International Agency for Research on Cancer

World Health Organization

Scientific Council **SC/46/10**
Forty-sixth Session 15/12/2009

Lyon, 27–29 January 2010
Princess Takamatsu Hall

PURCHASE OF SCIENTIFIC EQUIPMENT

Introduction

1. As described in IARC Medium-Term Strategy 2010–2014, one of the priorities of the Agency is to perform interdisciplinary research, pioneering the integration of laboratory sciences and epidemiology.

2. Indeed such is the contribution now of laboratory-based techniques to epidemiology (in analysis of exposure, genetic susceptibility, diagnosis and early detection as well as establishing biological plausibility of exposure-disease associations) that these areas can no longer be considered as separate parts of the Agency's activities.

3. The above strategy requires high quality laboratory facilities, in turn implying a constant upgrade and update of the scientific equipment at IARC. Investment in recent years has been lacking and the correction of this situation is not possible through the regular budget alone. Obtaining funding for major items of equipment through competitive grant applications is also difficult. Therefore the Director would like to approach the Governing Council to consider investment from the Governing Council Special Fund for major items of scientific equipment at its 52nd session in May 2010. No such request was made by the new Director in 2009, given that the Medium-Term Strategy had not yet been established. This approach is first submitted to the Scientific Council for its consideration.

4. In 2009 the Director was able to invest in a fluorescent microscope and a flow cytometer financed using savings from the Director's regular budget allocation due to changes in priority. However, the available funds were not sufficient to cover the purchase of additional instruments that are required to accomplish IARC research objectives in line with the Medium-Term Strategy. These requirements are outlined below. In this context the Agency plans to develop biomarker research, including metabolomics, in conjunction with the recruitment of new scientific staff both in the Biomarkers Group in the Section of Nutrition and Metabolism (NME) and in Mechanisms of Carcinogenesis (MCA).

5. The Scientific Council is requested to advise the Director and the Governing Council on the proposed request to use funds from the Governing Council Special Fund to purchase the scientific equipment listed below:

1. Next-generation DNA sequencing instrument;
2. Gas chromatograph;
3. HPLC/MS/MS.

Next-Generation DNA sequencing instrument

6. The Agency currently has two sequencing machines. The first, based in GCS, is a Spectrumedix 96-capillary sequencer purchased in 2004 to support mutation screening activities. Whilst it is used for several research projects it has a limited throughput. In addition, the platform does not offer additional applications, e.g. transcriptomes. The second, based in MCA, is an ABI 16-capillary sequencer purchased in 2001 and is only really adapted to sequencing relatively small DNA fragments.

7. Next-generation DNA sequencing has the potential to dramatically accelerate biological and biomedical research, by enabling the comprehensive analysis of genomes, transcriptomes and interactomes in an inexpensive, routine and widespread manner. These analyses are key requirements in the multi-centre international studies that the Agency plans in the coming Medium-Term Strategy, with examples outlined below.

8. Not only will the next generation sequencing technologies lower the cost of DNA sequencing beyond what is possible with standard dye-terminator methods (Sanger sequencing method), it will also provide the high throughput required to enable large-scale epidemiological studies. Over the past three years, “massively parallel” DNA sequencing platforms have become widely available, reducing the cost of DNA sequencing by over two orders of magnitude. These new technologies are rapidly evolving, and short-term challenges include the development of robust protocols for generating sequencing libraries, building effective new approaches to data-analysis, and frequently a rethinking of study design in an interdisciplinary approach.

9. The next-generation sequencing instruments offer several specific areas of application ranging from whole genome sequencing, targeted re-sequencing of specific genes, detection of somatic mutations through to transcriptome analysis, miRNA discovery and methylation profiling. In addition, this platform is also used for studies of protein/DNA interaction by chromatin immunoprecipitation (ChIP-seq) and DNA sequencing. This enables the next phase of work to elucidate the functional significance of genetic polymorphisms identified in genome-wide association studies. Many studies have clearly demonstrated the high performance of ChIP-seq, which is increasingly replacing the ChIP-on-ChIP method. Thus the technology permits the integration of the Agency’s studies on mechanisms of carcinogenesis and identification of cancer risk factors in epidemiological studies drawing on biological specimens e.g. in the IARC Biobank.

10. A large number of IARC laboratory and epidemiology groups are performing or planning studies that require large scale DNA sequencing.

11. These programmes comprise:

(i) Whole genome sequencing approach of DNA from germ line cells as well as kidney, brain, colorectal and lung cancers in large case-control studies in high-risk populations, e.g. Central Europe for lung cancer and Asia/Latin America for head and neck cancers (GEN/GEP, QAS, MPA);

(ii) Targeted sequencing of melanoma (GCS) and tobacco-related (GCS/GEP) susceptibility genes in large-scale series;

(iii) Large-scale mutation analyses of cellular oncogenes and tumour suppressor genes (e.g. TP53, KRAS, CCNB1, LKB1, PTEN, CDKN2A, EGFR and HER2) in liver, breast, oesophageal, lung and brain tumours as well as Li-Fraumeni and related syndromes (MOC, MPA). Several of these studies will also analyse the DNA mutations in relation to histological diagnosis, risk factors (e.g. geographical areas) and therapeutic response;

(iv) Large-scale methylation and microRNA profile studies in a broad spectrum of cancers (EGE in collaboration with MCA, LCA, DEX, ICB, NME), e.g. (i) liver cancer in respect to HBV status and/or alcohol consumption, (ii) lung cancer and smoking habits, (iii) head and neck cancer and therapeutic response. In addition, other studies aim to assess the impact of diet and lifestyle on epigenetic changes and cancer susceptibility during childhood and adulthood;

(v) DNA sequencing of infectious agent genomes, e.g. *Helicobacter pylori,* HBV and HPV from healthy and cancer patients (ICB, ICE, MOC). Several multi-centric studies have been initiated to evaluate the potential role of genotypes and natural variants (or polymorphisms) in the carcinogenesis process.

12. As a result of the interdisciplinary approach taken and the need for the availability of this technology to permit a number of aspects of the Medium-Term Strategy to proceed, the establishment of this DNA sequencing platform is considered of high priority.

13. Some of the above mentioned research programmes have been already supported by competitive extra-budgetary funds, e.g. kidney cancer sequencing project (GEN) supported by EU-FP7. This situation will considerably facilitate the establishment and running of this platform at IARC.

14. Currently, there are three commercially available next-generation sequencing platforms:

- The 454 sequencer (Roche Applied Science; Basel, Switzerland);
- The Genome Analyzer (Illumina; San Diego, CA, USA);
- The SOLiD platform (Applied Biosystems; Foster City, CA, USA).

15. Roche and ABI have visited IARC and presented their DNA sequencing platforms whilst presentation of the Illumina Sequencer is planned. The selection of platform would be made following consideration by the Laboratory Steering Committee and the usual tendering practices of the Agency for items over 70 000 US$.

16. Due to (i) the large number of IARC research programmes, (ii) the key role of IARC in several multi-centric studies and (iii) the high versatility of this instrument, the establishment of this novel internal facility will be highly beneficial in terms of coordination, feasibility, rapidity and cost effectiveness of the different studies. In addition, generation of this common platform will not only positively impact on the individual scientific projects, but will also facilitate the establishment of new internal collaborations.

Chromatography and biomarkers

17. The Agency will place emphasis on the translation of knowledge on mechanisms of carcinogenesis into biomarkers which can be applied to epidemiological studies. A part of this strategy includes the recruitment of a senior scientist to lead a group on Biomarkers. The new appointee will be able to recruit a junior scientist to join the existing laboratory researchers in the NME Section and will work closely with the other laboratory groups at IARC. In order to succeed both in this recruitment and more generally in this key strategic area, significant investment is needed in chromatographic equipment adapted to the high-throughput analyses needed for epidemiological studies. Two complementary investments are required.

Gas chromatograph

18. As described in the Medium-Term Strategy in relation to NME, the scientific activities cover the measurement of several biomarkers, e.g. hormones and their metabolites, fatty acids, growth factors. The resolution of complex fatty acids mixtures in human blood samples, as biomarkers of dietary fatty acids and fatty acid metabolism, is becoming an increasingly important task in the analysis of fats for determining nutritional value and for the isolation of new compounds (e.g. industrial *trans* fatty acid isomers). The measurement of these biomarkers implies the use of a new generation of gas chromatograph systems that allows advanced blood fatty acid separation capabilities, powerful new enhancements in throughput and real-time self-monitoring instrument intelligence, which makes this machine suitable for application to large-scale epidemiological studies.

19. The gas chromatography methodology initially set up at IARC for fatty acid analysis was based on two chromatographs that are now more than 15 years old. Both gas chromatographs needed extensive repairs, but no spare parts for such instruments were available on the market, so this platform was dismantled. Therefore, the purchasing of a new instrument is urgently required.

20. The above purchase would allow the measurements of about 60 fatty acids in human blood samples, including endogenous (metabolism) and exogenous (diet) fatty acids in human blood samples. In addition, the latest generation of gas chromatographs coupled to an electron capture detector would, for example, also allow the measurement of food contaminants and environmental chemicals such as organochlorines, herbicides and pesticides in biological fluids. This investment therefore opens up potential collaborations with other epidemiologists, notably in the Section of Environment.

21. The re-establishment of this platform at IARC is considered a high priority and therefore the purchase of one gas chromatograph is requested.

HPLC/MS/MS

22. The Agency needs two distinct HPLC/MS/MS instruments to fulfill its Medium-Term Strategy. The Director requests support in 2010 for the purchase of one instrument following the rationale detailed below.

23. The first of the two research areas is ongoing and involves the quantitative analysis of small molecules best carried out with an HPLC coupled to an electrospray ionization (ESI) triple quadrupole MS/MS characterized by high sensitivity and specificity. The current instrument at the Agency was purchased a decade ago and although it is still functional it lacks the sensitivity of up-to-date triple quadrupole instruments. Ideally this instrument should be replaced with an upgraded model. Analyses include various biomarkers of exposure (adducts, metabolites, nutrients, hormones) and early biological effect (mutations).

24. The second research area is in relation to the development of metabolomics at the Agency. Evidence on the complexity of the etiology of chronic diseases, especially cancer, is accumulating, suggesting potential interactive associations between diet, lifestyle and genetics. In this context, many epidemiologic studies attempted to assess dietary intake together with genetic measures and other variables. However, given the multi-factorial complexities of dietary exposures, dietary intake assessment methods are associated with measurement errors which affect dietary estimates and may obscure disease-risk associations. Critically, traditional exposure biomarkers do not permit an understanding of the cellular and physiological effects of different diets, something important in establishing the biological plausibility of exposure-disease associations.

25. There is thus a clear need to identify new biomarkers for validation of dietary assessment and for disease risk estimation. The logical source of most dietary biomarkers is the human metabolome, i.e. the pool of metabolites detectable in people. Metabolic profiles have been shown to be useful probes of nutritional effects for many types of interventions in both experimental models and man, to deliver novel biomarkers of disease risk in large uncontrolled human population studies. Such metabolomic approaches require a distinct type of MS, notably with a time of flight (TOF) facility in order to obtain high resolution with exact mass information and hence unambiguously identify the spectrum of metabolites under analysis. The approach also requires highly specialized software.

26. Several studies (cross-sectional, as well as nested case-control studies) on metabolomics are ongoing/being planned by several groups at the Agency (DEX, LCA, MOC) with the support of a large 1H NMR spectroscopy platform based in Lyon (CRMN, Lyon). To complement this platform, and to meet the increasing demand for cancer biomarker research arising within the Agency (which foresees application to cohorts in developing countries, and mother:child cohorts), the setting up of a strong platform with high-throughput based on HPLC/MS/MS technology is required. This platform will be based in NME within the Biomarkers Group, which is currently under development.

27. The establishment of metabolomics at the Agency will depend on the recruitment of the Head of Biomarkers Group and the recruitment of a junior scientist with expertise in metabolomics and HPLC/MS/MS. Clearly it would be unwise to commit to the purchase of specific equipment for this approach in the absence of qualified scientists.

28. Therefore the Director requests that the Scientific Council consider the need for the Agency at this stage to invest in one HPLC/MS/MS instrument. If recruitment in the Biomarkers Group is successful then priority will be given to the new technology for metabolomics; the replacement of the current HPLC/MS/MS (see paragraph 23) would be deferred. However, if the recruitment process does not attract the necessary expertise in metabolomics then the purchase

of the replacement instrument would proceed in order to provide a much needed update of the existing equipment. In summary, the Director requests the flexibility to acquire the most appropriate HPLC/MS/MS instrument during 2010 based on the above rationale.

29. An indicative price for the HPLC/MS/MS and other equipment is given in the Table below. A decision as to which specific make of instrument to purchase would be made following consideration by the Laboratory Steering Committee and the usual tendering practices of the Agency for items over 70 000 US$.

Requested budget

Equipments	**Quantity**	**Approximate price (€)**
Gas chromatograph equipped with automatic on-column injectors, highly polar capillary columns and powerful gas chromatograph software (including gas generators)	1	80 000
New generation large scale DNA sequencer	1	525 000
HPLC/MS/MS	1	580 000
Total		1 185 000