

## Launch of the ComNet (comet network) project on the comet assay in human population studies during the International Comet Assay Workshop meeting in Kusadasi, Turkey (September 13–16, 2011)

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### Background

The alkaline comet assay, or single cell gel electrophoresis (1), is widely used in molecular epidemiology to assess effects of occupational/environmental exposure, to test the effectiveness of dietary modulation in counteracting DNA damage and to look at, for example, changes in background levels of DNA damage with age or disease (2,3). A less common application is the study of individual differences in the capacity to repair various kinds of DNA damage (4). Because of the variation in protocols, it is very difficult to compare results obtained in different laboratories, especially since we do not yet have a full understanding of which factors are critical in determining the results of a comet assay experiment. The incomplete information about these factors, together with the uncertainty about links between the results of the comet assay and the risk of disease in healthy subjects, are major gaps in our knowledge, limiting the validity of this assay and its applicability in human studies.

In an attempt to resolve these important outstanding issues, we are setting up a network of researchers using the comet assay in human biomonitoring, to promote collaboration between

scientists working in this field and to make full use of existing molecular epidemiological studies. The ultimate aim of the network, ComNet, is to establish the comet assay as a reliable trusted biomarker assay. The model for this exercise comes from large international collaborative studies, such as the HUMN (The International Collaborative Project on Micronucleus Frequency in Human Populations) project ([www.HUMN.org](http://www.HUMN.org)) which, in 10 years after its founding in 1997, achieved its stated aims of establishing a database of baseline micronucleus (MN) frequencies from human population studies, defining the methodological, demographic, genetic and exposure variables that determine MN frequency, publicising standardised protocols and establishing, through prospective studies, that MN frequency measured in peripheral blood lymphocytes is a predictive marker of cancer risk (5). Another example is the European Study Group on Cytogenetic Biomarkers and Health project, which contributed critical evidence linking the frequency of chromosomal aberration in healthy subjects to the risk of cancer (6).

To facilitate contact among interested laboratories, a website ([www.comnetproject.org](http://www.comnetproject.org)) has been created to explain the aims of ComNet, to receive comments, and especially to allow laboratories to register as participants in the project. The website will eventually be used for input into the database.

The idea of the project, proposed during the previous International Comet Assay Workshop meeting held in Perugia in the 2009, was developed by Andrew Collins (Oslo, Norway), Stefano Bonassi (Rome, Italy) and Maria Dusinska (Kjeller, Norway), who defined the basic strategy and invited a larger group of key persons in the field to join in coordinating the project. The help of Erdem Coskun (Ankara, Turkey) was critical in setting up the website and in selecting the long list of laboratories which have published data using the comet assay in human populations.

This founding group was enlarged to form a steering committee, with Diana Anderson (Bradford, UK), Alok Dhawan (Lucknow, India), Gudrun Koppen (Mol, Belgium), Marcin Kruszewski (Warsaw, Poland), Massimo Moretti (Perugia, Italy), Emilio Rojas (Mexico City, Mexico), Günter Speit (Ulm, Germany), Semra Sardas (Istanbul, Turkey), Peter Møller (Copenhagen, Denmark), Omar Garcia (Havana, Cuba), Iris Benzie (Hong Kong, China) and Vera Garaj-Vrhovac (Zagreb, Croatia). The composition of the steering committee may be further modified to include key persons and leading groups in the field.

The first meeting of the steering committee was held in Kusadasi, Turkey, during the 9th International Comet Assay Workshop, September 13–16, 2011. Here follows a report of the main issues discussed during the meeting, including the project's objectives and strategy. Present at the meeting were Andrew Collins (Chair), Stefano Bonassi, Diana Anderson, Erdem Coskun, Alok Dhawan, Gudrun Koppen, Marcin Kruszewski, Massimo Moretti, Emilio Rojas, Günter Speit and Mahara Valverde.

## Aims and priorities of ComNet

The main priorities of the ComNet project are

- to establish the comet assay as a reliable biomonitoring tool for human studies and
- to define the relevance of DNA damage (as measured with the comet assay) for human health and disease.

The practical objectives are

- (i) by means of a pooled analysis, to establish basal levels of DNA damage and to look for correlations of comet assay measurements with sex, age, smoking, nutritional/lifestyle factors and with other biomarkers, especially biomarkers causally associated with human health (such as oxidative stress and MN frequency);
- (ii) to determine the experimental factors affecting the performance of the assay and therefore its reliability and reproducibility and to encourage the use of standard protocols to facilitate the comparison of results from different studies and
- (iii) to create a pooled cohort of subjects screened with the comet assay to establish the links, if any, between the results of the assay and human health and disease.

## Strategy

- (i) As a first step, a database of all identifiable human population studies employing the comet assay will be assembled. As a start, emails were sent to all researchers known to us who have been involved in relevant projects, with a request to register on the website as a member of ComNet. To date, 100 partners from 31 countries have registered. Recruiting is continuing. We are keen to extend the network as widely as possible, and any readers who are or have been involved in human biomonitoring with the comet assay are encouraged to join, by visiting the website, [www.comnetproject.org](http://www.comnetproject.org).
- (ii) Information will be collected on the studies carried out by ComNet members; aim of study, design of study, types of samples analysed, assays used, numbers of subjects, selection criteria and other types of information collected (relating to anthropometry, nutrition, health, genetics, lifestyle and occupational exposure).
- (iii) Published (and possibly unpublished) data from these studies will then be compiled into a database in order to carry out pooled analyses to establish background levels of strand breaks and oxidised DNA bases, differences between the sexes, effect of age, smoking, etc. and correlations with other biomarkers.
- (iv) A set of guidelines for using the comet assay as a biomonitoring tool will be prepared, to ensure that in future, the comparison of different studies will be easier and more reliable. We will test different methods of sample preparation and storage, looking at cell types other than white blood cells as sample material.
- (v) Validation trials will be carried out, analysing identical samples in different laboratories.
- (vi) A prospective (cohort) study will be planned, from the ComNet database, including a cohort of healthy individuals from biomonitoring studies, with differing but known

levels of exposure to DNA damaging agents or to protective factors. They will be followed over a period of years to establish whether the extent of DNA damage or DNA repair capacity measured by the comet assay can predict the risk of cancer and other diseases in these subjects.

## Funding

At present, there is no core funding for ComNet, and we rely on self-financing of members. Substantial financial support will be essential if we are to secure our objectives.

## Future meetings

A half-day meeting of ComNet is planned as a satellite of the EEMS meeting in Warsaw in September 2012 ([www.eemseu.org](http://www.eemseu.org)).

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Conflict of interest statement: None declared.

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