UK Biobank cohorts'. Amino acid metabolism is dysregulated in colorectal cancer patients; however, it is not clear whether pre-diagnostic levels of amino acids are associated with subsequent risk of colorectal cancer. The speaker evaluated a potential association of pre-diagnostic amino acid concentrations with colorectal cancer risk, in the European Prospective Investigation into Cancer and Nutrition (EPIC) and UK Biobank cohorts. Higher levels of glutamine and histidine were shown to be associated with reduced risk of colorectal cancer in the two large prospective cohorts. As these associations were present several years before diagnosis the speaker concluded that amino acid dysregulation may reflect perturbed cancer-promoting metabolism or sub-clinical early-stage neoplasia. It was discussed whether the metabolically active microbiome may affect the amino acid levels of plasma – for example histidine decarboxylase is present in the gut. This is still under investigation.

Richard Beatson (Kings College London) presented 'Aberrantly glycosylated MUC1; the perfect cancer target?'.
The common upregulation of aberrantly glycosylated MUC1 in multiple carcinomas provides a specific and prevalent cancer target. However, over the past 30 years, multiple preventative and therapeutic approaches have failed; why is this, and what new strategies does the community have? The last 5-10 years have given us greater biological insight into the role of aberrant glycans, carried by MUC1 and other scaffold proteins, in shaping the tumour microenvironment through the engagement of lectins carried by immune cells. This greater depth of understanding, allied to improved and novel technologies, has enabled us to revisit the idea of MUC1 and glycan targeting using different or improved approaches. Methodological questions only following the presentation.

Robert Hillary (University of Edinburgh) presented 'Epigenomic prediction of common disease states and their risk factors'. Blood proteins can serve as important biomarkers for many common diseases. Blood protein levels are closely related to DNA methylation, an epigenetic mechanism that integrates genetic and environmental risk factors. Inter-individual variation in DNA methylation can be harnessed to generate predictors or surrogates of protein biomarker levels. DNAm-based surrogates may show more stable longitudinal trajectories than the protein itself and help to disentangle whether associations between biomarkers and disease represent cause or consequence. The speaker performed epigenome-wide association studies on blood levels of over 400 proteins, revealing 47 novel loci whereby methylation correlates with protein levels. In addition, these data were applied to causal analysis methods to identify causal pathways linking methylation, protein and disease. Associations were observed between 137 DNAm-based surrogates and 11 common disease states in ≤9,537 individuals from Generation Scotland. One measure of biological ageing (DNAm GrimAge), which incorporates DNAm-based surrogates of seven blood proteins, was also associated with brain health and complex diseases in the Lothian Birth Cohorts and Generation Scotland. The speaker concluded that these data show that DNAm-based signatures relating to proteins hold promise in predicting disease onset and inform us about biological

pathways involving blood proteins and common disease. Methodological questions only following the presentation.

Robert Bedford (Labcorp) presented 'A mechanistic insight into the lung-derived inflammatory response following in vitro exposure to cigarette smoke and next-generation nicotine delivery products'. *In vitro* models are able to demonstrate similar responses to those observed following aerosol exposure *in vivo*. Despite this, their application in understanding downstream effects of airway toxicity, such as macrophage recruitment, are at an early stage. The speaker carried out a study in which organotypic lung tissues (MucilAir™) were exposed to three aerosols known to induce different levels of toxicity. The ability of MucilAir™ recovery media to induce downstream inflammatory events was subsequently investigated with tissues exposed to cigarette smoke and heated tobacco product (HTP) inducing THP-1 polarisation – a marker of inflammation. In contrast, exposure to electronic nicotine delivery system (ENDS) aerosol did not induce this response. To understand the molecular initiating events driving this inflammatory response, cytokine, histological and RNA analysis of MucilAir™ tissues was performed. Increased levels of biomarkers linked to immune cell differentiation via the NLRP3 inflammasome pathway, including IL-1β, cleaved caspase-3 and cytochrome P450 enzymes was observed. The speaker suggest that as similar observations have previously been made in human airway inflammation the exposure platform could act as a representative model for studying such events *in vitro* to test the inflammatory risk posed by inhaled compounds.