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Publishable Summary Report (Month 48)

Project context and objectives:

For patients with end-stage renal disease, transplantation has become the preferred treatment, providing better survival than prolonged dialysis, in both adults and children. In renal allograft recipients, graft survival at one year post-transplantation has improved to more than 95%. However, at 10 years, it is only approximately 65-70%, with no major improvement with regards to the previous decade. Also, although acute rejection (AR) is a major risk factor for the development of chronic allograft nephropathy leading to graft loss, decreases in the incidence of AR in recent years have not translated into improved long-term outcomes. Both immune mechanisms (Tcell mediated and antibody-mediated rejection, de novo or recurrent glomerular disease, etc.) and nonimmune mechanisms (nephrotoxicity of calcineurin inhibitors, accelerated aging, epithelial mesenchymal transition, etc.) contribute to the progression of chronic histological damage and scarring of renal allografts. These injury processes jeopardize graft function and long-term graft survival. As only a subset of patients develop chronic injury and because at present physicians do not have the ability to reverse chronic fibrotic kidney damage, it is essential that the transplant community develops reliable and noninvasive approaches to predict which patients are most likely to develop graft failure, so that appropriate interventions can be instituted before this becomes clinically apparent.

The practical objectives of BIOMARGIN are to:

Discover, select and validate:
(1) blood and/or urine biomarkers, **at different omics levels**, of renal allograft lesions, with good diagnostic performance as compared to biopsy histological analysis ('Gold standard'); (2) mechanism-based classifiers of graft lesions, including intragraft mRNA or miRNA as well as lipid, peptide and protein localization within the graft, to help histological interpretation of the biopsy; and (3) early biomarkers of chronic graft dysfunction and ultimately graft loss, less invasive than graft biopsy and with improved predictive values of longterm outcome.

Provide clinicians with tools (analytical techniques, interpretation algorithms, a dedicated website) to obtain such information in a timely manner, and promote these innovations towards scientific societies and patient associations.

Set-up a research environment for further biomarker research in transplantation.

The BIOMARGIN consortium employs an innovative strategy tackling several complementary –omics and mass spectrometry imaging approaches. The biomarker candidates will be selected and integrated using molecular biology, computational biology, statistics and disease progression models.

Description of the work performed and main results since the beginning of the project (up to M36)

❖ **The objectives of the first 18-months period were mainly to:**

(1) Collect, distribute and analyze triplets of blood, urine and kidney biopsy samples from ca. 120 renal transplant patients, with normal biopsy, or histological signs of T-cell mediated rejection (TCMR), Antibody-mediated rejection (AbMR) or Interstitial Fibrosis/Tubular Atrophy (IFTA).

(2) Write the protocol, patient information and consent forms of the BIOMARGIN European Cohort Study (BECS), translate them into the different languages of the Partners' member state, submit them to the national authorities and national/regional/local ethics committee (as appropriate) and obtain the signatures of the transplant centers members of the consortium to participate in this study.

The plan was to select banked urine, blood and biopsy samples collected as usual practice in the 4 Biobanks participating in the project (APHP Necker #3, KU Leuven #6, MHH #9 and CHU Limoges #13). However, when reviewing the results of the first exchange of test samples for the purpose of quality control, an unexpected disparity of sample collection and preparation conditions was discovered, resulting in a risk for the reliability of analytical results. Then, after a long discussion among the consortium, a SOP was written to harmonize sample collection, preparation and storage. As a consequence, sample collection started late leading to an overall delay of 10 months which has impacted all the work-packages.

In parallel, all the activities planned by CARDINAL SYSTEMS #10 for the clinical trial 3×BIOS^2 were finalized:
- Regulatory and ethical application of the case-control & trans-sectional and cohort studies
- Central reading of the first set of preselected biopsy slides
- Anonymization and shipment of the first set of samples from the 4 biobanks to the 8 labs involved
- Development of an electronic Clinical Research File (eCRF)
- Preselection and final selection (after central reading by expert pathologists) of 134 sample triplets (urine, blood, biopsy core) collected in the standardized conditions
- Quality monitoring of the samples and of the clinical research files
- Sample shipment to the 8 laboratories on May 13, 2014

The analytical tasks performed for step 1 of 3xBIOS^2 study ("training set") for biomarker discovery are as follows:
- Urine protein and creatinine concentrations were measured in all samples by INSERM #1a
- Untargeted microRNA profiling in blood was completed by INSERM #1c.
- The extracted RNA samples were subsequently sent by INSERM #1c to KU Leuven #6 and= mRNA expression analysis is on-going
- Untargeted analyses of microRNA in urine: 119 samples underwent microarray analysis by INSERM #1b (11 were disqualified, as they did not meet the QC criteria). All raw data and QC reports were sent to CEA #4 for statistical analysis
- Untargeted analyses of mRNA in 120 urine samples by INSERM #1c had begun (10 samples disqualified after quality check)

- Untargeted analyses of peptides in urine was successfully performed in all samples except one by MOSAIQUES DIAGNOSTICS Gmbh #8 using CE-MS, and in 48 samples by INSERM #1a using MALDI-TOF/TOF. The rest of the analyses, as well as all analyses by NanoLC-ESI-HRMS by VITO #7, had begun.
- Untargeted analyses of proteins in urine: half of the proteomic analyses were performed by INSERM #1a, using NanoLC and MALDI-TOF/TOF.
- Untargeted analyses of lipids in urine: after method comparison, optimization and validation, UNIVERSITE PARIS DESCARTES #11 analyzed all samples and detected, annotated using an on-line database, and semi-quantified many lipids of different categories in a large range of concentrations.
- Untargeted analyses of metabolic biomarkers in plasma and urine: 3 different GCMS methods were developed. The development of scripts for MS data preprocessing was on-going. Due to missing the initial time-slot, analyses were postponed.
- Untargeted analysis of mRNA and miRNA gene expression in biopsy samples: KU Leuven #6 extracted 131 biopsy samples and evaluated RNA quality and concentration. mRNA of 118 good-quality samples was amplified and hybridized onto microarrays. Microarray gene expression profiles were then calculated and normalized. At month 18, the extracted RNA samples were subsequently sent for miRNA expression analysis to APHP Necker #3.
- Mass-spectrometry imaging of renal allograft biopsies: specialized staff training was delivered and reproducible and efficient methods developed and validated, for sample preparation and analysis by MALDI-TOF (lipids, proteins) and TOF-SIMS (lipids).

Data integration and disease prediction modelling:
The "Prototype “2BIO-DB” biomarker database for data and metadata collection" was built by CEA #4 and sent to INSERM #1a at M12. The database is currently being filled with 120 miRNA biomarkers from the literature by APHP Necker #3. Proteins and mRNAs will follow shortly. In parallel, the database is currently being implemented as an online resource by INSERM #1a. 2BIO-DB will now be appended up to M48 with the new biomarkers discovered during the project.
A genetic data mining algorithm was applied on a dataset previously published by MOSAIQUES DIAGNOSTICS GMBH #8. At M17, CEA #4 received the first Biomargin datasets, of miRNA and peptidomics in urine. Preliminary univariate and multivariate analyses of both datasets was performed.

Longitudinal evaluation of the diagnostic and prognostic performance of selected biomarkers:
The Biomargin ambispective European Cohort Study (BECS) protocol was discussed with, and agreed upon, by all the clinical Partners and presented at the Consortium annual meeting in March 2014 to have a feedback from the other Partners and the Advisory Board. In parallel were written information and consent forms for adult patients, for parents/legal guardians of children and for the children of different age groups themselves. Each form was translated into the languages of the clinical Partners, who further corrected them.

Finally, the protocol and associated consent and information forms were submitted for regulatory approval in France, Germany and Belgium between May and August 2014. However, as BECS includes a retrospective part for patients with samples already banked as part of usual clinical care, the pre-screening since month 6 of BIOMARGIN identified approx. 50 such patients who were eligible to enrolment as soon as approvals are obtained.

The Clinical Research File for BECS study was finalized in July 2014 and its electronic CRF was in preparation.

- **The Objectives of the 2nd part of the project were mainly to (M18-M36):**

1. Collect, distribute and analyze a second and third sets of triplets of blood, urine and kidney biopsy samples, corresponding to steps 2 and 3 of 3×BIOS^2.

2. Obtain regulatory approval for the BIOMARGIN European Cohort Study (BECS), enroll 450 adult and 50 pediatric patients and collect urine and blood samples at predefined times post-transplantation, as well as prior to any graft biopsy (together with a biopsy core for biomarker analysis).
3. Measure the expression of mRNA, miRNA, peptides, proteins, lipids and metabolites of the "extended list" in blood (mRNA, miRNA and metabolites), urine (all) and biopsy (mRNA, miRNA, lipids and proteins) of the selection set (step 2) and trans-sectional set (step 3) of the 3×BIOS^2 study
4. Set up of quantitative, validated reference techniques for the determination of the candidate biomarkers in the relevant matrices.
5. Propose an extended list of biomarker candidates through pathway analysis; validated methodology and corresponding software tools for biomarker consolidation; predictive models using a restrictive list of biomarkers and clinical covariates

❖ **Description of the work performed and main results:**

(1) Clinical transfer logistics and management: step 2 followed the same case-control design as step 1, where samples were collected from ca. 120 renal transplant patients, with normal biopsy, or histological signs of T-cell mediated rejection (TCMR), Antibody-mediated rejection (AbMR) or Interstitial Fibrosis/Tubular Atrophy (IFTA). Step 3 was a trans-sectional study where the first consecutive 450 triplets of samples accrued to the consortium's biobanks after a predefined starting date had to be analyzed. All the activities planned by CARDINAL SYSTEMS #10 for the clinical trial 3×BIOS^2 were finalized:

- Regulatory and ethical application of the case-control & trans-sectional (3xBIOS^2) and cohort (BECS) studies
- Central reading of the second and third sets of biopsy slides
- Anonymization and shipment of the second and third sets of samples from the 4 biobanks to the 8 labs involved
- Development of an electronic Clinical Research File (eCRF) for the steps 2 and 3 of 3×BIOS^2
- Preselection and final selection (after central reading by expert pathologists) of 138 sample triplets (urine, blood, biopsy core) collected in the standardized conditions at step 2 of 3×BIOS^2
- Shipment of 458 unselected sample triplets (urine, blood, biopsy core) collected in the standardized conditions at step 3 of 3×BIOS^2 (with central reading by expert pathologists in parallel)
- Quality monitoring of the samples and of the clinical research files
- Sample shipment to the 8 laboratories on months 26-27 for step 2 samples and on months 33-35 for step 3 samples

(2) Longitudinal evaluation of the diagnostic and prognostic performance of selected biomarkers:

- BECS regulatory approval was obtained in France, Germany and Belgium between 6 June 2013 and 30 Aug 2013.
- At the end of this second study period, 574 adult patients, but only 15 pediatric patients had been enrolled. The time-period of children enrolment was prolonged, as well sample and clinical data collection in all patients over the third study period.

(3) The analytical tasks performed for step 2 of 3xBIOS² study ("selection set") for biomarker discovery are as follows:

- Urine protein and creatinine concentrations were measured in all samples by INSERM #1a
- mRNA and microRNA profiling of blood samples: after arrival of step 2 samples on month 26-27, partner #1c extracted the 138 Paxgene® blood tubes selected, verified RNA quality and concentration using the Nanodrop technology and an Agilent 2100 BioAnalyzer. All samples were ready on month 36 for the quantification of blood mRNA and miRNA biomarker candidates from the extensive list defined in WP7. However, the final

assessment of miRNA and mRNA biomarker candidates (extensive list) in samples from the Selection set was only scheduled for Month 40. 458 Paxgene® tubes from the trans-sectional study (step 3) were sent to partner #1c between Month 33 and Month 35. RNAs were purified and their quality was assessed as described on Month 37. Aliquots of RNA samples from both Step2 and Step3 were sent to Partner #6 on Month 37.

- mRNA and microRNA profiling of urine samples: similarly, on month 26-27 138 urine samples were received by partner #1b and 135 by Part #1c; miRNAs and mRNA extraction was completed by month 28. miRNAs and mRNAs were to be quantified by Month 40. 456 urine samples from the cross-sectional study (step 3) were received by Partner #1c between months 33 and 35. RNA purification of these samples was ongoing on month 36.
- Untargeted analyses of peptides in urine: Since the evaluation of statistical data of the Training Set (3xBIOS^2 step I) was lagging behind and in order to avoid postponing analysis of the independent Selection Set (step II), it was decided by all WP4 partners at the beginning of the second reporting period that Part 1a (INSERM Limoges) and 7 (VITO) use also untargeted shotgun peptidomics/proteomics instead of switching to their targeted MRM techniques. All partners of WP4 therefore used the full data sets of the Selection Set samples to validate the peptide and protein marker candidates included in the extensive list of step I that was previously submitted to the EC as Del. 4.1. Untargeted analysis of the Selection Set was accomplished by all WP4 partners and proteomic data sent to partner 4 (CEA) for data integration and disease prediction modelling, as part of WP7. Moreover, each WP4 partner started its own evaluation to identify the most valid peptide/protein biomarkers indicative of the different biopsy-defined renal allograft injuries.
- Untargeted analyses of lipids in urine: because step 2 samples were received before the statistical results of step 1 it was decided, like in other WPs, to perform a second untargeted lipidomics analysis on this second series of samples. LC-MS analyses of the 137 urinary extracts were performed in one batch. The complete set of data files was sent to partners 4 (WP7) for statistical analysis on month 33.
- Untargeted analyses of metabolic biomarkers in plasma and urine was performed by partner 12 on urine and plasma samples from the selection set (step 2) using both gas- and liquid- chromatography mass spectrometry (GCMS and LCMS). Standard operating procedures were used for metabolite extraction, MS analyses and data processing. Separate extraction methods were developed for plasma and urine. Data processing was performed using in-house mass spectral libraries and custom scripts. Multivariate data analyses were performed. As the most discriminative metabolic differences were found using GCMS, it was decided that GCMS should be used to analyze plasma and urine samples from the selection set (step 2). No clear Biomarker candidates were found in urine, while in plasma a number of pathways were identified that need further support from the proteomics studies. After validation using proteomics, the development of targeted methods for a number of specified biomarker candidates related to the identified pathways still had to be done.
- messenger RNA and microRNA profiling of biopsy samples: Allprotect® samples from the Selection set (step 2) were sent to Partner #6 on month 26-27. Partner #6 extracted all Allprotect® tubes that were selected for step 2 (N=137). After extraction, RNA quality and concentration were evaluated using the Nanodrop technology. All samples were ready on month 36 for the quantification of biopsy mRNA and miRNA biomarker candidates from the extensive list defined in WP7. The assessment of miRNA and mRNA biomarker candidates (extensive list) in samples from the Selection set could not be done before the end of this period and was scheduled for month 40. The 319 Allprotect® samples from the trans-sectional study (Step3) were received by Partner #6 between months 33 and 35. RNAs were purified and their quality was assessed as described. Aliquots of RNA samples from Step2 and Step3 had to be sent to Partner #1c on Month 37.

- Mass-spectrometry imaging of renal allograft biopsies: During this second reporting period, 10 human kidney biopsies (one control sample, two normal transplant kidneys and 7 rejection samples) were evaluated using ToF-SIMS (Time of Flight-Secondary Ion Mass Spectrometry) and MALDI-TOF/TOF (Matrix Assisted Laser Desorption Ionization- Time of Flight) in parallel. The first provides the localization of elements and lipids, and the second of lipids, peptides and proteins. The data showed interesting preliminary findings.

(4) Data integration and disease prediction modelling: Period 2 was mainly devoted to the following tasks: biomarker consolidation and extended list of biomarkers and statistical data integration and restricted list of biomarkers. CEA (Part 4) received the step 1 datasets between July 2014 (M17) and February 2015 (M24) and then performed statistical analyses using the first version of the pipeline mainly developed during the first phase of the project. The first results presented during the annual meeting in Hannover (March 2015) and the discussion with the clinicians showed that it would be better to take into account a mixed phenotype definition of the samples for the statistical analysis, instead of the "single diagnosis category" since a proportion of samples exhibited mixed phenotypes. Therefore, a second version of the pipeline was developed and the statistical results for each dataset presented during the statistical analysis Workshop held in Paris in October 2015. Since then, Partner 4 received eight step2 datasets, the statistical analyses were carried out with the same pipeline and comparisons were made between the relevant variables of step1 and step2 in order to generate a restricted list of biomarkers.

Objectives, work performed and main results of the third period (12-month period from M36-M48):

The European Commission has granted the BIOMARGIN programme a one-year prolongation, as per their decision in August 2016. This third report is thus an intermediate description of the achievements obtained between months 37 and 48.

❖ **The objectives of the third period were mainly to:**

- finalize the tasks related to step 3 of 3xBIOS² study ("Trans-sectional study") and validate the biomarkers previously selected in the first two steps of the 3xBIOS² study,
- advance the tasks related to the BECS study (step 4) and the longitudinal validation of the different sets of candidate biomarkers.

❖ **Description of the work performed and main results:**

(1) Clinical transfer logistics and management and longitudinal evaluation of the diagnostic and prognostic performance of selected biomarkers

With regards to the trans-sectional study (3xBIOS² step 3): the results of blind centralized histological reading of graft biopsies by expert pathologists (after adjudication of discrepant results by consensus between the experts) was reported to the Partners on month 46; the clinical data was cleaned and the clinical database sent to all partners on month 47. The biopsy slides were returned to the clinical sites at months 47-48. Monitoring visits for the 3xBIOS² study step 3 were performed at CHU Limoges on month 42, and at Necker hospital on months 40 and 44. After this, the 3xBIOS² study was considered as completed but no close-out visit was performed on the 4 clinical sites as the same participate in the BECS study.

Enough adult patients have been enrolled in the BECS study and recruitment was stopped, while recruitment of paediatric patients is prolonged to try and reached the target of 50 individuals. A new version of the CRF and eCRF with minor modifications was provided by Partner #10 on month 44. As 116 biopsies from adult patients eligible to BECS (retrospective part) were used in 3xBIOS² steps 1, 2 or step 3, the corresponding histological data were entered in the BECS e-CRF by Partner #10. A specific Investigator Study File (ISF) for the BECS study was prepared and provided by Partner #10, following regulatory approvals, to each participating site (adult and pediatric teams).

Based on the results obtained at the first 3 steps, it was decided that the laboratory Partners involved in BECS (step 4) would be: Inserm Limoges #1a, Inserm Paris #3, KU Leuven #6, MOS #9 and possibly AcureOmics #12 if they wish. As per month 48, 2313 sets of blood and urine sample doublets and at least 1000 biopsy samples have already been collected. Dispatching of these samples had not yet begun by month 48. They are planned for months 51 and 57. In the meantime, more samples will be collected from the 645 adult patients and the 26 pediatric patients enrolled in the study up to Month 57 and from the next pediatric patients to be enrolled, and their clinical follow-up will be pursued up until end of December 2017.

(2) The analytical tasks performed for step 3 of 3xBIOS² study ("trans-sectional study") for biomarker discovery are as follows:

messenger RNA and microRNA profiling in blood and urine samples

miRNA biomarker candidates (restricted list) were determined in 458 blood samples by Partner#1c and data were subsequently submitted to partner #4 (CEA) for further data analysis on Month 48. Messenger RNA profiling was assessed in the same samples by Partner #6 and the raw and normalized gene expression data were subsequently submitted to partner #4 (CEA) for further data analysis on Month 47.

456 urine samples were received by Partner #1b on month 39 from partner 8 (MOS) for determination of 4 miRNAs, but had not yet been analyzed by month 48, as new amplification protocols and robotized analysis was being developed. Analyzes are planned for M51. The same urine samples were received by Partner #1c between Month 33 and Month 35. RNA purification was completed by Month 38. The samples were pre-amplified using a new pre-amplification protocol and 24 RNAs (including additional mRNA candidates identified from other platforms) were quantified by qPCR in all of them. Gene expression data were subsequently submitted to partner#4 (CEA) for statistical analysis on Month 48.

messenger RNA and microRNA profiling of biopsy samples

Step2 (Selection set): Aliquots of RNA samples from Step2 and Step3 were sent to Partner #1c on Month 37. The quantification of biopsy mRNA and miRNA biomarker candidates from the extensive list defined in WP7 was subsequently performed in samples from the Selection set between Month 39 and Month 44. The miRNA expression data were submitted to partner #4 (CEA) on Month 42, and the mRNA expression data on Month 44.

Step3 (trans-sectional study): Allprotect® samples from the trans-sectional study (N=319) were sent to partner #6 between Month 33 and Month 35. RNAs were purified and their quality was assessed as described. Measurement of miRNA and mRNA expression in the trans-sectional study samples is scheduled between M48 and M60.

Proteins and peptides in urine samples

By M48, all WP4 partners (#1a, #7 and #8) had completed the analyses of the selection set (3xBIOS², step 2) and provided absolute or relative concentrations in this urine sample set for the proteins and peptides of the extensive lists of biomarker candidates. During this third period, the WP4 partners improved their technological platforms (i.e. partner 1a by establishing a Q-TOF SWATH method for peptidomic analysis), or their raw proteome data processing workflow (i.e. partner 7 (VITO) by introducing the SWISS algorithm to fill up the gaps in the protein data matrix). The decision made at the beginning of the second reporting period that partners 1a (INSERM Limoges) and 7 (VITO) use also untargeted shotgun proteomics/peptidomics instead of switching to a targeted MRM technique turned out to be the right choice, since it was observed that the disease groups in this sample set were somewhat different from the respective disease groups of step 1 with respect to their proteomic/peptidomic composition. Subsequently, all WP4 partners analyzed the proteins and peptides of the restricted list in the trans-sectional study (step3) samples. The results were submitted to the EC and sent to Partner #4 for statistical analysis shortly after the end of this third reporting

period (M50). A LC-triple quadrupole method is now being developed by Partner #1a for the analysis of targeted biomarkers in the BECS prospective cohort samples.

Lipidomics and metabolomics analysis in blood and urine samples

Different adjustments were decided and approved for the period covering Months 36 to 48. Partner 11 designed a reference LC-HRMS technique for the quantification of 80 putative lipid biomarkers and analysed the 454 samples of the "trans-sectional study". The quantitative data are now being processed and will be used for assessing the diagnostic value of the 80 candidates by crossing them with the clinical and histological information.

For partner 12, a method for quantitative amino-acid analysis was implemented for the simultaneous quantification of 26 amino-acids in plasma. In addition to this, quantitative methods were developed for endogenous metabolites that might be used as rejection biomarkers. Furthermore, a draft priority application for a patent has been filed.

MALDI-TOF and TOF-SIMS imaging of kidney graft biopsies

In situ identification of peptide/protein/lipid biomarker candidates by MALDI-TOF imaging of kidney graft biopsies (in a subset of the Training Set) will be reached at Month 58.

(3) Data integration and disease prediction modelling:

The 3rd period of the project was mainly devoted to task 7.C "Statistical data integration and restricted list of biomarkers". At the end of the 2nd period, CEA had received 8 step 2 datasets from Partners #1a, #7, #8, #11 and #12. These datasets had been obtained from untargeted analyzes which means that the same set of variables was available in both step 1 and step 2 data. The first strategy was to apply the statistical pipeline developed during step 1 on step 2 datasets, in order to identify the variables that were selected in both steps as a restricted list of biomarkers. This strategy that corresponds to the intersection of results from step 1 and step 2 was used as a first approach.

Partner #4 also received step 2 datasets corresponding to targeted analyses, where the variables measured were the best identified during step 1. The strategy presented in the case of untargeted data is obviously not adapted for targeted analyses, so that different approaches were successfully developed. At this stage, the main objective was to test the restricted list of biomarkers on step 2 datasets, but also to identify relevant groups of variables as biomarkers signatures. As a further development, predictive models will be constructed on those signatures and evaluated on step 3 datasets (task 7D).

On Month 47 CEA received from Partner #10 the phenotype definition of the step 3 samples, as provided by the BIOMARGIN clinicians, as well as the first step 3 datasets. The next period of the project will be devoted to the last tasks of the work package.

Expected final results and their potential impact and use

The final goal is to provide renal transplant clinicians with innovative biomarkers enabling closer, more accurate, more predictive and/or less invasive monitoring of renal transplant patients than serum creatinine or graft biopsies. Such currently unavailable biomarkers will likely improve patient care and will have several positive impacts.

- Diagnostic tools:

The whole purpose of BIOMARGIN is to develop new biomarkers that will enable a closer monitoring of the graft in order to detect acute or chronic injuries earlier, which will translate into a more rapid intervention and hopefully better long-term outcome.

- Improve treatment outcome for transplanted patient:

Early and specific diagnosis of immunological or non-immunological allograft injuries is a major prerequisite for a successful intervention. The earlier therapy can be started, the greater the chances are to stop, or even reverse, the injury process and prevent irreversible scarring of the renal tissue. Based on this, we expect to better conserve renal tissue and function, thus prolonging allograft survival, which is currently limited to approximately 12 years on average. This is particularly important for pediatric patients, who are expected to need several transplantations during their lifetime to avoid prolonged dependence on dialysis.

- Better understanding of the mode of action of existing or potential treatments:

So far, the pathophysiology of progressive loss of allograft function has been poorly understood. There are only a few clearly defined allograft injuries, such as acute T-cell mediated rejection and acute/chronic antibody-mediated rejection, but the underlying causes of graft loss appear to be much more diverse. The use of different 'omics' technologies in BIOMARGIN holds the promise of delineating specific molecules and pathways in these processes, of immunological or nonimmunological origin, which can serve to define therapeutic targets.
Moreover, biomarkers may bring a new understanding of drug effects on the renal intra-cellular pathways and on biomarker levels that should help transplant clinicians select the best therapeutic option in a given situation and monitor its effects, using biomarkers. In addition, by unravelling signalling pathways involved in graft lesions such as fibrosis, BIOMARGIN should also open new paths for therapeutic interventions.

- Impact on graft outcome, patient survival and quality of life:

Owing to closer monitoring of the graft and faster reaction regarding the adaptation of individual patient treatment, the rates of renal graft function deterioration and of graft loss should be reduced.
At the present time, the therapeutic arsenal does not allow for the reversal of chronic antibodymediated rejection or allograft fibrosis, for instance. This is the reason why biomarkers with a strong predictive value would be important, as the most efficient therapeutic measures nowadays are preventive in nature. The general condition of the patients will improve, resulting in a better quality of life for both the patient and his/her entourage. Also, prolonged graft survival should translate into prolonged patient survival, as patients on dialysis have a shorter life expectancy than transplanted patients. Less kidney allograft recipients will come back on the waiting list for kidney transplantation each year, and consequently this will increase the total number of patients being transplanted and shorten the time patients spend on the waiting list, which in turn will also increase their life expectancy, as recently demonstrated.
In Europe, 50,000 to 100,000 patients have end-stage renal failure. Compared to dialysis treatment, for most patients kidney transplantation is better suited to regain health, quality of life, and the ability to pursue an individual and self-sufficient lifestyle. Approximately 18,000 kidney transplantations are performed annually in Europe. However, this figure is far exceeded by the number of patients on the waiting list for renal transplantation. Improving the success rate of transplantation by prolonging the allograft survival would contribute to have less second or third renal transplantations, hence more new patients transplanted, instead of being dependent on dialysis.

- Socio-Economical Impact:

The BIOMARGIN non-invasive biomarkers will reduce the need for, and the costs related to, graft biopsies. Extended graft survival will also result in less patients returning to dialysis, the cost of which is clearly much higher than the cost of transplant patient medical care, especially years after

transplantation. Also, shortening the time patients spend on a waiting list is cost saving. Finally, patients with a functioning graft are much more likely to be able work and to earn their living. A second important economic impact expected is for the European industry, especially the SME sector, through the commercialization of key-in-hand techniques for biomarker analyses and interpretation.
Finally, the involvement of patient associations (through the external advisory board and active communication actions planned) will help spread the results of this research quicker and to a wider community.

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