European Cooperation in the field of Scientific and Technical Research - COST -

Secretariat

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MEMORANDUM OF UNDERSTANDING

Subject : Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action BM0803: A European network of the HLA diversity for histocompatibility, clinical transplantation, epidemiology and population genetics (HLA-NET)

Delegations will find attached the Memorandum of Understanding for COST Action BM0803 as approved by the COST Committee of Senior Officials (CSO) at its 171st meeting on 18-19 June 2008.

MEMORANDUM OF UNDERSTANDING

For the implementation of a European Concerted Research Action designated as

COST Action BM0803

A EUROPEAN NETWORK OF THE HLA DIVERSITY FOR HISTOCOMPATIBILITY, CLINICAL TRANSPLANTATION, EPIDEMIOLOGY AND POPULATION GENETICS (HLA-NET)

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 270/07 "Rules and Procedures for Implementing COST Actions", or in any new document amending or replacing it, the contents of which the Parties are fully aware of.
2. The main objectives of the Action are, through networking European research teams working on Human Leukocyte Antigen (HLA) molecular diversity in human populations, to standardize protocols and procedures for sampling, handling, storing and processing data and to develop a user-friendly bioinformatics platform accessible to scientists in different fields.
3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 31 million in 2007 prices.
4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.
5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter V of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

The molecular characterization of the HLA (Human Leukocyte Antigen) polymorphism in human populations represents a crucial step in several disciplines concerned by public health (histocompatibility/transplantation and epidemiology) and also constitutes a main research focus in human molecular evolution (molecular population genetics). While needing similar requirements at the different levels of their analysis (good quality of sampling, high resolution HLA typing, powerful biostatistic analyses adapted to complex HLA data, easy access to specific population databases and understandable computer tools), the investigators working in these different fields are currently limited in their interactions. HLA-NET offers an innovative framework by which those scientists will put their expertise in laboratory work, clinical work, ethical issues, population genetics, biostatistics and/or computer science into contribution to elaborate consensual standards, define common procedures and share high quality data and tools. Highly significant scientific, technological and societal benefits are expected through the Action with immediate applications in donor-recipient matching, case-control studies and population genetics research. The network will expand and disseminate its results through an electronic platform starting with more than 15 laboratories throughout Europe. The workshops supported by this Action will permit essential transfers of knowledge and expertise at the European scale and will foster collaboration throughout Europe and beyond.

Keywords: HLA, histocompatibility/transplantation, epidemiology, population genetics, European network

B. BACKGROUND

B.1 General background

Knowledge of the genetic diversity of the human species has improved considerably in recent decades thanks to the rapid progress of genomic research. The possibility of genotyping individuals at high-resolution over the entire genome has been crucial in addressing major issues related to biomedicine and molecular biosciences, such as the assessment of disease susceptibilities; the control of stem cell and organ transplantation; the appraisal of the genetic structure of human

populations and its meaning; the understanding of human genome evolution and its interaction with the environment, among other essential topics. A common goal of these studies is to estimate genetic diversity within and among human populations and to describe it through the molecular typing of population samples.

A main challenge of tissue typing (histocompatibility) laboratories involved in clinical research (donor-recipient matching) is indeed to produce molecular HLA (Human Leukocyte Antigen) data of high quality. Such laboratories address a crucial health problem in modern societies, the need for hematopoietic stem cell transplantation involving the search for HLA compatible donors. Stem cell volunteer donors are generally recruited randomly, although a few registries try to improve the recruitment among their ethnic minorities in order to increase the HLA diversity of their cell collections and hence the probability of finding an appropriate donor for a given patient. Knowledge of the distribution of alleles and haplotypes in many different population groups as determined by high resolution typing may indeed allow the design of more efficient recruitment algorithms.

But the accurate description of allelic and haplotypic HLA profiles and the identification of rare HLA variants in human populations are not only crucial to recipient-donor matching and research projects on histocompatibility. In addition, researchers in at least two other disciplines share related objectives. Firstly, as HLA genes play an essential role in susceptibility or resistance to serious human diseases, such as HIV, their meticulous molecular analysis underpins epidemiological research. Statistically reliable comparisons between case and control population samples are needed to assess the susceptibility (or resistance) conferred by specific HLA alleles. Knowledge of the prevalence of a susceptibility allele in a given population is crucial to evaluate the genetic risk provided by several different HLA alleles in autoimmunity, infectious diseases or allergic reactions to drugs.

Secondly, HLA genes are of particular interest from a population genetics point of view to study the mechanisms of molecular evolution. Different human populations exhibit different HLA genetic profiles. This is partly explained by the geographic dispersal of modern humans throughout the world, and partly by an influence of natural selection. Indeed, the evolution of HLA may be driven, as stated above, by an advantage of specific alleles, but also by an advantage conferred to heterozygous individuals against a large variety of pathogens. Knowledge of the distribution of

allele frequencies in many different populations may help to understand human peopling history and the interaction of populations with their environment in a pathogenic context.

Why a COST Action?

Launching HLA-NET - a European network of laboratories involved in the study of HLA for histocompatibility, epidemiology and/or population genetics - is highly relevant in the present research context. Despite their different goals and applications, all these laboratories are united by a common research task, the description of HLA molecular diversity in human populations, to get accurate reference material for their own studies in different disciplines, and to provide pan-European materials to research groups working internationally. Moreover, those laboratories are concerned with similar types of methodological problems raised by the complexity of the HLA polymorphism: how can a population sample for different applications be defined accurately? How gene frequencies and other statistics with highly complex data be estimated? How can data be generated that is comparable to those of other laboratories? What legal and ethical rules should be followed to harmonise with national requirements? HLA-NET is designed to answer those questions via standardization of protocols and procedures and the development of an electronic platform to collect, handle, store and process HLA data and share them among European laboratories.

Most importantly, different research groups and/or investigators possess varying levels of expertise in laboratory work, clinical work, ethical issues, population genetics, biostatistics and/or computer knowledge related to the different disciplines involved in such studies, and are therefore not always capable of addressing all their questions accurately. This justifies launching the creation of the network as a COST Action. Indeed, COST offers the appropriate framework whereby researchers of different laboratories and disciplines, while pursuing research funded by their country, are able to meet, put their own experience and know-how into contribution, and discuss the optimum solutions to solve problems linked to the study of this complex polymorphism. COST is oriented towards networking basic research and multidisciplinarity, which fit precisely the nature of activities shared by the groups involved in the Action. Moreover, COST encourages researchers mobility and scientific exchange more than other research frameworks and applies to a particularly wide spectrum of European States.

B.2 Current state of knowledge

Histocompatibility: HLA matching in allogeneic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) is a recognized therapy for a variety of hematologic, immunologic and congenital disorders. Unfortunately only 30% of patients eligible for this therapy do have a HLA-identical sibling donor. In related and unrelated HSCT, disparity for HLA antigens increases the risk of graft rejection and of graft-versus-host disease (GVHD) thereby influencing transplant outcome. Comprehensive HLA class I and II matching between donor and recipient is aimed at reducing the risk of post-transplant complications.

Population analyses on HLA-A/B/(C)/DRB1 and DR/DQ haplotypes at high-resolution level are essential in view of the observation that the availability of several potential donors in HLA-A/B/DR matched at a low-resolution level does not guarantee compatibility at high-resolution (i.e. allelic) level because of the extreme allelic diversity of HLA genes. Based on the data from the NMDP (National Marrow Donor Program) registry, a recent study has computed HLA allele and haplotype frequencies in donors from different ethnic backgrounds in the US population.

Such a measure of the overall diversity in the HLA haplotype composition of a given population, as well as discrimination between common and rare alleles in different populations is extremely useful for the design of unrelated donor search algorithms and clinical histocompatibility practice, given that sampling schemes are rigorously controlled (for sample sizes in particular) and that ethnicity is accurately defined on historical backgrounds. The huge collection of HLA data produced by European laboratories combined to the rich background of historical, anthropological and cultural knowledge existing for this continent represents an exceptional potential for the constitution of such reference data sets in Europe.

Epidemiological studies

HLA exhibits specific associations with infectious, parasitic, and autoimmune diseases. HLA markers have been extensively studied in relation to diseases such as rheumatoid arthritis or type I diabetes, and are now being uncovered in resistance to HBV or HIV infections. Such applications have become crucial for public health.

As for population genetic studies, the success of these studies depends on high-quality population data, in particular when a sample of patients has to be compared to a healthy population (case-control studies). Reliable and standard criteria have to be set to define accurate control populations for all studies concerned by a given disease (minimal sample size, typing technique used to describe the sample, origin of the individuals included in the sample, etc), because to use heterogeneous definitions compromises the comparability of the results. Moreover, the progress of epidemiological studies depends upon a reliable appraisal of the statistical power of the tests used to detect disease associations. A continuous validation of the statistical methods used is therefore essential.

Population genetic studies

The HLA molecules of the major histocompatibility complex (MHC) in humans are encoded by several highly polymorphic genes (A, B, C, DRB1, DQB1 and DPB1) that have been widely used to describe the global genetic diversity of human populations. An increasing number of population samples have been tested at DNA level for these loci within the scope of the Biostatistics/Anthropology component of successive International Histocompatibility Workshops. Joint reports on those data, including analyses at both allelic and haplotypic levels, have shown that HLA genetic differentiations among populations are relevant indicators of human population history, i.e. past human migrations and demographic events linked in great part to the dispersal of Neolithic farmers and/or the main linguistic phyla throughout the world.

The success of those studies, where population samples from different sources are compared, depends on the accuracy and continuous standardisation of the HLA typing, and on the permanent adaptation of statistical techniques and computer programs. Molecular typing regularly leads to the discovery of new mutations, and hence to the definition of an ever-increasing number of HLA alleles recorded in the public databases like IMGT/HLA (http://www.ebi.ac.uk/imgt/hla/). Depending on the typing methods used, HLA genotyping also suffers from the occurrence of allelic ambiguities, either because a part of the genetic variation is not covered by the DNA probes used in the protocols, or because the profile of reactivity of a given individual authorizes several interpretations in terms of genotypes. Until now, the problem of ambiguities has generally been “solved” by empirical procedures where rare alleles, and hence, genotypes, are eliminated in ambiguous outcomes. Such methods inevitably hamper the description of genetic variation existing in each population and the results of the statistical analyses done on those data. To avoid false or oversimplified results, the computer tools developed to handle data and estimate gene frequencies

and other statistics (like linkage disequilibrium among different loci) have to take into account the continuous updating of the data due to the definition of new alleles and ambiguities. More generally, they have to face a growing disparity between the increasing level and complexity of observable HLA genetic variation and the typically low sample sizes available for the populations tested.

Innovation of the Action

This Action proposes a framework where chief investigators with expert knowledge in different fields related to the study of HLA molecular diversity in human populations are brought together to work on the standardization of protocols and procedures needed at all stages of their analyses. In addition, the Action develops a bioinformatic platform accessible to researchers in these different fields and providing user-friendly access to standards and consensual protocols, networked population databases and computer programs to analyse complex HLA data. The Action encourages and supports bi-directional transfers of knowledge between laboratory and computer researchers, and boosts research projects with a strong multidisciplinary component.

B.3 Reasons for the Action

An increasing number of European HLA laboratories asks for collaboration with teams involved in population genetic studies in order to be able to handle more and more complex HLA data and get biostatistical support, either in clinical medicine (donor-recipient matching in transplantation) or in epidemiology (disease prevention through association studies involving genetic markers in autoimmunity and other diseases). To network those laboratories is all the more imperative because both scientific advances in biomolecular sciences and societal needs in biomedical domains related to public health are addressed. Rapid progress is expected through the promotion and coordination of inter-disciplinary education and expertise sharing (laboratory, statistical and computer work).

Immediate scientific/technological benefits:

– clinical applications: improvement in the search for donor-recipient matching in transplantation needing a precise knowledge on the repertoire of HLA alleles and haplotypes defined at high resolution in different populations and biostatistical knowledge

– epidemiological applications: easy access to reference populations, deep knowledge on how to

use statistically powerful algorithms, possibility to compare to related data according to concerted ethical rules

- molecular population genetics: characterization of the HLA human diversity at standardized resolution, access to comparable data according to concerted ethical rules, knowledge on how to used biostatistical and computer tools for complex data

- bioinformatic applications: improvement of computer algorithms adapted to HLA, needing a deep knowledge on typing methods, allelic definitions and ambiguities.

Future scientific /technological benefits:

- Significant enhancement of laboratory, biostatistical and bioinformatic expertise of European researchers involved in HLA studies, allowing them to generate results of high quality with a greater independence, save research time and get larger financial supports

- Crucial improvement of scientific knowledge on the HLA genetic diversity of local and immigrant European populations, providing reference data for population genetics, epidemiology and transplantation

- Development of competitive bioinformatic tools for the study of human molecular diversity

- Development of crucial European synergies whereby European laboratories will reach a highly competitive rank for population data analyses at an international level. Through this Action, European laboratories will take a large part to the organisation and success of future European and international histocompatibility and immunogenetics workshops by presenting a cohesive network for data handling and analysis as well as a coherent synopsis of the HLA genetic diversity in European and related countries.

Future societal/economic benefit:

- Improvement of the situation in terms of clinical transplantation: patients should benefit of a more rapid access to transplantation because of more efficient identification of a suitable matched donor in the international registry.

– Improvement of the situation in terms of autoimmune and infectious diseases: patients should benefit of a better evaluation of the susceptibility conferred by specific HLA alleles for some pathologies.

– European tissue typing companies will take advantage of the definition of consensual standards and references to update their technologies and adapt their services to current needs.

B.4 Complementarity with other research programmes

This Action does not overlap with any current or planned European research. On the contrary, it pursues highly complementary goals to those followed within other frameworks linked to the study of the human MHC, and more particularly:

– the EFI (European Federation for Immunogenetics), aiming since 1985 at promoting the development of immunogenetics in Europe, with numerous medical applications. EFI is also promoting interactions with other immunogenetics societies such as ASHI and ASEATTA. EFI has initiated a professional laboratory accreditation program that has revealed quite successful with over 200 European laboratories now accredited.

– the POSEIDON (Procedures for Organisation of Secure European Integrated DONation chain in unrelated hematopoietic stem cell transplantation: technical, organisational, legal, ethical and social aspects) project, recently proposed, whose main task is to analyse the current situation of donor registry and cord blood banking in European countries for legal, ethical, technical and economical aspects. EFI is involved in the POSEIDON project for the optimisation of technical and medical procedures in laboratories involved in bone marrow transplantation.

The focus of these organisations is different from that of the Action. Indeed, HLA-NET is completely oriented towards the characterization of human population molecular diversity and its relevance in different disciplines. On the other hand, the activities of these organisations or projects may be advantageously related to the Action, for example to coordinate decisions taken concerning legal and ethical rules in each domain of investigation.

C. OBJECTIVES AND BENEFITS

C.1 Main/primary objectives

The main objectives of the Action are, through networking European research teams working on Human Leukocyte Antigen (HLA) molecular diversity in human populations, to standardize protocols and procedures for sampling, handling, storing and processing data and to develop a user-friendly bioinformatics platform accessible to scientists in different fields.

C.2 Secondary objectives

1. Elaborate reference guide(s) for HLA population data analysis to be used by tissue typing laboratories and research groups in epidemiology and population genetics. Such guide(s) will include :

   - information about HLA typing techniques
   - information about appropriate statistical methods to analyse different datasets,
   - detailed description of consensual standards defined by the network, and including

     - criteria to constitute reliable population samples depending on the aim of each study: donors for transplantation, patients and control populations for epidemiology, ethnically-defined populations for population genetics, etc, with appropriate questionnaires;

     - standard data formats (multi-locus genotypes, DNA sequences, etc) for data analysis, with recommendations on the levels of resolution and ambiguities for data comparison;

     - legal and ethical rules to work with (e.g. to obtain informed consent of the sampled individuals and accurate rules for respecting their dignity, rights, safety and well-being) and exchange population data and/or biological samples.

   Such guides will be essential to define common strategies for the generation of new data, take common decisions on HLA typing, and choose appropriate statistical methods and computer programs to analyse the data, leading to harmonise the genetic analysis of human populations.

2. Establish an inventory of existing HLA genetic data in local and immigrant European populations that can be shared between laboratories. Most important is the possibility to reuse the blood or DNA of previously sampled populations to test additional loci or investigate additional DNA regions according to appropriate ethical rules, as a non-invasive way to increase the potential of different teams. Data sharing will allow performing coordinated, complementary and/or new genetic analyses related to the study of HLA in population genetics and epidemiology (non-classical HLA genes, KIR genes, etc.).

3. Improve multi-disciplinary expertise. One objective of the Action is to educate all researchers to a minimum level in the discipline in which they do not have their primary level of expertise, to make them more independent.

4. Forster dialogue (and possibly share data and/or competences) with laboratories concerned by the study of other human polymorphisms in Europe. Such interactions will be largely facilitated by the high visibility of the HLA network launched as a COST Action. A further objective is to promote scientific relationships with isolated research groups located in other continents (e.g. Africa, South East Asia, etc), leading to international collaborations.

C.3 How will the objectives be achieved?

Development of a network of human expertise

A number of primary experts working in the different disciplines concerned by the Action (population genetics, biostatistics, computer science, tissue typing, transplantation, epidemiology, ethics) will contribute their knowledge and experience to propose activities and coordinate the achievements of the objectives. Different tasks will be distributed among several Working Groups (WG) including appropriate expert(s). The activities of the WG will be networked through an electronic project organizer. The HLA-NET electronic platform will publish specific comments by experts on consensual decisions of each WG to inform and exchange views with other scientists. A core of experts will be ready to discuss with other scientists and students on the main topics related to HLA diversity in human populations in different disciplines. At least two scientific workshops will be organized, where researchers will explain the progress of research in these domains.

Development of a network of data, documents and computer facilities

Access to data, information and tools will be achieved through the creation of an electronic platform where computer tools and databases, guides and other documents agreed by the Working Groups will be brought together, continuously updated and made available. Such a platform will play a central role for HLA population data analyses conducted within local European research programmes. The network will include both a public area (with announcements and public documents) and a password-protected area to access more confidential data.

At least two starting institutions will be involved in the development and networking of the population databases and computer program platform, using one computer server and employing one or two computer experts each. All laboratories will then use their own computer resources to work for the Action, i.e. at least one personal computer connected to the Internet per scientist.

Development of an educational programme

Scientists and students will be educated in both laboratory work and biostatistics/computer program use.

All laboratories will use their infrastructure and laboratory material to welcome early-stage researchers and students involved in Short-Term Scientific Mission to learn either computer or laboratory techniques. One or two experts or technicians per laboratory will train visiting scientists.

At least one educational meeting (in the form of a Training school) will be organized, but students will also be invited to attend the scientific workshops.

Total manpower

The network of human expertise will involve all scientists (mostly HLA laboratory heads) currently interested by the Action (currently 15 Institutions among 13 European countries). The development of the network of data, documents and computer facilities will involve at least 4-5 computer scientists (to start) belonging to 2 Institutions and PhD or post-doc students. The educational programme will involve between 15 and 30 technicians(1-2 per Institution) for the training of young scientists, students and/or other technicians.

Total equipment

The network will use (to start) 2 servers of 2 Institutions and at the personal computers of all partners. The educational programme will use the laboratory infrastructure and material of all partner Institutions.

C.4 Benefits of the Action

The Action is expected to provide considerable scientific, technological and societal benefits.

Scientific benefits:

The elaboration of consensual standards and protocols and the access to data, biostatistical and computer tools will catalyze the improvement of research in several disciplines:

1. In histocompatibility and clinical transplantation, European registries of HLA-typed hematopoietic stem cell donors to be linked to precious information on the HLA molecular diversity in human population, thereby improving the quality of the data used in clinical medicine. The Action will lead to an improvement and/or acceleration of the procedures to search compatible donors, as it hardly depends on precise knowledge on the inventory of HLA alleles and haplotypes defined at high resolution in different ethnically- or geographically-defined populations; research in donor-recipient matching also depends on a good biostatistical knowledge and practice, which will be stimulated by the development of the HLA-NET platform.

2. In epidemiology, researchers will get an easier access to reference populations, biostatistical tools and computer programs to analyse their data will high statistical power and possibility to compare with other related studies.

3. In molecular population genetics, the data record of human populations tested for HLA will considerably improve as it will include standardized and comparable data for population genetics analyses. Also, the continuous adaptation of computer tools to complex HLA data (and the definition of new alleles) will not represent an obstacle any more to such studies.

4. In all these disciplines, scientists will have the guarantee that they use and share data according to concerted ethical rules. This respect of rules will be acknowledged at the international level.

5. Highly significant synergies among European laboratories will allow them to reach a highly competitive rank for population molecular data analyses at an international level. European teams will then be invited to take a large part in the organisation of both European and international meetings and conferences.

6. Many non-European countries like United States, Australia and South American countries will also benefit from this network, as the Action will provide valuable references to describe the genetic profiles of their immigrant as compared to their native populations.

Those benefits will allow improving techniques, saving time on research, building new ideas to innovate, updating work, filling gaps through inter-disciplinarity, and training students and young researchers. Local research is then expected to improve in quality and become more visible and competitive.

Technological benefits:

The Action will boost the development of laboratory typing techniques, biostatistical tools and computer algorithms, which will stimulate technological development in these domains. The Action will also lead to the development of publicly available tools for handling and analysing complex genetic data, an issue that will be acknowledged by the scientific community.

Societal benefits:

Patients waiting for compatible transplantation should benefit of a more rapid access to health care thanks to more efficient identification of a suitable matched donor in the international registry. Also, patients suffering from specific autoimmune or infectious diseases should benefit of a better evaluation of the susceptibility conferred by specific HLA alleles for some pathologies.

Economic benefits:

The Action will also promote the development of European tissue typing technologies by companies involved in the commercialisation of HLA typing kits. Those companies will take advantage of the progress made through the Action in the definition of standards to adapt their performances. Due to the importance of the European market in the field, the European bioengineering industry will get a competitive advantage.

C.5 Target groups/end users

– The end users of the results expected under the Action are mainly academic researchers and physicians/clinicians working in Hospitals and related Institutions:

  - Researchers in the different fields concerned by the Action: population geneticists, epidemiologists, immunogeneticists
  - Clinicians and physicians concerned by transplantation
  - Practical workers in tissue typing laboratories

– Patients needing hematopoietic stem cell transplantation or suffering from several diseases known to be linked to HLA variability

– PhD and post-doc students who will participate in the educational programme and open workshops

– Teachers in biology and medicine

– Private tissue typing companies

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

The Action will coordinate **three main research tasks**:

1. Establishment of standardized protocols, questionnaires and procedures for collecting and databasing HLA-typed population samples and defining representative control samples for important research tasks in epidemiology, transplantation and population genetics.

   This main task is crucial to fill in the current lack of comparability among different studies pursuing similar goals, like a reliable estimation of HLA gene frequencies in samples of healthy individuals to compare with patients suffering from severe diseases (HBV, HIV, rheumatoid arthritis, etc), or the identification of rare HLA multi-locus haplotypes in ethnically or geographically well-defined populations to optimise the search of potential donors in haematopoietic stem cell transplantation. This problem is due to currently heterogeneous recruitment procedures leading to poor statistical inference and insufficent statistical power.

2. Establishment of standards for describing and handling HLA genetic data (typing results) including the definition of acceptable levels of resolution and ambiguities and the adoption of universal and user-friendly formats, and taking into account the different typing strategies currently in use.

   This task involves careful comparisons of genetic typing methodologies and their ability to produce results at comparable resolution levels; it also addresses the search for strategies to handle ambiguous data and interpret heterogeneous HLA genotypes due to the very high level of complexity of this polymorphism.

3. Construction of a bioinformatics platform for HLA population data storage and analysis and establishment of procedures for its continuous development adapted to the complexification of the HLA nomenclature and the improvement of computer algorithms.

The complexity of the HLA polymorphism is due to the existence of hundreds of different alleles at various loci and the uninterrupted discovery of new ones. As a consequence, HLA population data are neither stored nor analysed in an efficient and comparable way in different laboratories. An optimized use of the large amount of data produced by different labs needs a common and public access to very specific computer facilities, continuously updated in relation to the development of the HLA allelic record and the methodological advances in data analysis.

Summary of work plan

HLA-NET will work thanks to the involvement of leader scientists with thorough expertise in the different fields concerned by the main tasks described above. As a strongly multidisciplinary Action, it involves immunologists, epidemiologists, molecular biologists, biostatisticians, population geneticists and computer scientists. Besides those human needs, it requires laboratory infrastructure with a wide range of HLA typing technologies (PCR-SSO, PCR-SSP, Luminex, etc) as well as powerful computer resources (servers, personal computers and network facilities).

As described in detail below, four Working Groups (WG) will be constituted to achieve the different tasks, and will meet regularly to synthesize and carry on their work. The WGs will be open to dialogue with laboratories and potential partners during scientific meetings and short term scientific missions in order to integrate innovative proposals and include new collaborators. Such meetings and missions will also allow disseminating the results of the Action on a regular basis and educate young scientists and students.

D.2 Scientific work plan – methods and means

During the first meeting, the scientists involved in the Action will join different WGs according to their specific interest, skills and availability. As mentioned above, four WGs are envisaged to work on the main tasks of the Action:

WG1: Working on the standardization of population data and the definition of sampling strategies

WG2: Working on the definition of standards to produce comparable genetic data

WG3: Working on the development of a bioinformatics platform to store and analyse population genetic data

WG4: Working on the standardization of ethically correct rules for population sampling and data sharing and dissemination

Each WG will assume a specific task and will include members having expertise in a specific field: population genetics, field and hospital work (WG1), tissue typing (WG2), bioinformatics (WG3), field and clinical work, ethics (WG4). In addition, different WG will have to interact together to achieve their goals. For example, the data information collected by WG1 and the standards defined by WG2 will have to be passed on to WG3 to design and/or adapt database fields and file formats for their adequate storage, extraction and use with computer programs. WG4 will have to disseminate its expertise to WG1 and WG3 to design ethically correct sampling, storing and diffusion of data. WG1 and WG2 will have to work closely in order to evaluate the situation in terms of available data and promote additional sampling and typing for the optimisation of the database contents. During the work of the Action, other WG may be added or current WG groups may be modified to include new members with deeper and/or innovative competences and ensure the integration of additional European teams.

WG1: Working on the standardization of population data and the definition of sampling strategies

WG1 will address crucial issues related to the optimization of the population data record used by scientists working on HLA:

1. Generating new data may consist either on sampling new populations or on using already collected material to type loci not included in previous studies. Depending on the financial situation of each laboratory, a priority has often to be given to one among several possible plans, and such a decision is intimately related to the significance of the upcoming data in the context of current research. WG1 will design a desirable framework for the laboratories to evaluate the appropriateness of their projects: the data updates achieved through the Action will allow an evaluation of whether some populations or some loci are more important to type at a given stage of their project.

2. New data may be produced by other laboratories not yet involved in the Action. One important task of WG1 will be to contact all European laboratories planning to sample new populations and/or generate HLA data on already collected material and coordinate their analyses through

the Action.

3. The quality of population data depends on the application of an accurate sampling scheme allowing a precise description of the populations and a reliable estimation of gene frequencies and other statistics. Recommendations, guidelines and appropriate questionnaires will be defined and distributed through the Action by WG1. It will be most important to define clearly whether population samples are defined on an ethnic or geographic basis or whether they represent control data for disease association studies.

WG2: Working on the definition of standards to produce comparable genetic data

WG2 will address crucial issues related to the standardization of the HLA genetic data produced by different laboratories:

1. The genetic data produced by HLA laboratories should have an acceptable level of resolution and minimal ambiguities. In case of ambiguities, one may decide to perform additional molecular tests, but this may be too costly or time-consuming when numerous genotypes are concerned. WG2 will analyze the current situation of each laboratory taking into account the wide range of typing technologies to reach a consensus on resolution levels and ambiguities and establish standard rules and formats.

2. Consensual decisions on data formats and standards have to take into account the improvement of typing technologies. WG2 will maintain useful information on technical progress and coordinate the choices made by the different laboratories to analyse their data.

3. WG2 will also establish and maintain contacts with HLA typing companies interested in the development of tissue typing kits.

WG3: Working on the development of a bioinformatic platform to store and analyse population genetic data

WG3 will address computational issues related to the storage, access, analysis and diffusion of the HLA population data produced by different laboratories:

1. A main objective of most HLA laboratories is to compare the data they produce to available

data, either at a local scale (e.g. across a specific geographical region or a continent) or at the global scale. Several population databases have been or are currently developed locally by European teams involved in Action (e.g. Allelefrequencies.net, Geneva.unige.ch). WG3 will develop a network of existing databases which will be connected to a platform of computer programs and tools, as described below.

2. Computer programs are needed for many purposes related to the study of HLA in human populations: to convert data formats in order to adapt them to existing programs (either software to download or programs running online); to convert genotypic data according to different levels of resolution; to interpret HLA typing profiles in order to produce genotypic data and code ambiguities; to estimate allelic and haplotypic frequencies from genotypic data including ambiguities or not; to estimate and test the significance of linkage disequilibrium; to test Hardy-Weinberg equilibrium; to estimate gene diversity; to estimate inbreeding coefficients; to test selective neutrality; to test associations with diseases; to test the homogeneity among different samples; to compute genetic distances among populations; to compare different populations and groups of populations (analysis of variance, multivariate analyses); to analyse the data in relation to other information (e.g. geography) and other useful analyses. A central task of WG3 is to make all currently available computer programs accessible to laboratories through a user-friendly platform. This group will also elaborate useful guidelines to use such programs and work continuously on the integration of new softwares.

WG4: Working on the standardization of ethically correct rules for population sampling and data sharing and dissemination

WG4 will address legal and ethical issues related to collection and diffusion of data:

1. Informed consent by sampled individuals must be obtained in all population studies, and the research groups have to respect the dignity, rights, safety and well-being of the persons participating in a given study. A main task of WG4 is to gather information related to legal and ethical regulations in different countries and discuss them in an attempt to reach a consensus on ethical issues and examine possible suggestions to address to European countries.

2. This WG will also establish contacts with governmental experts.

The network of resources developed by the four Working Groups, as described above, will be completely new in Europe and will contribute to building a real European landscape of excellence in this domain. The task of the Action is indeed to bolster the visibility of Europe in the international context of research, amongst which its participation to the International Histocompatibility and Immunogenetics Workshops, where United States and other non-European regions like Japan are currently the main leaders.

The means for carrying out the Action is through WG meetings, cross-group meetings and scientific workshops. Those workshops will be open to potential partners of the Action and to bioengineering companies. In addition, short-term scientific missions will be organized with the aim to educate young researchers and students (either educate researchers working in a wet laboratory to computer analyses, or educate computer scientists to laboratory techniques).

Finally, the results of the Action will be disseminated through the web, through published reports and other publications.

E. ORGANISATION

E.1 Coordination and organisation

Management Committee

HLA-NET will be coordinated by the Management Committee (MC) consisting of scientists of participating countries and having a Chairperson, a Vice-Chairperson, and also involving one scientist responsible for each WG (WG coordinators).

The MC will be in charge of the following tasks:

1. Plan and coordinate the different types of meetings (MC, WG and cross-group meetings) and scientific missions of the Action
2. Organize the distribution of funds to the different activities
3. Establish contacts with potential partners of the Action
4. Establish contacts and collaborations with other European programs and organisations

5. Synthetize the results of the different WG and produce Action reports
6. Promote the dissemination of the results within and outside Europe
7. Establish contacts with private bioengineering companies interested in HLA typing.

MC meetings

The MC will meet once or twice a year to check the progress of its own tasks and the tasks of each WG, to discuss essential issues related to the Action, to evaluate the remaining work to be done for the achievement of the milestones, to establish priorities to continue the Action and to write drafts of Action reports. Those meetings will be grouped in time and space with WG meetings (e.g. one-two days for the WG, and one day for the MC), and will be held in different places depending on the location of the involved laboratories. During the meeting periods, MC members will be in close contact with all WG members (not only coordinators) for useful discussions. If possible, the MC-WG meetings will also be coupled with European or International meetings (in particular, EFI conferences and IHIWC workshops) where COST events will be proposed to disseminate the results of the Action.

During inter-meeting periods, the MC will coordinate its activities through an electronic project organizer allowing all MC members (but safely protected from non-members) to access the reports and other documents or information necessary for their activities.

WG group coordination and organisation: these are described in Section E2.

Short term scientific missions (STSM)

All laboratories will use their infrastructure and laboratory material to welcome early-stage researchers involved in Short Term Scientific Mission (STSM) to learn either computer or laboratory techniques. One or two experts or technicians per laboratory will train visiting scientists.

To use specific computer programs and understand their outputs is not always easy for people working with tissue typing in a laboratory, even when precise guidelines are provided through electronic manuals. To promote the coordination of HLA genetic analyses at the population level, both direct contact with a qualified person demonstrating the use of these tools and effective training are necessary. A main task of the Action is to educate researchers working in a wet laboratory to data handling and computer analyses. This will be achieved by using the HLA-NET

platform developed through the Action.

Also, young scientists trained in biostatistics, bioinformatics and population genetics need to learn laboratory techniques to understand better the problems that arise from HLA typing and better adapt the analytical tools and programs. Those scientists will also participate to Short Term Scientific Mission (STSM).

At least one educational meeting (for example in the form of a summer school) will be organized to integrate laboratory and computer knowledge.

Milestones

The most important milestones (related to the work plan presented in Section D1) are listed below:

- Create an operational website for the Action
- Establish standardized protocols and procedures for collecting and sharing population data
- Establish standardized rules and formats for handling HLA genetic data
- Network available databases and link them to the defined standards
- Network useful computer programs and link them to the networked databases
- Overall integration of the HLA-NET platform
- Educate and train scientists and students in handling and analyzing data using the HLA-NET platform

E.2 Working Groups

Four WGs are envisaged, in harmony with the objectives of the Action and based on the workplan explained in Section D2:

WG1: Working on the standardization of population data and the definition of sampling strategies.

WG1 is mainly composed of experts in population genetics and field or hospital work.

WG2: Working on the definition of standards to produce comparable genetic data.

WG2 is mainly composed of experts in tissue typing.

WG3: Working on the development of a bioinformatics platform to store and analyse population genetic data.

WG3 is mainly composed of experts in biostatistics and/or bioinformatics. This WG may organize specific visits of its computer experts to laboratories needing to learn how to use databases and programs or to assess computer facilities in different Institutions.

WG4: Working on the standardization of ethically correct rules for population sampling and data sharing and dissemination.

WG4 is mainly composed of experts in field and clinical work and ethics.

These WGs will meet at least once a year to report their activities and achievements. Like MC members, WG members will work through an electronic organizer during inter-meeting periods.

As explained in Section D2, different WGs will have to interact together to achieve their goals. For example, WG3 (bioinformatics) may want to know all possible outcomes of a given typing technique to update the database and needs to meet the experts of WG2. Therefore, cross-meetings will take place between appropriate WG during the MC-WG meeting periods.

E.3 Liaison and interaction with other research programmes

Liaison and interaction with other research programmes will be essential to improve the efficiency of the WG during their work. Several connections will be automatically established:

1. WG1 and WG3 with the Anthropology/Biostatistics component, the HSCT component, and the Rare alleles component of the 15th International Histocompatibility and Immunogenetics Workshop (IHIW).

2. WG2 with the EFI organization (e.g. Standards and Quality Assurance Committee) to define standards.

3. WG4 with the POSEIDON programme for the establishment of legal and ethical rules.

Contacts with the components of 15th IHIW will occur through e-mails and meetings organized during the workshop. Contacts with EFI and POSEIDON will occur through e-mails and meetings

organized during the EFI meeting. Also, because most participants of the Action are involved either in IHIW or EFI components, or both, liaison and interaction with these organisations will occur during component meetings.

As mentioned in Section E1, the MC will also propose to include COST events in EFI and IHIW meetings to promote liaison and interaction with other research programmes,

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to considerably involve early-stage researchers. This item will also be placed as a standard item on all MC agendas.

This COST Action will take appropriate measures to ascertain the gender balance in all its activities. About half of the scientists who have declared their interest for the Action are women (including the launching investigator). An accurate gender-balance is now a priority in most Academic Institutions and the applicants are aware of the importance of including women in research activities. Therefore central emphasis will be given to the involvement of both women and men in working groups, educational activities and meetings. This need to respect an appropriate gender balance will be recalled at each MC and WG meeting.

Early-stage researchers, like master and PhD students, will be educated through the Action in different fields of expertise. This will give them the necessary background for their PhD and the possibility of being involved in multi-disciplinary research projects. PhD students will also get more autonomy to handle new research projects in their own countries.

F. TIMETABLE

The HLA-NET Action will last 4 years with the following schedule. The Action will start with a meeting at the beginning of the first year where members will be appointed to the WG, and a preliminary Action schedule is decided. The first milestone will be the creation of the Action website after 6 months of activities. At the end of the first year WG1 and WG2 will have provided

preliminary standards and these will be updated continuously during the whole period, with annual checkpoints. WG3 will network databases by the end of the 2nd year and computer programs by the end of the 3rd year. WG4 will work in close connection with the other WGs during the whole period. The final integration of the HLA-NET platform (including connection between standards, databases and programs) will occur in the middle of the 4th year. MC-WG meetings occur once a year but optionally more often and the Action's report will be produced annually as per the COST guidelines. Short-Term Scientific Missions will start at the beginning of the 2nd year and last until the middle of the last year. One educational meeting will be organized at the end of the 3rd year (optionally two) and two open scientific meetings at the end of the 2nd and the 4th years.

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, BE, BG, CH, DE, FR, IT, NL, NO, PT, SE, SI, UK . On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at EUR 31 million for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

1. The target audiences for the dissemination of the results of the Action include fellow researchers working in immunogenetics and transplantation. About 900 of the European investigators were members of the EFI organisation in 2007. Those scientists are interested by the definition of standards, the accessibility of comparable data and computer programs and the technical recommendations in both HLA typing and biostatistical analyses. Participants to the POSEIDON framework will also be interested in relating their activities in the domain of ethics to those of the Action.

2. Moreover, most research groups working in population genetics in Europe and in the world, either with HLA markers or with other molecular polymorphisms will acknowledge the coordination of European research activities in this domain. Indeed, they will follow the progress achieved in the genetic characterization of European and related populations and either compare their own HLA results or those of other markers to understand the genetic history of this continent.

3. Beyond Europe, the past and future International Histocompatibility and Immunogenetics Workshop gathers hundreds of researchers in immunology, epidemiology, transplantation and anthropology/biostatistics who will be interested by the achievements of the Action, in particular the accessibility of data, useful computer tools and standards.

4. European tissue typing companies will also be interested in getting information in order to adapt their technologies according to the findings (e.g. list of rare alleles only found in Europe or outside Europe) and recommendations (levels of resolution and ambiguities, data formats) of the Action.

H.2 What?

1. The Action website will be open to both scientists and the public with announcements of the Action activities and more particularly the open workshops.

2. The HLA-NET platform will be accessible with a password and will publish electronic versions of the main documents elaborated by the WG:

a) Guidelines for HLA typing, use of standards, legal and ethical rules

b) Technical manuals on computer programs and population databases

c) Meeting reports

d) Proceedings of the open workshops.

3. Regular announcements and short reports of HLA-NET activities in existing newsletters like those published by EFI and IHIW organisations

4. Scientific publications in peer-reviewed journals on both specific and multidisciplinary research topics related to the Action and whose results have been generated thanks to the scientific

achievements of the Action.

H.3 How?

The website of the Action will be developed to present clearly its objectives and its most recent achievements, as well as announcing public workshops and educational meetings. A permanent call to join the Action will be disseminated through the website, with a list of contacts and an electronic forum to encourage dialogue.

Publications will be disseminated both electronically (guidelines, technical manuals available on the website) and through several journals. The EFI Newsletter has a high impact among immunogeneticists and will be useful to disseminate regularly the Action reports. Moreover, joint reports will be sent to Tissue Antigens, and research articles related to the Action to other, more general, peer-reviewed journals like the European Journal of Immunogenetics and the European Journal of Human Genetics.

The midterm and final workshops will be open to both scientists and bioengineering companies. Paper versions of the main publications will be available during those workshops to disseminate the results to potential industrial partners.