GAPAID
genes and proteins for autoimmunity diagnostics

GAPAID Final publishable summary report

Executive summary.

GAPAID was a two-year project started on September 1st, 2012, under the "Research for small-medium enterprises" FP7 program, and successfully concluded on August 31st, 2014. The project was aimed at developing new reliable diagnostic tools for rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), two autoimmune diseases that affect ca. 1.537.000 and 1.272.000 patients in Europe, respectively.

RA is one of the most common autoimmune disorders worldwide. Around half of RA patients are unable to hold down a full-time job within 10 years of onset. In RA the objective of therapeutic intervention is to control symptoms, prevent disability and avoid joint damage. The course of RA is highly variable and the commonly used step-up pharmacological strategies imply that a proportion of newly diagnosed RA patients could be inadequately treated for a specific period of time.

SLE is a multisystem disease affecting mostly women in childbearing age, and leading to impaired function of several organs, including the kidney. In SLE, the main goal of treatment is to prevent disease relapses and preserve organ function. The diagnosis of SLE must be based on the proper constellation of clinical findings and laboratory evidence. Management depends on disease severity and organ involvement. Periodic follow-up and laboratory testing are imperative to detect signs and symptoms of new organ-system involvement and to monitor the response or adverse reactions to therapies.

RA and SLE are multifactorial diseases caused by environmental stimuli acting on genetically predisposed subjects. The clinical diagnosis of RA and SLE is assisted by the use of in-vitro diagnostic tests aimed at evaluating the presence/level of several antibodies circulating in the patient's serum. However, this diagnostic approach is unsatisfactory because it can only assist in the diagnosis after the initial disease onset, is not useful for evaluating disease susceptibility for early prevention, and does not provide any information for monitoring disease progression for the setup of personalized therapeutic treatments.

GAPAID project contributed to the challenging task of improving the diagnosis of these two diseases by:

- identifying and setting up a diagnostic method based both on genetic and on serological markers specifically and significantly associated with the disease;
- developing two novel diagnostic products, one for RA and one for SLE, composed by a serological array, a genetic array and a software exploiting the acquired scientific insights.

The GAPAID prototype of the in-vitro diagnostic tool for the diagnosis of RA contains:

- a semi-quantitative array-based immunoassay for the determination in human serum or plasma of antibodies directed against Citrullinated Peptides and Rheumatoid Factor;
- a genetic array for the genotyping in human serum of five Single Nucleotide Polymorphisms (SNPs) associated with the predisposition to develop the disease;
- a software combining the genetic and the antibody results to predict the probability to develop RA.

The GAPAID prototype of the in-vitro diagnostic tool for the diagnosis and the follow up of SLE contains:

- a semi-quantitative array-based immunoassay for the determination in human serum or plasma of complements and of antibodies directed against dsDNA, ssDNA, nucleosome, histon, Sm, Ribosomal P proteins, SSA, nucleosome, CENP-B, C1q, collagen, and cardiolipin;
- a genetic array for the genotyping in human serum of two SNPs: one SNP is associated with the predisposition to develop the disease and the second SNP correlates with the probability to belong to a more severe subset of disease;
- a software combining the genetic and the antibody results to predict the probability to develop SLE and the probability of belonging to a more severe subset of SLE.

Summary description of the project context and the main objectives.

GAPAID project was aimed at developing new reliable diagnostic tools for two autoimmune diseases: rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

Rheumatoid arthritis

RA is a chronic inflammatory disease that leads to progressive joint deformity, disability, and sometimes to premature death. It is a complex disease with a high degree of clinical heterogeneity, as assessed by genetics, environmental risk factors, autoantibody patterns, cytokine expression, clinical course and response to therapy. RA may affect many tissues and organs but it principally attacks the joints, producing an inflammatory synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints. RA can also produce diffuse inflammation in the lungs, pericardium, pleura, and sclera, as well as nodular lesions, most common in subcutaneous tissue under the skin. The causes of RA are still unknown. However, human autoimmunity and some genetic and environmental factors have been suggested to play a major role in the occurrence and progression of RA. Because of the chronic nature of the disease, the societal cost is enormous and continues to rise. RA is a common illness of global significance for public health.

RA affects almost 1% of the adult population world-wide. It is more prevalent in women than in men and increases with age (5% in women over 55). Although the aetiology of RA is still largely unknown, certain demographic factors associated with an increase in RA risk have been identified. These are sex, age and, possibly, family history.

The diagnosis of RA combines patient history of joint pain and stiffness, physical examination, documentation of symmetric polyarticular joint swelling (synovitis), and laboratory tests including radiographs, inflammatory markers [erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)], and autoantibodies [rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP)].

Currently used biomarkers for diagnosis of RA are mostly clinical. The first biomarker used for RA was the presence of RF. However, this marker lacks specificity and has been supplemented by the detection of antibodies against CCP, now referred to as anti-citrullinated peptide/protein antibodies (ACPA). It has long been recognized that RF plays a pivotal role in the differential diagnosis. For this reason, testing for RF has been one of the classification criteria for RA since 1987 (1) and, although many years have passed since their identification, their crucial role in classifying RA has been confirmed by the updated criteria (2). ACPAs have been recognized as a hallmark of RA (3) since the beginning of the century. ACPAs can be detected years before onset of disease and play a role in the pathogenesis of RA (4). ACPA-positive disease is associated with an unfavourable outcome. Anti-CCP antibodies may be detected in roughly 50-60% of patients with early RA at ‘baseline’ (e.g., at their initial encounter with a specialist, usually after 3-6 months of symptoms). In about 33% of patients with an established RA diagnosis, however, ACPA or RF cannot be detected and recent findings suggest that in about half of them, novel autoantibodies may be present (5).

Systemic Lupus Erythematosus

SLE is a systemic chronic autoimmune disease with widespread inflammation, tissue damage and wide array of clinical manifestation. The most common organ system presentations are cutaneous, musculoskeletal, and renal. The classical pattern of SLE is characterized by periods of illness and remissions, but some patients have chronic activity. Diagnosis of SLE can be difficult as the symptoms of SLE can be similar to those of other autoimmune disease, therefore it takes a long time to set up the right diagnosis. The mortality of this autoimmune disease is high and the survival could be improved by the recognition and diagnosis of early and mild cases.

The prevalence of SLE is 15 to 50 cases per 100.000 persons in Europe but it changes regarding sex (female-male ratio is 9:1) and racial groups (incidence is higher in Afro-Americans than Caucasians). SLE

affects mostly women in childbearing age. The incidence of SLE is 3.3 to 4.8 cases per 100.000 persons in Europe.

There is no one single test or methods for SLE diagnosis. The rheumatologists use the ACR's "Eleven criteria of Lupus" to diagnose SLE (6). Four or more of these eleven criteria must be presented to make the diagnosis including skin rash, arthritis, neurological disorder, renal disorder and serological tests measuring autoantibody against cell nucleus or changing of the serum complement level. The tests carried out in laboratories are the assays for the determination of complement level, and the immunoassays for the detection of antibodies to nuclear antigens (ANA) and other antigens related to disease activity (such as Sm and dsDNA).
The basic test, represented by ANA determination, is not itself specific for SLE. In fact, while it is true that almost all SLE patients are ANA positive, on the other hand there are many diseases that have positive ANA test (e.g. Sjögren's syndrome, autoimmune thyroiditis, rheumatoid arthritis, primary biliary cirrhosis primary).

Genetic factors in RA and SLE
Several genome-wide studies in these two complex disorders have highlighted the impact of genetic polymorphisms on the risk of developing the disease, on its severity and on the response to therapy. As common pathogenic mechanisms are shared by different autoimmune disorders, it is conceivable that the same set of genes may predispose to multiple autoimmune diseases.

The first genetic marker found for RA is in the HLA-DR region. HLA-DR molecules associated with RA are HLA-DRB1 0401, 0404 and 0101, referred to as the shared epitope. Several studies have reported prognostic factors in RA with conflicting results (7-10). From a practical standpoint, clinical and laboratory variables are insufficient to predict the disease outcome in individual patients (11). An easy to use tool, based on reliable markers that can be determined early in the disease course and provide a rational basis for appropriate treatment, would be very helpful to clinicians (12).

In SLE, linkage studies and genome-wide association studies have confirmed the risk conferred by *HLA-DR2* (*DRB1*1501*) and *HLA-DR3* (*DRB1*0301*) genes in many European populations. Outside the MHC region, *IRF5* (encoding interferon regulatory factor 5) is one of the most strongly and consistently SLE-associated loci; *STAT4,* encoding the signal transducer and activator of transcription 4 protein, has been found to associate with SLE in multiple studies in European or Asian populations (13, 14). Only a few studies have addressed the influence of disease-predisposing genes on SLE severity and outcome. No study has so far combined clinical, serological and genetic data to build up a model for predicting disease outcome in SLE.

GAPAID main objectives
In this context the GAPAID project was aimed at improving the diagnosis of these two diseases by:

- identifying and setting up a diagnostic method based both on genetic and on serological markers specifically and significantly associated with the disease;
- developing two novel diagnostic products, one for RA and one for SLE, composed by a serological array, a genetic array and a software exploiting the acquired scientific insights.

In the case of RA, the achievement of the final target depended on the Background already present in the GAPAID Consortium and on the Foreground to be identified through the GAPAID activities, which are summarized as follows:

1) the genetic profile associated with the disease is performed by the use of selected SNPs (Progenika Background and Foreground);
2) the disease-specific serological markers are detected by proprietary antigenic molecules (Toscana Biomarkers Background);

3) the array technology for the detection of the serological markers (Diagnosticum Background);
4) the algorithm for the combination of the clinical, genetic, and serological data identified during the GAPAID project (Foreground).

Therefore, the main scientific objectives for RA were aimed at:

- identifying a diagnostic method based on serological and genetic biomarkers significantly and specifically associated with the disease;
- evaluating the clinical relevance of the prototypes by determining their diagnostic performances, in the case of the serological array, and the significant association with the disease for the genetic prototype.

The technological objectives for RA were aimed at:

- adapting the antigenic molecules to the array technology and setting up a multiplex protocol for the development of the serological prototype;
- exploiting the scientific results acquired though the identification of a diagnostic algorithm by developing a software and a genetic prototype to complete the set up of the final diagnostic product.

For SLE the achievement of the final target depended on the Background already present in the GAPAID Consortium and on the Foreground to be identified through the GAPAID activities, which are summarized as follows:

1) the genetic profile associated with the disease is performed by the use of selected SNPs, which were identified during the GAPAID project (Foreground);
2) the disease-specific serological markers are detected by proprietary molecules (Toscana Biomarkers and Diagnosticum Background);
3) the array technology for the detection of the serological markers (Diagnosticum Background);
4) a serological array for the detection of 8 biomarkers specific for SLE (Diagnosticum Background);
5) the algorithm for the combination of the clinical, genetic, and serological data identified during the GAPAID project (Foreground).

Therefore, the main scientific objectives for SLE were aimed at:

- selecting and identifying the SNPs associated with the disease and with different subset of the disease;
- identifying a diagnostic/prognostic method based on serological and genetic biomarkers significantly and specifically associated with the disease;
- evaluating the clinical relevance of the prototypes by determining their diagnostic performances, in the case of the serological array, and the significant association with the disease for the genetic prototype.

The technological objectives for SLE were aimed at:

- adapting the antigenic molecules of TBM to the array technology of DIAG and setting up a multiplex protocol for the development of the serological prototype;
- exploiting the scientific results gained though the identification of a diagnostic algorithm by developing a software and a genetic prototype to complete the set up of the final diagnostic product.

References

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Description of the main S & T results/foregrounds.

The overall objectives of the GAPAID project were aimed at improving the diagnosis of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) by:

- identifying and setting up a diagnostic method based both on genetic and on serological markers specifically and significantly associated with the disease;
- developing two novel diagnostic products, one for RA and one for SLE, composed by a serological array, a genetic array and a software exploiting the acquired scientific insights.

These objectives were achieved during the GAPAID project thanks to the scientific activities performed by the RTD performers and to the demonstration activities completed by the SME/OTH partners. The great cooperation among the GAPAID Partners allowed to achieve the following results:

- the main project results corresponding to six in-vitro diagnostic prototypes;
- the main scientific and technological results.

GAPAID main project results

In the GAPAID project the following main project results were obtained:

1) genetic prototype for rheumatoid arthritis;
2) serological prototype for rheumatoid arthritis;
3) prototype of the starting package for rheumatoid arthritis;
4) genetic prototype for systemic lupus erythematosus;
5) serological prototype for systemic lupus erythematosus;
6) prototype of the starting package for systemic lupus erythematosus.

The genetic and the serological prototypes are comprised in each starting package of the final in-vitro diagnostic tool. All the six main project results can be classified as in-vitro diagnostic medical devices. The market sector corresponds to the in-vitro diagnostics and the medical application refers to the devices for autoimmunity.

Description of GAPAID main project results for the diagnosis of Rheumatoid Arthritis

The prototype of the final starting package is an in-vitro diagnostic kit that is designed for the diagnosis of RA by the evaluation of different parameters. The final starting package contains:

- a semi-quantitative array-based immunoassay (Figure 1), for the determination in human serum or plasma of IgA+IgG class antibodies directed against Linear Citrullinated Peptides (CP), and of IgA+IgM class antibodies directed against Rheumatoid Factor (RF);
- a genetic array (Figure 2), for the genotyping in human serum of five Single Nucleotide Polymorphisms (SNPs) associated with the predisposition to develop the disease;
- a software (Figure 3), combining the genetic variables, the antibody results and some clinical parameters to predict the probability to develop RA.

The array-based immunoassay (i.e. serological prototype) is a multiplex assay that provides with one evaluation the titer of the serological antibodies, CP and RF, which are requested by the European League Against Rheumatism (EULAR) classification criteria for RA diagnosis. The genetic array is a multiplex assay that provides the genotype of an individual to five SNPs that GAPAID project confirmed to be associated with RA. The software combines the serological and the genetic results with some clinical variables according to the diagnostic algorithm developed during the GAPAID project.

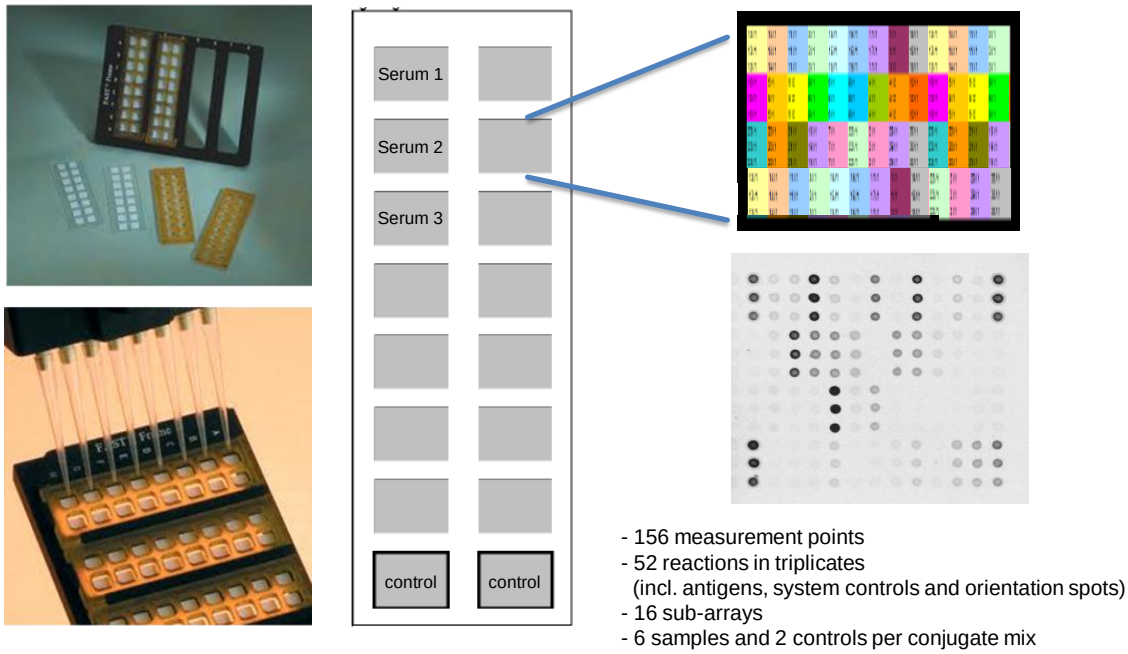

Figure 1. Serological prototype consisting of a microarray containing the printed antigens and controls. The substrate of the arrays is a nitrocellulose coated glass slide with 16 pads. The detection is performed with a mixture of 2 fluorescent probes using a suitable reader.

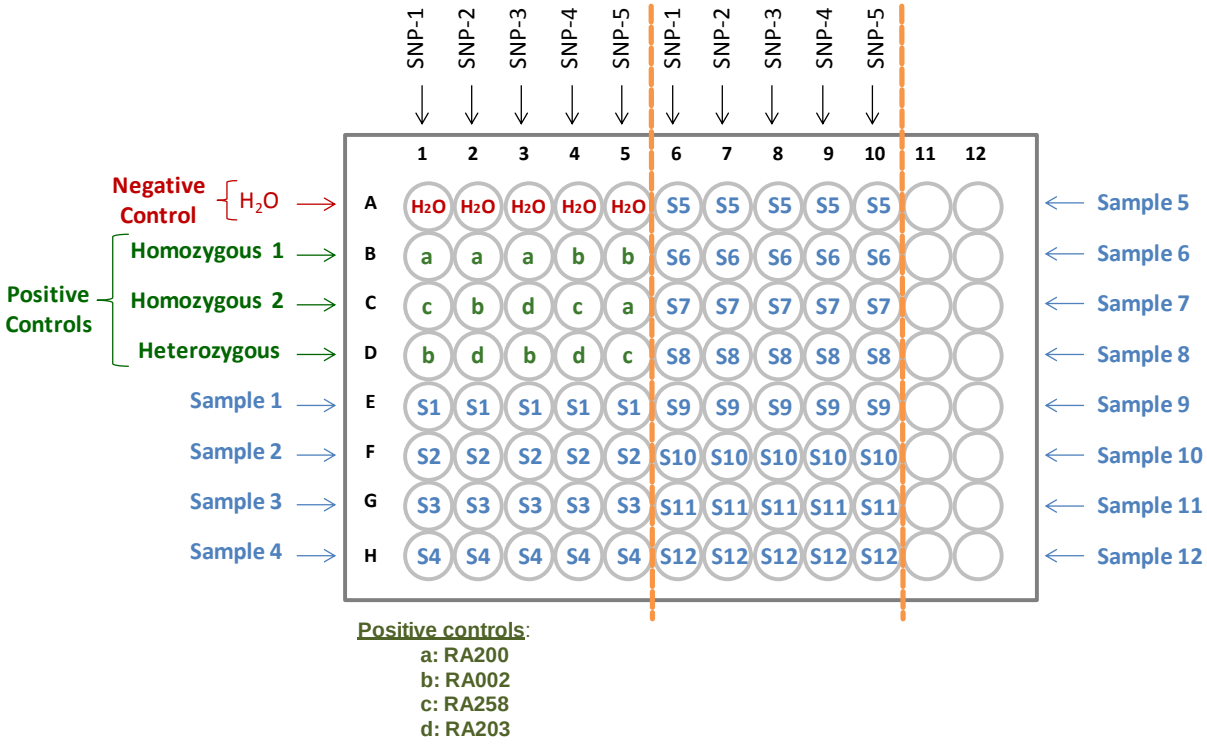

Figure 2. Genetic prototype for the detection of five SNPs associated with RA predisposition.

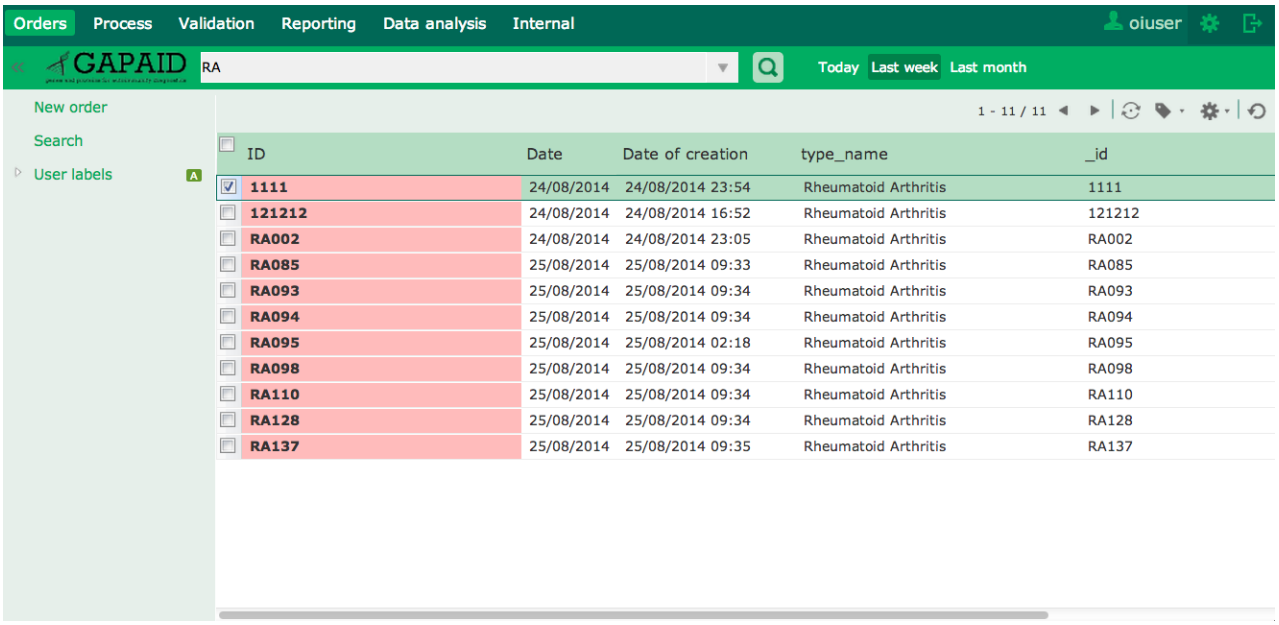

Figure 3. GAPAID software was set up to process automatically data received from genetic and serological tests on the basis of the diagnostic algorithm developed for RA. GAPAID software is a web-based solution, which comprises a powerful search engine and can be easily integrated with Laboratory Information Systems used in the hospitals.

The RA starting package as result of the algorithm is characterized by a 68.1% sensitivity at 98.3% of specificity as determined on a total population of 484 samples comprising 269 RA patients, 63 disease controls, and 152 healthy blood donors. All the analytical and diagnostic performances have been evaluated during the GAPAID project.

Description of GAPAID main project results for the diagnosis of Systemic Lupus Erythematosus

The prototype of the final starting package is an in-vitro diagnostic kit that is designed for the diagnosis and the follow up of SLE by the evaluation of different parameters. The final starting package contains:

- a semi-quantitative array-based immunoassay (Figure 1), for the determination in human serum or plasma of IgG+IgM class antibodies (i.e. dsDNA, ssDNA, nucleosome, histon, Sm, Ribosomal P proteins, SSA, nucleosome, CENP-B, C1q, collagen, and cardiolipin), and of C3+C4 class complement;
- a genetic array (Figure 4), for the genotyping in human serum of one SNP associated with the predisposition to develop the disease (diagnosis) and of another SNP that correlates with the probability of belonging to a more severe disease (prognosis);
- a software (Figure 5) combining the genetic variables, the antibody results and some clinical information to predict the probability to develop SLE and to predict the probability of belonging to a more severe subset of SLE.

The array-based immunoassay is a multiplex assay that provides with one evaluation performed on human serum the titer of most of the serological antibodies comprised in an Anti-Nuclear Antibody (ANA) test and specific for SLE diagnostic criteria. The genetic array is a multiplex assay that provides the genotype of an individual to two SNPs that GAPAID project confirmed to be associated with SLE. The software combines the serological and the genetic results with some clinical parameters according to the diagnostic and prognostic algorithms developed during the GAPAID project.

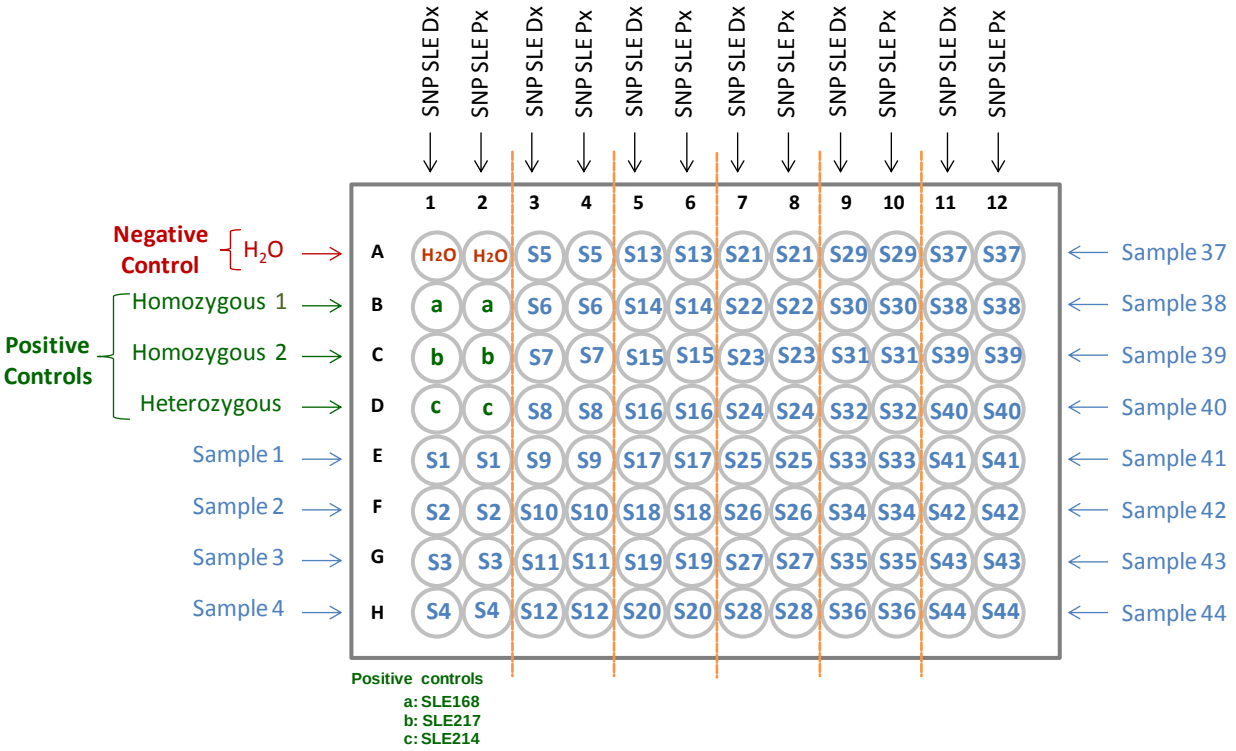

Figure 5. Genetic prototype for the detection of two SNPs, one is associated with SLE predisposition and the other one with the probability of belonging to a more severe subset of SLE.

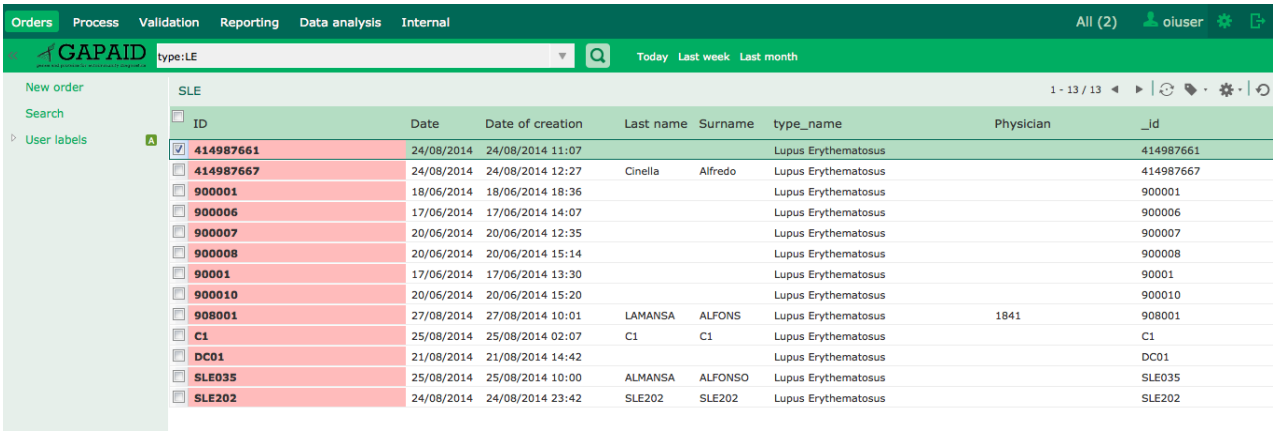

Figure 6. GAPAID software was set up to process automatically data received from genetic and serological tests on the basis of two algorithms developed for SLE. GAPAID software is a web-based solution, which comprises a powerful search engine and can be easily integrated with Laboratory Information Systems used in the hospitals.

GAPAID main scientific and technological results

To allow the successful development of the final products of the GAPAID project, the following scientific results were obtained:

1) Collection of human samples and of the whole set of clinical data on activity and severity of the disease for 269 RA patients and 211 SLE patients included in the study and recruited for serological and genetic analyses by the Department of Internal Medicine of the University of Pisa (Pisa, Italy), and the Department of Rheumatology and Immunology of the University of Pécs (Pécs, Hungary).

2) Collection of human samples for 152 healthy blood donors, 63 RA disease control samples, and 63 SLE disease control samples included in the study and recruited for serological and genetic analyses by the Department of Internal Medicine of the University of Pisa (Pisa, Italy), and the Department of Rheumatology and Immunology of the University of Pécs (Pécs, Hungary).

3) More than 50 out of the 64 analyzed SNPs were associated by the University of the Basque Country (Bilbao, Spain) with the susceptibility to RA or with several related clinical features. In the case-control analyses, several well-established RA risk loci were found. In addition, the association of one locus was replicated in the present study for the first time in an independent cohort of European origin. A functional SNP target of the biologic drug tocilizumab, which shows to be an effective treatment for RA, was also detected significantly associated with RA disease. In the case-case analysis focused on the SNP association with ACPA: one ACPA negative and three ACPA positive-specific loci were also replicated for the first time in this study. The role of three SNPs in the development of erosive RA was also confirmed, validating their clinical relevance. Moreover, the analysis identified three additional new erosive RA-associated loci.

4) Regarding SLE susceptibility genes, previously detected associations have been replicated in GAPAID project, even analysing the Italian and the Hungarian populations separately. Furthermore, the association detected between one locus and the risk to nephritis constitutes the first replication in a European population. The study performed by the University of the Basque Country (Bilbao, Spain) identified also newly associated SNPs to the different clinical subphenotypes explored, such as central-nervous system involvement or antiphospholipid syndrome occurrence in SLE.

5) Diagnostic sensitivity and specificity of the RA serological array set up by the University of Budapest (Budapest, Hungary) and containing citrullinated peptides from Toscana Biomarkers (Siena, Italy) printed on Abc technology developed by Diagnosticum (Budapest, Hungary), were assessed on the whole cohort of 475 RA samples and controls. ACPA sensitivity was 65-70% at 95% of specificity, which was found comparable to current CCP2 tests in this cohort of samples.

6) Diagnostic sensitivity and specificity of the SLE serological array set up by the University of Budapest (Budapest, Hungary), containing reagents from University of Firenze (Firenze, Italy), Toscana Biomarkers (Siena, Italy), and Diagnosticum (Budapest, Hungary) printed on Abc technology of Diagnosticum, were assessed on the whole cohort of 429 SLE samples and controls. The sensitivity of the test is around 70-75% with the specificity being set at 95%, which is comparable to ANA screening tests.

7) The algorithm matching the serological and genetic data for RA diagnosis was set up by Progenika (Bilbao, Spain). The GAPAID diagnostic algorithm combines two clinical, two serological, and five genetic variables detecting RA patients with a really high accuracy (Specificity 98.3% and Sensitivity 68.1%).

8) The algorithms matching the serological and genetic data for SLE diagnosis and prognosis were set up by Progenika (Bilbao, Spain). The GAPAID SLE diagnostic algorithm combines one clinical, five serological, and one genetic variable, and is able to detect SLE patients with a really high accuracy (90% Specificity and 73.6% Sensitivity). The GAPAID SLE prognostic algorithm combines two

serological, two clinical, and one genetic variable, and is able to predict severity of SLE patients with a high accuracy (AUC=0.75).

To allow the successful development of the final products of the GAPAID project, the following technological results were obtained:

1) A prototype device for the evaluation of genetic markers for RA was developed by Progenika (Bilbao, Spain).
2) A prototype device for the evaluation of genetic markers for SLE was developed by Progenika (Bilbao, Spain).
3) A serological array for the evaluation of RA biomarkers was developed by Diagnosticum (Budapest, Hungary).
4) A serological array for the evaluation of SLE biomarkers was developed by Diagnosticum (Budapest, Hungary).
5) A computer-based workstation to match the genetic and serological data with the clinical status of RA patients was developed by Omnilab Iberia (Madrid, Spain).
6) A computer-based workstation to match the genetic and serological data with the clinical status of SLE patients was developed by Omnilab Iberia (Madrid, Spain).

Description of the potential impact, the main dissemination activities and the exploitation of results.

Description of the potential impact of GAPAID results

GAPAID project was aimed at developing new in-vitro diagnostic tools based on the evaluation of genetic and serological markers to improve the diagnosis of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). These conditions require expensive life-long treatment and care. Timely diagnosis and appropriate treatments can slow the progression of the disease and improve the patient's quality of life.

RA is one of the most common autoimmune disorders worldwide. Around half of RA patients are unable to hold down a full-time job within 10 years of onset. In RA the objective of therapeutic intervention is to control symptoms, prevent disability and avoid joint damage. The course of RA is highly variable and the commonly used step-up pharmacological strategies imply that a proportion of newly diagnosed RA patients could be inadequately treated for a specific period of time.

SLE is a multisystem disease affecting mostly women in childbearing age, and leading to impaired function of several organs, including the kidney. In SLE, the main goal of treatment is to prevent disease relapses and preserve organ function. The diagnosis of SLE must be based on the proper constellation of clinical findings and laboratory evidence. Management depends on disease severity and organ involvement. Periodic follow-up and laboratory testing are imperative to detect signs and symptoms of new organ-system involvement and to monitor the response or adverse reactions to therapies.

RA and SLE are multifactorial diseases caused by environmental stimuli acting on genetically predisposed subjects. The clinical diagnosis of RA and SLE is assisted by the use of in-vitro diagnostic tests aimed at evaluating the presence/level of several antibodies circulating in the patient's serum. However, this diagnostic approach is unsatisfactory because it can only assist in the diagnosis after the initial disease onset, is not useful for evaluating disease susceptibility for early prevention, and does not provide any information for monitoring disease progression for the setup of personalized therapeutic treatments.

The GAPAID scientific results allowed to identify genetic markers associated with the disease and with the serological markers, thus leading to an improvement of the capacity of diagnosing RA and of the capacity of diagnosing and monitoring SLE. In fact, the GAPAID scientific activities succeeded to detect the association of several genetic markers with the susceptibility to develop RA and SLE, or with related clinical features. The set up of the algorithms allowed to integrate the genetic and serological markers with clinical data. The algorithm defined for RA has a diagnostic predictive value. For SLE two algorithms were identified: one with a diagnostic predictive value and the other one able to predict the probability to belong to a more severe subset of the disease.

The GAPAID prototypes will provide to the clinicians a new instrument for the diagnosis of these diseases and for a deeper comprehension of the genetic markers. The proper evaluation of the genetic markers is extremely important for both RA and SLE that are multifactorial diseases whose etiology is based on environmental and genetic factors. An improvement in diagnosis of RA and SLE and treatment management in the case of SLE will avoid unnecessary tests, will limit adverse reactions and improve the overall outcome of patients, thus optimizing resources in Health Services and contributing to EU societal objectives.

The technological results allowed to set up a final prototype based on the innovative and cost-effective multiplex technologies and comprising a software for the easy adaptation to hospital information systems. Kits to be marketed will be supplied in an array-based multiplex format for the evaluation of the genetic and serological information. The multiplex format offers several advantages for reducing the costs for local public health. In fact, by this technology is possible to evaluate the presence of 9 biomarkers for RA and of 10-11 biomarkers for SLE in up to 14 patients in a single serologic assay. This will result for the local public health in money and/or time saving, depending on the clinical protocols internally set up for the diagnosis of such diseases. The use of multiplex assays will result also for the local public health in lower amounts of waste to

be disposed. In addition, the multiplex format allows the clinic to reduce the needed amount of blood, thus limiting the risk of infection in manipulations for the involved operators.
The set up of a software designed for the hospitals successfully translated the scientific results into a marketable product. GAPAID software is a powerful tool that allows the integration of the GAPAID diagnostic platforms in a clinical environment. The software helps the hospital laboratory end user with a clear workflow for controlling sample processing through all steps and assists clinicians to extract valuable information on the data collected for each patient.

Description of the exploitation of GAPAID results
The GAPAID scientific results were exploited by the development of two in-vitro diagnostic tools for RA and SLE. For each disease, the final prototype contains: a serological kit, a genetic array, and a software.
The GAPAID companies that will be responsible of manufacturing the single components are:

- Diagnosticum (Budapest, Hungary) for the serological array;
- Progenika (Bilbao, Spain) for the genetic array and the software.

The 3 components will be assembled in a unique kit by Progenika.
Possibly, the serological and the genetic arrays will be separately provided.

In the case of RA, the diagnostic test is intended mainly for rheumatologists, in order to help them make an early and proper RA diagnosis in the clinical practice, enabling the patient to begin treatment earlier. Targeted customers will, thus, include major specialized hospitals, both public and private, as well as major testing labs. Trained technicians and specific instrumentation is required.
There are currently quite a few players in this segment, with companies based throughout the world. None of these companies, however, commercializes any product that comprises the simultaneous detection of serological, genetic, and clinical factors.

Regarding SLE, the diagnostic test is intended mainly for rheumatologists, in order to help them make an early and proper SLE diagnosis in the clinical practice, enabling the patient to begin treatment earlier, and in order to recognize between mild or severe cases, enabling personalized treatments. Targeted customers will, thus, include major specialized hospitals, both public and private, as well as major testing labs. Trained technicians and specific instrumentation is required.
Recently there are several ANA screen or dsDNA-IgG ELISA tests on the market (AELUSKU, IBL, IMMCO, Eurodiagnostica). BioRad has a multiplex test (Bioplex 2200) for ANA screen based Luminex technology. Nor the ELISA IVD test neither the Luminex-based test combine the serological, genetic and clinical data to diagnose SLE and recognize the mild or sever cases. GAPAID may be a unique on the market in the field of SLE diagnosis.

Before any marketing efforts are undertaken, the two final GAPAID products will require a clinical validation since the kit has been developed using a limited population of samples from only two countries (Hungary and Italy). To do this, Progenika is pursuing agreements with major rheumatology referral centers in Europe, North America and Asia to validate the tool in these populations. Progenika has a wide network of research and healthcare centers that collaborate in the clinical validation process. These centers supply Progenika with well characterized human samples relevant for the overall research procedure. Cooperation reaches a wide range of areas that go from the overall design of the validation process to data interpretation. Progenika's international network includes hospitals in France, Germany, Holland, Italy, Spain, Sweden, UK, Austria, Czech Republic, USA, Mexico and Japan.

Progenika aims to adapt its distribution strategy for each product and geography in which the Group is present. Progenika's sales and marketing effort comprises several distant but related fields, from institutional marketing effort to distribution agreements with large pharmaceutical and diagnostic companies.

A description of the Progenika and Diagnosticum that will manufacture the GAPAID products is herein provided.

Progenika Biopharma, S.A. is a Spanish biotech company devoted to the identification and validation of genes and gene products involved in human disease, and to the development of new diagnostic and prognostic methods for their detection. The company, headquartered in Derio, Bizkaia (Spain), was founded in 2000 and has a subsidiary in Medford, Massachusetts (USA). Since 2013, it is part of the GRIFOLS Group.

Progenika focuses on complex diseases such as cancer, inflammatory, and cardiovascular diseases. It also develops comprehensive genetic-based products in highly demanding areas such as blood typing. Its technology facilitates the simultaneous analysis of multiple genes, proteins and environmental factors with the following advantages:

- provides an early diagnosis
- optimizes time and costs for all parties involved
- allows a better prognosis
- predicts and monitors the response to different drug treatments

With a staff of more than 80 highly qualified academic and scientific professionals, Progenika has extensive experience in design, development and manufacturing of products following highest quality standards, and maintains a considerable effort on investment in R&D for new innovative applications. Progenika´s R&D facilities are located in the Technological Park of Bizkaia, where the company holds over 1000 m^2 that comprise laboratories for functional genomics and proteomics. Equipment includes Affymetrix® platforms, Fluidigm®, Infinium® HD Beadchips, Illumina®, and TaqMan® technologies, GS Junior System, Genome Analyzer II, HiScan™SQ System, Luminex® 200, and HS 4800™ hybridization stations. The facilities include array printing equipment in which microarrays are produced under high quality standards ISO9001, ISO13485 and TUV certification, and the procedure complies with CE requirements, laws and regulations. These technologies have been used to develop new diagnostic and prognostic products, of which some examples include: BLOODchip® reference, a solid array for the genotyping of human blood groups, the first extensive blood group genotyping test obtaining CE mark; IDCORE+®, IDCORE® and IDHPA®, liquid arrays (xMAP® technology) for the identification of RBC and HPAs antigens, respectively; or LIPONext®, for routine clinical diagnosis of Familial Hypercholesterolemia by detecting mutations involved in cardiovascular diseases using High-Scale Massive Sequencing. The following table shows line of products currently under commercialization.

Area	Description	Products
Blood Group Genotyping	Genetic identification panel for the main allelic variants of RBC Groups and Human Platelet Systems	IDCore XT IDCore HPA BloodChip Reference BIDS XT Blood Services (CLIA Lab)
Cardiovascular	Genetic Diagnostic of cardiovascular genetic disorders like FH or LPL deficiency	Seq Pro Lipo RS LPLChip
Autoimmunity (monitoring of biologicals)	Protein-based test to monitor efficacy of Biological Drugs by measuring drug levels and ADA appearance	Promonitor IFX Promonitor ADL Promonitor ETN Promonitor RTX
Pharmacogenetics	Genetic Panel to detect polymorphism associated with the transport and metabolism of certain drugs	PharmaChip BrainChip
Preventia	Genetic Analysis to evaluate predisposition to different diseases (Cardiovascular, oxidative stress, etc)	Healthia Cardia

Diagnosticum

The company was established in 1989 as a Swiss-Hungarian Joint Venture Company to manufacture biochemistry reagents and immunology-based kits.

Diagnosticum is focused on full laboratory service and offers innovative chemistry solutions to meet the consumer demands.

Diagnosticum activities are:

- manufacturing and distribution of biochemistry reagents including control and calibrator
- distribution of clinical chemistry analyser
- production of turbidimetric kits for determination of serum proteins
- immunodiagnostics for veterinary use
- food allergy and intolerance tests
- distribution of ELISA tests for hormones, serum proteins
- distribution of laboratory equipment and consumables.

Diagnosticum distributes product on central-east Europe and also Far East such as Vietnam and Sri Lanka.

The R&D laboratory of Diagnosticum is focused on the research area of development, validation and production of new serological protein immunoassays. The members of the R&D laboratory of Diagnosticum have expertise in the production of monoclonal antibodies, development of immune-based methods and tests, validation of IVD products (ELISA kits, protein microarray).

The main research topics are:

i. development and validation of IVD ELISA tests,
ii. development of multiple protein (antigen) microarrays,
iii. development of point-of-care diagnostic medical devices (based on microfluidics and SPR methods).

The R&D laboratory has a service-based part for diagnosis allergy for drugs.

Based on the results of our previous research projects 8 CE marked human IVD ELISA tests and 2 CE marked veterinary diagnostics kits are manufactured and marketed.

IVD kits are manufactured under quality standards ISO 14001:2004 and ISO 9001:2008.

Description of the main dissemination activities of GAPAID results

Since the beginning of the project, the dissemination activities were aimed at ensuring the correct exploitation of the industrial results of the project only after the protection of the intellectual property rights (IPRs). In fact, the priority of the GAPAID dissemination strategy was first to protect the IPRs of the Foreground generated during the project, and then to proceed with an appropriate dissemination of the project outcomes. The first step of verification of any IPR to be protected was planned to guarantee to the SME partners the market benefits of the inventions produced by the GAPAID project. Only after the IPR assessment, the GAPAID Consortium proceeded with the disclosure of the scientific and/or technological results by means of an appropriate dissemination tool.

Some results of the GAPAID projects will be kept confidential for 3 years to adequately protect the IPRs and to ensure the product commercialization.

During the GAPAID project, two workshops were organized in Bilbao and in Budapest by Omnilab Iberia (Madrid, Spain) to train the personnel of the SMEs involved in the project and to explain to the end users the characteristics and the specificity of the developed software.

The dissemination of the GAPAID results proceeded and will continue after the end of the project through the publication of articles on relevant international peer reviewed scientific journals and by oral or poster presentations at scientific meetings, congresses, and trade fairs.