Final publishable summary report

Executive summary

The central focus of the BIO-DrIM project is the implementation of biomarker-driven strategies for personalizing immunosuppression (IS) in order to improve the long-term outcome and to decrease the adverse effects (graft toxicity, diabetes, cardiovascular events, opportunistic and community acquired infections, bone loss, and malignancies) and costs of chronic IS in solid organ transplant patients. The mission of BIO-DrIM reflects the new strategy in the field of solid organ transplantation: leaving the path of one-size-fits-all weaning strategies and administration of new drugs in favour to a more personalized approach.
The biomarker-guided management of immunosuppressive therapy is an ambitious program that started time ago with other projects (IOT and RISET) in which a set of promising biomarkers was identified and developed, but the methodical validation was missing. Implementation of these biomarkers into the clinical routine requires multiple cross platform tests, high quality standardization, multicenter clinical validation and with this an international network. All these requirements meet up in the BIO-DrIM project, that includes 5 innovative clinical trials (~2.000 patients in screening phase and ~800 patients enrolled in trials).

More in detail, the BIO-DrIM project addresses several highly innovative issues:
- First IS withdrawal study in long-term liver transplant patients based on the presence of a molecular tolerance signature (personalized withdrawal – study 1);
- Two novel trials on systematic partial IS withdrawal in selected highly stable long-term kidney transplant patients for validation of decision making biomarkers to detect operationally tolerant patients (personalized withdrawal - studies 2+3);
- First controlled biomarker-driven perioperative stratification of kidney transplant patients into low/high responders to prevent high-dose standard IS in low responders (personalized minimizing IS – study 4)
- Novel approach to target selectively activated (allospecific) memory/effector T cells (to increase the proportion of low responders after kidney transplantation suitable for low-dose monotherapy – validation by biomarkers – study 5

Safety is a key issue of the clinical trials: all studies are performed according to the international rules and all drugs are used according to their applications.

In addition to the well-validated set-1 biomarkers used for decision-making, all trials are accompanied by a panel of standardized biomarkers (set-2a) and a panel of exploratory biomarkers (set-2b):

i) to further improve the predictive value of the biomarkers for patient stratification
ii) to learn more about the mechanisms behind success/failure of IS minimizing.

Gaining knowledge about the mechanisms behind successful weaning (regulation/effector balance) is also part of the project. Well-defined *in vitro* and experimental mouse/rat transplant studies are used to address questions regarding the mechanisms of success/failure of IS minimizing. In

particular, the scientists within BIO-DrIM address the interaction between regulatory pathways and donor-reactive memory/effector T cells.

The research and trials in BIO-DrIM are strongly translational. The involvement of SME´s and pharmaceutical industry guarantees a fast commercialization of promising product candidates.
Furthermore, health-economic studies push forward the translation into products used at the market as reimbursement strategies can be early developed and discussed with the health insurance companies and the government authorities.

Summary description of project context and objectives

FIVE MULTICENTER CLINICAL STUDIES

LIFT Study (WP1a; biomarker-based weaning of liver transplant patients): Biomarker-guided IS weaning strategy in long-term (>3yrs) liver transplant recipients by identification of operationally tolerant patients

Previous weaning studies have demonstrated that about 25-40% of selected liver transplant patients after year 3-6 post transplantation develop operational tolerance and can have their maintenance immunosuppression discontinued. However, in the majority (60-75%), standard immunosuppression weaning fails and they develop rejection. The biomarker being developed and validated by the BIO-DrIM consortium, which is a gene expression test using liver tissue, is key to accurately stratify patients before considering immunosuppression withdrawal.
The **LIFT study**, a biomarker-driven trial coordinated by KCL, involves long-term stable liver transplant patients who are randomized to immunosuppression weaning without taking into account the result of the biomarker (Arm A) or to biomarker-guided weaning (Arm B). The trial is currently open in 12 centres in 4 EU countries and now, there are 190 consented patients, which are or have undergone screening, and 105 of them have been randomised. The first interim analysis of the trial took place in 2018 and confirmed the clinical utility of the 5-gene transcriptional biomarker. The final analysis of the primary outcome is expected to be completed in 2021.

WEANING trial (WP1b; weaning of long-term stable kidney transplant patients): **Targeted partial/complete weaning of standard IS in long-term stable kidney transplant patients characterized as low-responders and identified as putative "operationally tolerant" by recently developed biomarker panel.**

The fully blinded **WEANING** trial, coordinated by ITUN (Nantes), aimed at improving the graft function in clinically selected Highly Stable (HS) patients following complete weaning of calcineurin inhibitor (CNI). In December 2014, the group decided to stop the study due to insufficient patient recruitment. The results of the WEANING Study were published in 2016 (*Am J Transplant* **2016**; 16: 3255–3261).

GAMBIT study (WP1c):
the group of KCL defined and validated a new gene expression signature that is independent of drug effects and also differentiates tolerant patients from healthy controls (cross-validated area under the

receiver operating characteristic curve [AUC] = 0.81). In a prospective cohort, it was demonstrated that the new signature remained stable before and after steroid withdrawal. In addition, a validated and highly accurate gene expression signature, that can be reliably used to identify patients suitable for IS reduction (approximately 12% of stable patients), irrespective of the IS drugs they are receiving was reported. The results of the studies were published in December 2016 (*Am J Transplant.* **2016;** 16: 3443–3457, PMID: 27328267).
Data obtained will be merged for ontology analysis to define the best final test format.

CELLIMIN trial (WP2; Prospective donor-specific cellular alloresponse assessment for Immunosuppression minimization in de novo renal transplantationtrial using a biomarker as stratificator for IS).
The **CELLIMIN** trial has been approved by the **Voluntary-Harmonization Process (VHP)**. CELLIMIN is a non-inferiority trial with enrichment design aiming to demonstrate the utility of the IFN-γ ELISpot marker for the stratification of kidney transplant recipients into "low" and "standard-of-care" IS regimen.
All clinical sites participated in extensive lab and interlab comparision to validate the robustness and correctness of the IFN-γ ELISpot assay, the biomarker used as stratification tool.
184 patients have been screened with pre-transplant donor-specific IFN-γ ELISpot. 102 (55.43%) displayed a negative result and therefore underwent randomization. After randomization, 49 (48%) of patients received the "low" immunosuppressive regimen and 53 (52%) received the "High" immunosuppressive regimen. At 30th November 2018, 94 patients have achieved 12-month of follow up, 74 6-month follow-up and 16, 3-month follow-up. **Despite not having achieved the number of patients pre-specified in the trial, the relevant number of transplant recipients recruited in this study is already unique and thus, will allow for a relevant evaluation of the impact of using a novel biomarker to stratify patients in different immunosuppressive regimens. Also, the analysis of patients recruited but not randomized to different immunosuppressive regimens, this is, those showing a positive donor-specific IFN-γ ELISpot will enhance the understanding of the impact of cellular alloimmunity in the context of kidney transplantation.**

We will conduct a preliminary analysis of the whole recruited study population by next April 2019, when the last recruited patient will have been randomized. We will analyze the primary end-point with a minimum follow-up of six months after transplantation. Furthermore, we plan to perform the final analysis with the total number of patients randomized by next October 2019, when all patients will have achieved 12 months of follow up.

RIMINI trial (WP3, Tacrolimus after rATG and infliximab induction immunosuppression): Shifting kidney transplant patients to low-responders suitable for early IS minimization
RIMINI is an international multicenter open-label single-arm Simon's two-stage Phase II clinical trial aiming to estimate confidence interval for the observed efficacy of the induction regimen with rATG and infliximab and a go/no go rule for further clinical development. A total of 75 patients will receive the proposed induction regimen. To introduce IS-based on tacrolimus/steroids as early as possible without losing control of acute/chronic rejections would be of great benefit and could

reduce adverse effects and costs. However, this is only possible in a minority of patients yet. Memory/effector T cells are a major challenge. Therefore, increasing the pool of low-responders by selective targeting effector/memory T cells would have a big advantage.
Rationale: T-cell depletion is not sufficient to control allo-memory. ATG/Thymoglobulin treatment leads to a reduction of the clonal size of donor-reactive memory/effector T cells. Antigens, present in vivo, induce a bias of lymphopenia-induced proliferation LIP (preferential expansion of surviving alloreactive, Ag-specific Tem/Teff), which is an undesired effect. Here the combination of Thymoglobulin/Infliximab will help to reduce freshly activated naïve Tem/Teff to enlarge the pool of patients suitable for low IS.
Using tolerance/rejection biomarker monitoring we aim to prove shift from high responders to low responders while using infliximab as co-induction agent to target recently activated memory/effector T cells.
The initiation meeting was held in Berlin, on November 15th 2016. Enrolment into the RIMINI trial started in January 2017. At the end of 2018 56 kidney transplant recipients were enrolled into the RIMINI trial, 33 from Prague, 19 from Berlin and 4 from Barcelona. Thus, the recent enrolment rate is 75%. Not all intended clinical partners are currently enrolling patients for the RIMINI trial, which proved to be the main obstacle to reaching the expected numbers of patient enrolment. The principal reason is the enrolment into concurrent CELLIMIN trial. The study enrolment is still open and 3 participating centres enrol patients and will continue to do so beyond the end of the BIO-DrIM consortium. This is critical as only 25% patients (n=20) need to be enrolled into the trial to reach target population. Full set 12-months data evaluation is thus delayed and expected on June 2020 as we anticipate last patient to be enrolled in the trial in June 2019. Recently 20 patients completed the study with 12 months follow-up. 7 patients prematurely terminated the study. Recent number of efficacy failures (defined primary endpoint of the study) is 20 which anticipates acceptable efficacy and safety of the protocol and suggests non inferiority to the standard of care regimen.

VALIDATING SET-1 BIOMARKER TESTS READY FOR DECISION MAKING FOR THE ON-SITE PATIENT STRATIFICATION WITHIN THE CLINICAL TRIALS DESCRIBED IN WP1-3

Validated biomarkers (set-1) – as decision-making markers applicable for guiding minimizing IS:
- Molecular “tolerance signature” in liver biopsy of liver transplant patients
- Molecular “tolerance signatures” in peripheral blood of kidney transplant patients
- Donor-reactive IFN-γ ELISpot

The markers were selected in our previous studies performed in the EU funded projects IOT, RISET. The tests are well methodical validated now and already applied in the ongoing clinical trials.

Establishment of the ELISpot technique as stratification tool clinical trial.
10 ELISpot Readers were installed by Gen-ID at each local laboratory in the clinical sites of Berlin, Barcelona, London, Amsterdam, Regensburg, Hamburg, Prague, Nantes, plus Santander and Oviedo. Establishment at the local sites took place by extensive technology transfer, combined with training sessions and technical support.

VALIDATING RECENTLY ESTABLISHED SET-2 BIOMARKERS AND IMPLEMENTING NEW BIOMARKER CANDIDATES FOR IMPROVING PERSONALIZED IS WITHIN THE BIO-DRIM CLINICAL TRIALS

Methodically validated biomarkers (set-2A) – applicable for prospective validation concerning their clinical value:

- Molecular signatures in urine of kidney transplant patients
- Gene expression signatures diagnosing balance of innate and adaptive immune responses
- Immune cell subset composition profiling (Multiparameter flow cytometry)

New biomarkers (set-2B) – markers applicable for retrospective validation: e.g. quantification of new immune cell cell subsets (DC subsets, T-cell subsets, Treg subsets) applying our standardized multiparameter flow cytometry. Using our established platform, we have implemented such markers into the trials of BIO-DrIM for exploring their putative value in retrospective analyses.

Set-up and validation of Mulitparameter Flow Cytometry

Technical advice and equipment were provided and installed by Beckman Coulter - Immunotech at the clinical sites of six European countries. More precisely, 10-color Navios cytometers were provided by Beckman Coulter - Immunotech to the clinical sites of Berlin, Nantes, London, Barcelona, Prague, Amsterdam, Regensburg and Hamburg,

In a collaborative effort between Beckman Coulter – Immunotech and the Charite antibody panels in a ready-to-use dry format (DuraClone) were established, tested and validated. These panels are now commercially available.

ANALYSING THE HEALTH-ECONOMIC IMPACT OF BIOMARKER-GUIDED PERSONALIZED IS

Health-economic data demonstrated the usefulness of implementing biomarkers into the management of IS (personalized IS) concerning the cost/benefit ratio, performing health-economic analyses by using Micro Costing. The method used attempts to measure costs and benefits of service as accurately as possible, by including all fixed and variable costs of care at local prices, given the institutional structure within which service and care are being given. It also takes into account country-specific rules, such as fixed prices for diagnostic procedures (point system), drugs, and in/out-hospital service.

STUDYING THE MECHANISMS BEHIND SUCCESSFUL WEANING

The groups from Oxford, Nantes and Berlin worked together to develop a humanized mouse model of « Experimental system to investigate 'high responder' recipients who have pre-existing donor reactive memory cells ». This project contains two aims. The first one is to set up and validate in the three centres the human skin transplant model in humanized mice using the protocol developed by the Oxford group. Human immunoregulatory cell types have been administrated to humanized mice in this human skin transplant model to evaluate the efficacy of these cell types to control graft rejection. The second aim, performed by the Oxford group is to set up a new experimental system to investigate 'high responder' recipients who have pre-existing donor reactive memory cells in humanized mice. Oxford, Nantes and Berlin teams investigated the potency of their different human

immunoregulatory cell types to control graft rejection in 'high responder' recipients using this model.
In parallel, Nantes group continued their work on the potential of new therapeutics to control accelerated heart rejection in rats, a model recently developed by this team.

DISSEMINATING THE RESULTS TO SCIENTIFIC, PATIENT AND PUBLIC COMMUNITY (AND DEVELOPING COMMERCIALIZATION STRATEGIES BY PARTNERING WITH SMES/INDUSTRIES

The aim is spreading the knowledge and the results generated by the project to wider community of end users and to the scientific community. The results have been shared with the different communities and commercialization strategies have been put in place (diagnostic products, drug and biomarker diagnostic combinatory products, novel targets of IS drugs). GenID developed and meanwhile market several EliSpot IVD kits e.g. EBVSpot, CMVSpot and TransSpot as outcome of the project. The QC kit GenID established together with the partners is a fixed size in offering standardized controls in routine use. Moreover GenID set up a sample bank with different donors to offer this in clinical trials. These kits are CE IVD marked according European guidelines.
With the experience and the obtained expertice from BIO-DrIM project to run clinical multicenter studies with EliSpot, GenID decided to set up a GLP / CRO lab to offer this service to pharmaceutical companies.
Due to the project, GenID has hired two more scientists and secures the jobs of 25 people employed in production and development. In addition, another position in Regulatory Affairs is planned for 2019.
Sales in the EliSpot area (especially EBV and CMV kit) have increased continuously over the last few years by more than 15 % and (rouphly two million) account for more than half of GenID's sales.

For Milenia Biotec the BIO-DrIM project served as a networking platform, resulting in a Co-Development-Agreement with the big pharma for the development of tests to be used within the area of transplantation.

Regarding Beckman Coulter – Immunotech, the collaboration within the BIO-DrIM consortium has led to the commercialization of the 1st multicolor panels in the DuraClone format. Hence, a model of reagent development at Beckman Coulter has been built following the successful collaboration with the BIO-DrIM. Collaborators are directly involved in the development of reagents which led to the commercialization of nine additional DuraClone products following this approach.

Thus, an effort has been devoted to implementation activities in support of the SMEs and industrial partners involved for understanding the early prospective commercialization strategies for the biomarkers of the Consortium. As consequence a good collaboration between the groups ended up with exploitable results.