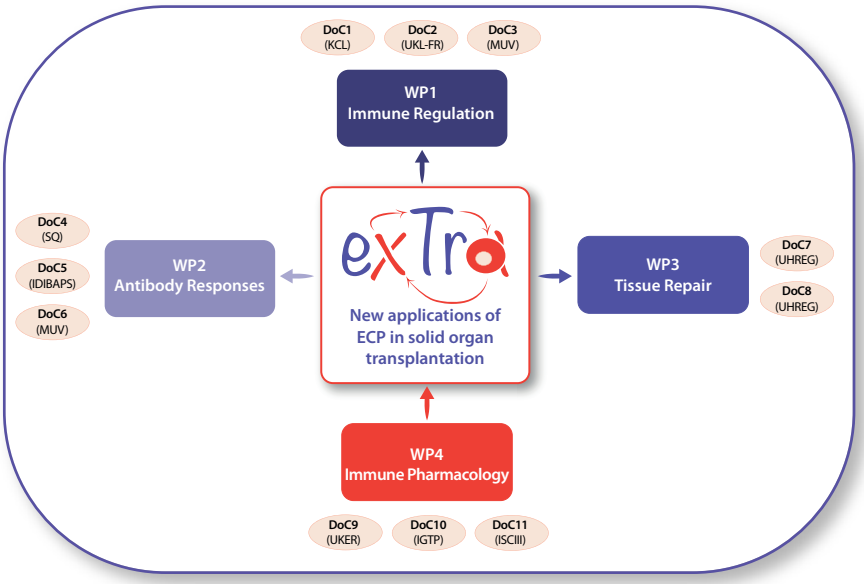
Marie Sklodowska-Curie Doctoral Network (MSCA-DN)

Innovative Applications of Extracorporeal Photopheresis in Solid Organ Transplantation

Call for applications for 11 Doctoral (PhD) Training Positions in basic and translational immunotherapy

Offer Description

exTra is a Doctoral Network funded by the European Union Horizon Europe Programme. The exTra Consortium is a research network of leading European scientists from academia and industry, experts in clinical transplantation, immunology, pharmaceutical development and medical device manufacture to address key questions on extracorporeal photopheresis (ECP) through a coordinated, interdisciplinary effort. exTra proposes 11 independent doctoral research projects with the ambition of providing its trainees with a comprehensive understanding of basic and translational immunology, especially relating to development and licensing of new immunotherapies. Through its research and training activities, the exTra project will contribute to scientific advancement and innovation in Europe, ultimately leading to societal and economic benefits stemming from clinical innovations in transplant immunotherapy and beyond.



Graduates of exTra will be well-prepared to enter the workplace with an innovative and beyond state-of-the-art view on fundamental and translational immunology especially relating to development and licensing of new immunotherapies.

Participating in exTra offers doctoral candidates many unique opportunities, including:

- A project as Marie Skłodowska Curie trainee in one of the participating institutions with the objective of receiving a doctoral degree (PhD).
- State-of-the art, exciting research in an international consortium with highly integrated research projects.
- Expert training in preclinical and clinical development of cell-based medicinal products.
- At least six weeks of research training in the lab of another consortium member, mostly in a different EU country than the country where most of the project will take place.
- Training in both academic and commercial research environments.
- Salary according to EU guidelines for Marie Skłodowska Curie trainees, including mobility payments and family allowances where applicable.

Skills, Qualifications and Project-specific Requirements

Project-specific criteria are detailed for each individual project.

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Benefits

- extra Doctoral candidates will be employed according to the rules for doctoral candidates in MSCA-DNs and the general regulations of each host institution.
- The financial package will include the monthly researcher allowances subdivided into 1) a living allowance of €3,400 (adjusted according to the relevant country correction coefficient), 2) a mobility allowance of €600, and 3) a family allowance of €660, if applicable. Employer costs and other deductions depend on recruiting host.
- Doctoral candidate will be given an EU-funded employment contract for 36 months by their host institution and will be entitled to full employee benefits and inclusion in social security schemes of the host country.

Eligibility criteria

Experience eligibility requirement

Eligible candidates must:

- hold a Master's degree or equivalent in a field of science relevant to their chosen project (see below)
- demonstrate a history of academic excellence
- demonstrate an affinity for **Basic Immunology** and **translational research**
- not already be in possession of a doctoral degree at the date of recruitment.

Mobility eligibility requirement

The fellow must not have resided in the country where the research training activities will take place for more than 12 months in the 3 years immediately prior to the recruitment date (and not have carried out their main activity (work, studies, etc.) in that country).

Other requirements

- Applicants must speak and write fluently in English
- Applicants should be available to start their project preferably not later than 1st October, 2023
- Applicants must be eligibile to work in the European Union.
- Other eligibility criteria may apply depending on the recruiting beneficiary.

Application Process

exTra will select Doctoral Candidates through a 2-step recruitment process.

The selection procedure will be open, transparent, and merit-based, fully aligned with the Code of Conduct for the Recruitment of Researchers. Although the selection will be based on the quality of applications, gender balance will also be considered.

Candidates can apply for **maximum three PhD projects** and the applications need to be submitted separately. Applications (in English) must include:

1) a **cover letter** which will also include the motivation for the position, emphasizing the candidate's strength regarding the project and the requirements (max 3 pages)
2) a **CV** (max 2 pages),
3) a scanned **copy of all relevant diplomas or certificates** that formally entitle the candidate to embark on a doctorate. Typically, these documents will include Batchelor's and Master's Degree certificates. In case the Master's Degree has not been obtained yet at the closing date for application, the candidate has to submit a declaration signed by their supervisor or University official stating that the degree will be obtained by the time of PhD enrolment.
4) **Letter of Recommendation** from two appropriate referees or contact details of two referees

Application documents in **a single pdf file** should be sent by email to Mrs. Christine Bayer, Project Administrator (christine.bayer@ukr.de) and to the relevant project supervisors (**see email address in individual project descriptions**). The subject line of the email must be in the following format: "exTra: application for Project#_Title of PhD project".

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The closing date for applications is 6th September, 2023

Applicants are advised to familiarise themselves thoroughly with the projects, for which they apply and be ready to answer questions on their chosen topics. After reviewing all project applications, supervisors of individual projects will contact selected applicants to organise an initial screening interview by telephone or videoconferencing. The most promising candidates may then be invited to a personal interview at the host institution or a further videoconference, potentially with several other project supervisors.

Research projects offered by exTra

ESR	Project Title	Primary Supervisor	Institution	EU State
1	Immunometabolic impact of ECP in transplantation	SAFINIA, Niloufar niloufar.1.safinia@kcl.ac.uk	King's College London (KCL)	UK
2	Modulation of NK cell and MRC responses after ECP therapy	ZEISER, Robert robert.zeiser@uniklinik-freiburg.de	Univ. Hospital Freiburg (UKL-FR)	DE
3	ECP induction therapy after lung transplantation	WEKERLE, Thomas thomas.wekerle@meduniwien.ac.at	Medical University of Vienna (MUV)	AT
4	Prevention of de novo donor-specific antibody production by ECP	TEN BRINKE, Anja a.tenbrinke@sanquin.nl VAN HAM, Marieke m.vanham@sanquin.nl	Stichting Sanquin Bloedvoorziening (SQ)	NL
5	ECP as add-on therapy in high-risk kidney transplant recipients	DIEKMANN, Fritz FDIEKMAN@clinic.cat	Barcelona Institut d'Investigacions Biomèdiques (FRCB-IDIBAPS)	ES
6	ECP therapy to prevent CLAD in patients with persistent de novo DSA	BENAZZO, Alberto alberto.benazzo@meduniwien.ac.at	Medical University of Vienna (MUV)	AT
7	Treatment of ischaemia-reperfusion injury after liver transplantation	EGGENHOFER, Elke Elke.Eggenhofer@klinik.uni-regensburg.de	Univ. Hospital Regensburg (UHREG)	DE
8	ECP as a bridging therapy before liver transplantation	HUTCHINSON, James James.Hutchinson@klinik.uni-regensburg.de	Univ. Hospital Regensburg (UHREG)	DE
9	Pharmacodynamic markers of ECP therapy in transplantation	MARTÍNEZ, Eva emmartinez.germanstrias@gencat.cat	Germans Trias i Pujol Research Institute (IGTP)	ES
10	Soluble mediators elicited by ECP that promote transplant tolerance	HACKSTEIN, Holger holger.hackstein@uk-erlangen.de	Univ. Hospital Erlangen (UKER)	DE
11	ECP preconditioning to reverse trained immunity in kidney transplantation	OCHANDO, Jordi jochando@isciii.es	Instituto de Salud, Carlos III (ISCIII)	ES

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DoC1	Immunometabolic impact of ECP in transplantation
Host Institution	King's College London (KCL)
Primary Supervisor	SAFINIA, Niloufar
Email address	niloufar.1.safinia@kcl.ac.uk
Planned duration	**36 months**
Subject Area	Molecular Immunology; Cellular Metabolism

Introduction: It is widely reported that ECP enhances Treg responses, although the mechanism of this effect is unresolved. The focus of this proposal is dissecting the impact of ECP on T cell metabolism, exploring both effector T cells (Teff) and CD4+ FoxP3+ Tregs. In liver disease, our recent studies revealed that Treg stability and immunosuppressive function are influenced by systemic and cellular metabolism. Notably, Tregs are mainly reliant on fatty acid oxidation and lipid metabolism, whereas Teff are glycolytic. We hypothesize that incorrect metabolic remodelling underlies many aberrant immune responses and that manipulation of metabolism can beneficially enhance or temper immunity. Here, we ask how ECP affects metabolic profiles of Teff and Tregs, and whether the therapeutic action of ECP can be explained by its direct or indirect effects on regulatory T cell metabolism.

Aims: **A1:** To investigate the impact of ECP on metabolic programming of Teff and Tregs. We will characterize circulating Tregs from patients before and after ECP treatment using a 31-parameter deep immunophenotyping panel on the Cytek Aurora, as well as deep immunometabolic phenotyping using our established CyTOF metabolic panel. We will isolate Teff and Treg for gene expression profiling and FOXP3 TSDR demethylation studies. We will also investigate Treg suppressor function (CFSE dilution assay), effector T cell function (effector cytokine production, intracellular staining and ELISA), proliferation (Ki67) and mitochondrial (Seahorse Analyzer) function. In collaboration with the imaging facility at KCL, we will image mitochondrion in Teff and Tregs by electron microscopy and conduct global metabolomics (LC-MS) and dynamic fluoxomics using stable isotope labelling. **A2:** To investigate the acellular fraction of photopheresates and its impact on Teff and Treg metabolism in two stages: (i) Investigation of the acellular component of photopheresates using Meso Scale Discovery (MSD) for measurement of pro-/anti-inflammatory cytokines (collab. DoC10) and LC-MS to determine the polar and non-polar lipid content; (ii) Teff and Tregs from healthy donors will be cultured with fractionated photopheresates to investigate the effects on cellular metabolism using methods listed in A1. **A3:** To correlate findings from A1&2 with clinical effects observed in patients undergoing ECP treatment. Samples will be collected before and after ECP treatment (at timepoints decided in light of results from A1&2) to confirm metabolic and functional consequences in patients.

Expected Results: **R1:** We expect to create the first comprehensive dataset from ECP-treated patients that captures information about immune phenotype, T cell function and metabolic status. Using this key resource, we will define metabolic changes in Teff and Tregs associated with ECP treatment. **R2:** We expect to model in vivo effects of ECP treatment by exposing cultured T cells to components of photopheresates. This knowledge should allow network partners (collab. Mallinckrodt) to optimize ECP procedures to generate more potent photopheresates, which could address an unmet need in liver transplantation (Safinia et *al.* EJI. 2021). **R3:** We expect to be able to predict metabolic changes in Teff and Treg of ECP-treated patients by measuring components (eg. lipid species) of photophesates. Our results will lead to development of innovative assays for ECP potency or therapeutic effect (collab. DoC9).

Secondments: **(1)** 2 weeks to Mallinckrodt (M14) will give the DoC an insight into the economics of developing new therapies; **(2)** 6 weeks to Quell (M20) to conduct an in-depth immunophenotypic characterisation of Treg and effector T cells.

Enrolment in Doctoral degree(s): Faculty of Medicine, King's College London (KCL)

Project-specific selection criteria: Candidate must have a masters degree in Immunology/ Biomedical sciences or equivalent. The candidate should have laboratory experience in immunology (cell culture, flow cytometry) and molecular biology. Candidate must be familiar with data analysis and statistics. Preferred candidate will have experience in techniques to study metabolism (mass spectrometry/ seahorse, flux dynamics).

Recommended reading: Candia P et al. Regulatory T cells as metabolic sensors. Immunity 2022. PMID: 36351373
Artyomov MN et al. Immunometabolism in the Single-Cell Era. Cell Metab. 2020. PMID: 33027638

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DoC2	Modulation of NK cell and MRC responses after ECP therapy
Host Institution	Univ. Hospital Freiburg (UKL-FR)
Primary Supervisor	ZEISER, Robert
Email address	robert.zeiser@uniklinik-freiburg.de
Planned duration	**36 months**
Subject Area	Molecular Immunology; Cellular Metabolism

Introduction: ECP is a safe and effective approach for treating graft-versus-host disease (GVHD) after bone marrow transplantation (Maas-Bauer *et al.* 2021. BMT). GVHD shares common immunopathological features with immune-related adverse reactions (irAEs) that occur after immune checkpoint blockade (ICB) in cancer patients, especially in the distribution, phenotype and function of neutrophils, monocytes and microglia. This insight led us to the recent discovery that ECP is a successful treatment for irAEs (Apostolova *et al.* 2020. NEJM) acting through changes in NK cells and myeloid regulatory cell (MRC) populations. Specifically, we showed that neutrophils produce reactive oxygen species (ROS) to inhibit pathologically activated T cells and to cause exhaustion. In this project, we will build on this experience to characterize the phenotypic and functional changes of myeloid cells after ECP in mice with tumours and/or solid organ transplants.

Aims: A1: To characterize MRC after ECP therapy in mice by single cell RNA sequencing. These studies will help to generate a list of candidate molecules transcribed by the MRCs that are immunosuppressive such as arginase-1, CD39, CD73 and IDO-1/2 (collab. DoC8). **A2:** To transfer MRC or NK cells that were generated in mice by ECP into secondary recipients in order to prove their immunoregulatory activity can be adoptively transferred. In a second step MRC and/or NK cells will be derived from transgenic mice lacking the specific effector molecules (e.g. Arg-1 KO mice) identified A1 (collab. DoC9). **A3:** To validate findings made using mouse models in the human setting, we will analyze peripheral blood of ECP-treated patients at multiple time points. The phenotype of MRC, NK cells and T cells will be characterized using CyTOF technology (collab. DoC3,5,8).

Expected Results: R1: We expect to generate preclinical evidence showing that ECP induces MRCs and modified NK cells in mice. **R2:** We expect the suppressive activity of ECP-induced cell populations can be adoptively transferred into secondary recipients to suppress transplant rejection and/or irAEs. We expect that ECP will allow reduction in immunosuppression while maintaining protective immunity. **R2:** We expect to identify molecules expressed by MRC that mediate their anti-inflammatory effects. **R3:** We expect to find a correlation between MRC frequencies and response to ECP in patients.

Secondments: (1) 2 weeks to Mallinckrodt (M14) will provide the DoC with an insight into the economics of developing new therapies; **(2)** 2 weeks to Amedes (M22) to learn about the four key concepts of Quality Management and their implementation; **(3)** 2 weeks to Beckman Coulter (M32) to gain an understanding of market potential assessment and of specific requirements for ISO9001- and IVDR-compliant flow cytometry analysis with special emphasis on analysis of immune-regulatory cells of the human immune system.

Enrolment in Doctoral degree(s): Faculty of Medicine, University of Freiburg (ALU-FR)

Project-specific selection criteria: experience in Flow cytometry, cell culture, interest in models of GVHD.

Recommended reading: N Engl J Med. 2017 Nov 30;377(22):2167-2179. doi: 10.1056/NEJMra1609337. PMID: 29171820
Hemasphere . 2021 Jun 1;5(6):e581. doi: 10.1097/HS9.0000000000000581. eCollection 2021 Jun

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DoC3	ECP induction therapy after lung transplantation
Host Institution	Medical University of Vienna (MUV)
Primary Supervisor	WEKERLE, Thomas
Email address	thomas.wekerle@meduniwien.ac.at
Planned duration	**36 months**
Subject Area	Cellular and molecular immunology, translational research

Introduction: Despite major advances in recent years, outcomes after lung transplantation remain are inferior to other types of organ transplants. High rates of acute and chronic rejection, as well as infectious complications, limit graft survival. Preliminary evidence from small, uncontrolled trials suggest beneficial effects of ECP in lung transplantation, particularly with regard to chronic rejection processes (Jaskch, P. *et al.* J Heart Lung Transplant. 2012). Therefore, a prospective, randomized, controlled single center trial is being performed at MUV to test prophylactic ECP therapy added onto standard immunosuppression (tacrolimus, MMF, steroids) in de novo lung transplant recipients with COPD as underlying disease. In the study group, 8 cycles of ECP treatment are administered within three months post-transplant. From this RCT cohort of 62 patients, samples have already been acquired and biobanked for the first 6 months of post-transplant follow-up. A comprehensive phenotypic profiling of leukocyte subsets using polychromatic flow cytometry and standardized antibody panels is ongoing. The randomized setting offers a unique opportunity to investigate mechanisms of action of ECP using biobanked samples.

Aims: **A1:** To analyze soluble factors released from (stimulated) PBMC of lung transplant recipients having received prophylactic ECP treatment in comparison to randomized controls without ECP (collab. DoC8). **A2:** To investigate gene expression profiles in PBMC of lung transplant recipients after prophylactic ECP treatment and compare them to profiles of randomized controls without ECP (collab. DoC9). **A3:** To correlate factors and genes identified in A1 and A2 with long-term outcome after lung transplantation, especially the occurrence of chronic lung allograft dysfunction (CLAD) (collab. DoC6).

Expected Results: **R1:** We expect to identify soluble factors that are induced or inhibited by ECP therapy. In particular we expect T cell-, B cell- and DC-related cytokines to be affected. **R2:** We expect to find genes that are up- or downregulated by ECP add-on therapy in lung transplant recipients receiving conventional immunosuppression. **R3:** We expect that soluble factors and genes identified in A1 and A2 correlate with the occurrence of chronic rejection (CLAD). If validated in an additional cohort, such biomarkers could serve as therapeutic targets for CLAD prevention in the future.

Secondments: **(1)** 2 weeks to Mallinckrodt (M16) will give the DoC an insight into the economics of developing new therapies; **(2)** 2 weeks to Amedes (M28) to learn about the four key concepts of Quality Management and their implementation; **(3)** 2 weeks to UHREG (M22) for data analysis.

Enrolment in Doctoral degree(s): Medical University of Vienna (MUV)

Project-specific selection criteria: Background in immunology, interest in translational research

Recommended reading: https://doi.org/10.1016/j.healun.2012.05.002

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DoC4	Prevention of de novo donor-specific antibody production by ECP
Host Institution	Stichting Sanquin Bloedvoorziening (SQ)
Primary Supervisor	ten BRINKE, Anja and van HAM, Marieke
Email address	a.tenbrinke@sanquin.nl m.vanham@sanquin.nl
Planned duration	**48 months**
Subject Area	Cellular Immunology

Introduction: ECP is an established treatment for ABMR in cardiothoracic transplantation and it is well-established that ECP can attenuate T cell-dependent antibody responses. Differentiation of antigen (Ag)-specific B cells into class-switched, high-affinity, antibody-secreting cells provides protection against invading pathogens but is undesirable when antibodies target allogeneic transplanted organs. Protective and pathogenic Abs are produced by Ab-secreting plasmablasts or plasma cells (ASCs) that originate from B cells. T cell help consisting of CD40L/CD40 costimulation and the secretion of specific cytokines is essential for the generation of long-lived plasma cells that produce high-affinity, class-switched Abs. T cell-dependent B cells differentiate in secondary lymphoid organs after being activated by their cognate Ag and undergoing so-called germinal center (GC) reactions. We previously found that compounds of dying or activated cells, such as sFASL and platelet releasate can directly affect B cell differentiation and antibody production (van Asten *et al.* 2021. *J Immunol.*); however, effects of ECP on B cells might also occur via modulation of T cells, since they play such an important role in the GC dependent B cell development. In this project we want to gain more insights into the effects of photoapheresates on B cell responses and antibody production.

Aims: **A1:** To develop a 3D B cell culture system that resembles physiological conditions as much as possible, including relevant oxygen levels (collab. Doc3,5). **A2:** To investigate the direct effect of ECP treatment on B cell phenotype and B cell activation, differentiation and antibody production using in vitro models, both in minimalistic cultures as well as in T-B cell co-cultures and 3D cultures. Also the indirect effect of ECP on B cells will be investigated (collab. DoC5,6). **A3:** Components of photopheresates will be separated and compared to elucidate if apoptotic or living leucocytes, platelets and/or secreted soluble factors are responsible for an effect on B cells (collab. DoC8,10). **A4:** The effects of photoapheresate fractions on naïve and memory B cells will be compared.

Expected Results: **R1:** We expect to adapt existing 3D culture systems to study B cell differentiation in more physiological settings. **R2:** We expect to get insights into the effects of ECP upon B cell differentiation, antibody production. In particular, we expect to separate direct effects on B cells from effects of ECP mediated through CD4+ T cells. **R3:** We expect to define what components of photoapheresates affect B cells (eg. roles for apoptotic cells or soluble factors). **R4:** We expect to gain insights into whether naïve or memory B cell responses are more sensitive to ECP treatment. These results will inform us about potential future clinical indications for ECP in solid organ transplantation, especially whether ECP only prevents de novo antibody production or should be effective for already ongoing antibody responses.

Secondments: **(1)** 2 weeks to Mallinckrodt (M16) to learn about the economics of developing new therapies; **(2)** 2 weeks to Amedes (M22) to learn about Quality Management; **(3)** 4 weeks to IDIBAPS (M24) for method and data sharing, and to gain insights into translational research.

Enrolment in Doctoral degree(s): Faculty of Science, University of Amsterdam (UvA)

Project-specific selection criteria: 1) A Master degree in Biomedical Sciences or a similar education level.
2) Knowledge of Immunology is required and a proven track record in research in cellular immunology is a prerequisite.
3) Team player with good communication skills.

Recommended reading: 1) Verstegen NJM *et al.* Single-cell analysis reveals dynamics of human B cell differentiation and identifies novel B and antibody-secreting cell intermediates. doi: 10.7554/eLife.83578. 2) Koers J *et al.,* Oxygen level is a critical regulator of human B cell differentiation and IgG class switch recombination. doi: 10.3389/fimmu.2022.1082154. 3) Steuten *et al.* Distinct dynamics of antigen-specific induction and differentiation of different CD11c+ Tbet+ B cell subsets. doi: 10.1016/j.jaci.2023.02.020. 4) Van Asten SD *et al.* Soluble FAS Ligand Enhances Suboptimal CD40L/IL-21-Mediated Human Memory B Cell Differentiation into Antibody-Secreting Cells. doi: 10.4049/jimmunol.2001390.

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DoC5	ECP as add-on therapy in high-risk kidney transplant recipients
Host Institution	Fundació de Recerca Clínic Barcelona-Institut d'Investigacions Biomèdiques August Pi i Sunyer (FRCB-IDIBAPS)
Primary Supervisor	DIEKMANN, Fritz
Email address	FDIEKMAN@clinic.cat
Planned duration	**36 months**
Subject Area	Transplant Immunology

Introduction: Kidney transplant recipients with a pre-operative calculated panel reactive antibody (cPRA) score of ≥ 90% stand a high risk of antibody-mediated rejection (ABMR) in the first year post-transplantation (Piñeiro *et al.* BMC Nephrology. 2018). It is well established in heart, lung, liver, and kidney transplantation that ECP reduces donor-specific alloantibody (DSA) titres and may be useful in managing otherwise treatment-refractory ABMR. The immunological mechanisms underlying this beneficial effect are not presently understood. In particular, we do not know what antibody-producing subpopulations are controlled by ECP. Nevertheless, clinical evidence suggests that ECP may be a valuable preconditioning or induction therapy for kidney transplant recipients with high cPRA. In this project, we will explore the potential application of ECP as an add-on therapy in high cPRA patients with the aim of reducing the risk of ABMR within the first year after transplantation (Xipell, M. *et al.* J. Clin. Apher. 2022). We aim to better understand the action of ECP in controlling humoral responses.

Aims: **A1:** Aims: A1: To analyse samples from a single-center, randomized clinical trial to assess the feasibility of using ECP induction therapy to prevent ABMR in high-risk (cPRA ≥ 90%) adult kidney transplant recipients with negative CDC and virtual cross-matches prior to transplantation (clinicaltrials.gov: NCT04414735). **A2:** To perform detailed immune monitoring studies of ECP-treated patients, focusing especially on B cells, Bregs and follicular helper T (TFH) cells. (collab. DoC4,6). **A3:** To collect blood samples for mechanistic investigations of T cells (collab. DoC1), B cells (collab. DoC4), NK cells (collab. DoC2), MDSC (collab. DoC9) and DCs.

Expected Results: **R1:** We expect to treat 15 high-risk patients with ECP induction therapy without serious adverse effects. This study is designed to draw conclusions about the efficacy of ECP in preventing ABMR. However, if we do not achieve significant results for the primary endpoint of the trial, we hope to demonstrate a reduction in alloantibody titers and cPRA as secondary endpoints. **R2:** We expect to observe changes in T cell-dependent B cell responses, but not antibody production by plasma cells. **R3:** We expect to find that control of alloantibody by ECP is primarily mediated by suppression of TH2 and T_{FH} cell responses.

Secondments: **(1)** 2 weeks to Mallinckrodt (M18) to learn about the economics of developing new therapies; **(2)** 4 weeks to SQ (M20) to learn about B cell culture techniques; **(3)** 2 weeks to Amedes (M24) to learn about the four key concepts of Quality Management.

Enrolment in Doctoral degree(s): Faculty of Medicine, University of Barcelona (UB)

Project-specific selection criteria: Candidates must have a Master's degree in Biomedical Sciences or a similar educational level with an immunological background and laboratory experience in basic techniques (flow cytometry, cell culture, and molecular biology). Candidates must be familiar with data analysis and statistics. Candidates should be a team player with good communication skills.

Recommended reading: Piñeiro GJ, *et al.* Extracorporeal Photopheresis Improves Graft Survival in a Full-Mismatch Rat Model of Kidney Transplantation. Transpl Int. 2023 Jan 12;36:10840. doi: 10.3389/ti.2023.10840.
Xipell M, *et al.* Immunogenic and immunotolerogenic effects of extracorporeal photopheresis in high immunological risk kidney recipients. A single center case series. J Clin Apher. 2022 Jun;37(3):197-205. doi: 10.1002/jca.21958.

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DoC6	ECP therapy to prevent CLAD in patients with persistent de novo DSA
Host Institution	Medical University of Vienna (MUV)
Primary Supervisor	BENAZZO, Alberto
Email address	alberto.benazzo@meduniwien.ac.at
Planned duration	**36 months**
Subject Area	Cellular and Molecular Immunology; Immunogenetics, Translational Research, Transplantation immunology

Introduction: Approximately 50% of all patients develop *de novo* DSA (dnDSAs) within the first year after lung transplantation (LuTx). In the majority of cases, dnDSAs disappear after 3-6 months; however, in 10-30% they persist longer than 6 months and represent a risk factor for worse graft (56% vs 72%, p=0.005) and patient survival (58% vs 75%, p<0.001) independent from any acute humoral complication. The exact pathogenic mechanism is still unknown. We hypothesize that dnDSA triggers a subclinical immune response against the allograft, which leads to chronic lung allograft dysfunction (CLAD). ECP is a standard therapy for CLAD in LuTx recipients and our institution routinely uses ECP as stabilization therapy after antibody-mediated rejection (Jaksch *et al.* J Heart Lung Transplant. 2012) We have initiated a prospective randomized trial to evaluate the therapeutic effect of ECP on dnDSAs and on long-term graft function. In this study, LuTx recipients with persistent dnDSAs (>6 months) within the first year after transplantation are randomized into two groups. The treatment cohort undergoes ECP, whereas the control group follows our watch-and-wait protocol. Our primary endpoint is 25% reduction in dnDSAs at 6 months after randomization. Secondary endpoints are reduction in antibody levels at 12 months, graft survival at 24 months post-transplant, ABMR rate and incidence of CLAD. Here, we propose to dissect the immunological mechanisms responsible for dnDSA suppression by ECP in our patients, leveraging local and network-wide expertise in transcriptomic, metabolomic and cellular analyses.

Aims: Using clinical data and samples from our trial (clinicaltrials.gov: NCT04792294) DoC6 will investigate the therapeutic potential of ECP to prevent or retard functional decline of LuTx in recipients with dnDSA. **A1:** To characterize the circulating miRNome in plasma of ECP-treated LuTx recipients compared to untreated controls (collab. DoC9). **A2:** To investigate the effect of ECP treatment on gene expression profiles in PBMCs from LuTx recipients (collab. DoC1). **A3:** To characterize the composition and level of activation of immune cell subsets over time in LuTx recipients receiving ECP compared to randomized controls (collab. DoC3). **A4:** To work with bioinformatics experts from MUV and UHREG in order to perform cross-platform computational analyses of data collected from our patients in A1-4 to identify immunological features that predict favorable clinical outcomes.

Expected Results: **R1:** We expect to identify an miRNA signature that is induced or inhibited by ECP therapy. In particular we expect T cell-, B cell- and DC-related miRNAs to be affected. **R2:** We expect to find genes that are up- or downregulated by ECP. **R3:** We expect an increase in Tregs (collab. DoC1), Bregs (collab. DoC4,5) and MDSC/Tol-DC (collab. DoC9), as well as an overall reduction in effector cell activation in ECP treated patients. **R4:** We expect a reduced dnDSA after ECP and improved graft survival with a lower incidence of CLAD. We expect to be able to predict such favourable outcomes using immune biomarkers, which could lead to commercially exploitable innovations.

Secondments: **(1)** 2 weeks to Mallinckrodt (M18) will provide the DoC with an insight into the economics of developing new therapies; **(2)** 2 weeks to Amedes (M26) to learn about the four key concepts of Quality Management; **(3)** 2 weeks to UKER (M32) for method and data sharing.

Enrolment in Doctoral degree(s): Medical University of Vienna (MUV)

Project-specific selection criteria: Candidates should have a Master's degree or similar education level in Immunology, Biology or Biomedical sciences. Knowledge of Immunology and a proven track record are required. The candidate should be familiar with basic techniques (flow cytometry, cell culture, and molecular biology). Candidates should be familiar with data analysis and statistics.

Recommended reading:
Iasella CJ, Ensor CR, Marrari M, et al. Donor-specific antibody characteristics, including persistence and complement-binding capacity, increase risk for chronic lung allograft dysfunction. J Heart Lung Transplant 2020;39:1417-25.
Benazzo A, Worel N, Schwarz S, et al. Outcome of Extracorporeal Photopheresis as an Add-On Therapy for Antibody-Mediated Rejection in Lung Transplant Recipients. Transfus Med Hemother 2020;47:205-13.
Keller M, Yang S, Ponor L, et al. Preemptive treatment of de novo donor-specific antibodies in lung transplant patients reduces subsequent risk of chronic lung allograft dysfunction or death. American Journal of Transplantation 2023;23:559-64
Baskaran G, Tiriveedhi V, Ramachandran S, et al. Efficacy of extracorporeal photopheresis in clearance of antibodies to donor-specific and lung-specific antigens in lung transplant recipients. J Heart Lung Transplant 2014;33:950-6.

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DoC7	Treatment of ischaemia-reperfusion injury after liver transplantation
Host Institution	Univ. Hospital Regensburg (UHREG)
Primary Supervisor	EGGENHOFER, Elke
Email address	Elke.Eggenhofer@klinik.uni-regensburg.de
Planned duration	**36 months**
Subject Area	Transplant Immunology; Cellular Metabolism; Translational Research

Introduction: Introduction: Ischemia-reperfusion injury (IRI) after organ transplantation may lead to impaired graft function, or worse, to massive organ damage by initiating fibrosis. There are two, linked causes: firstly, controlled cell death mechanisms and, secondarily, activation of innate immune cells. The complex relationship between cell death and immune cell activation in IRI is not fully understood and there is presently no clinically applicable therapy for IRI (Eggenhofer *et al.* IJMS. 2021). There is strong clinical evidence that ECP suppresses inflammatory pathologies and that the dying cells have effects on myeloid antigen-presenting cells. However, it is not known which cell death mechanisms are induced by ECP in detail (ie. apoptosis, necroptosis or ferroptosis) and how cell death by these alternative pathways might affect immune cells (Eggenhofer *et al.* J Hepatol. 2016). In this project, we will characterize which specific cell death mechanisms are evoked by ECP and how this affects the number and activation of IRI-induced effector cells (especially γδ T cells and NK-22 cells) using samples from ECP-treated patients. A comparison of *de novo* treated patients and long-term treated patients (or follow-up studies) will allow us to investigate the development of immune cell activation over time. With this approach, we aim to develop a cell death and/or immune cell activation assay panel that enables us to assess the consequences of ischemia-reperfusion after organ transplantation. Adoptive transfer of ECP treated leucocytes in a hepatic mouse model of IRI will enable us to characterize the direct effect of ECP on liver IRI.

Aims: **A1:** To characterize defined cell death mechanisms (apoptosis, necroptosis, ferroptosis) in apheresates before and after 8-MOP/UVA treatment. **A2:** To monitor immune cell status (especially γδ T cells and NK-22 cells) in lung, liver and kidney transplant recipients, which we will correlate with identified cell death markers in photopheresates (collab. DoC3,5,6,8). **A3:** To compare data from A1 and A2 with post-transplant clinical outcome from *de novo* treated patients with repeatedly treated patients and identify the correlation of cell-death/immune activation with fibrosis development in order to validate efficacy/potency markers of ECP after IRI. **A4:** To investigate the direct effect of photopheresates on liver IRI in a hepatic IRI mouse model (collab. DoC8).

Expected Results: **R1:** We expect to identify presently unknown cell death effects in ECP samples. We expect ECP therapy will induce cell-type specific cell death mechanisms that impact recipient's immune responses in different ways. **R2:** We expect to identify IRI-induced effector cell populations that respond to alternative cell death mechanisms. **R3:** We expect a different immune cell frequency and activation status in de novo vs. long-term ECP-treated patients, which will enable us to identify efficacy/potency markers for ECP mitigating IRI. **R4:** We expect to find an improvement of hepatic IRI in mice treated with photopheresates owing to modulated γδ T cells and NK-22 cell responses.

Secondments: **(1)** 2 weeks to Mallinckrodt (M20) to learn about the economics of developing new therapies; **(2)** 2 weeks to Beckman Coulter (M16) to gain an understanding of market potential assessment and of specific requirements for ISO9001- and IVDR-compliant flow cytometry assays with special emphasis on flow cytometry; **(3)** 2 weeks to Amedes (M24) to learn about the four key concepts of Quality Management.

Enrolment in Doctoral degree(s): University of Regensburg, Gradute School of Physiological Sciences (BioMediGS)

Project-specific selection criteria: Candidates should have a Master's degree in Biology, Biomedical Sciences or a similar educational level with an immunological background and laboratory experience in basic techniques (flow cytometry, cell culture, and molecular biology). Candidates should be familiar with data analysis and statistics and should be a team player with good communication skills.

Recommended reading: Eggenhofer E, *et al.,* Int J Mol Sci. 2021 Feb 18;22(4):2036. doi: 10.3390/ijms22042036.
Mishima E,.., Eggenhofer E, *et al.,* Nature. 2022 Aug;608(7924):778-783. doi: 10.1038/s41586-022-05022-3.

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DoC8	ECP as a bridging therapy before liver transplantation
Host Institution	Univ. Hospital Regensburg (UHREG)
Primary Supervisor	HUTCHINSON, James
Email address	James.Hutchinson@klinik.uni-regensburg.de
Planned duration	**36 months**
Subject Area	Molecular Immunology; Cellular Metabolism

Introduction: Clearance of apoptotic cells by macrophages is an essential process in normal tissue homeostasis and resolution of tissue after pathological challenge. Macrophages encountering apoptotic cells acquire anti-inflammatory and tissue-reparative properties. Various phagocytic receptors expressed by macrophages are implicated in direct or indirect (opsonic) recognition of apoptotic cell components, some of which deliver tolerizing signals. Clinical recovery after extensive liver damage, including transplant-related and toxic injuries, crucially depends upon repair processes mediated by monocyte-derived macrophages (Moroni et al. Nat Med., 2019). Apart from removing dead and dying cells, liver-infiltrating macrophages are responsible for secretion of trophic factors, promoting revascularization, driving fibrosis and suppressing local immune responses. The goal of this project is to better understand and exploit the tissue-reparative macrophage response by developing ECP as a pre-transplant "bridging" therapy for critically unwell patients on the liver transplant waiting list who fulfill the Kings College Criteria for acute liver failure, so have a mortality rate of ~85% unless transplanted (McPhail et al. Clin. Gastroenterol. Hepatol., 2016). To bring this highly innovative application of ECP to the clinic, we plan to demonstrate that macrophages treated with photopheresates promote hepatocyte growth and differentiation by (i) coculturing primary human macrophages with primary hepatocyte organoids, (ii) treating machine-perfused human livers with photopheresates, (iii) investigating blood monocytes after ECP in wait-listed patients with acute liver failure.

Aims: **A1:**To investigate whether exposing human monocyte-derived macrophages to photopheresates (or isolates from photopheresates) promotes growth and differentiation of primary human hepatocytes as organoids. We will treat monolayer cultures of human monocyte-derived resting macrophages with whole or fractionated photopheresates. These macrophages will then be overlaid with primary human hepatocyte cultures in matrigel. After 7 days' culture, we will assess hepatocyte numbers and phenotype. We will also analyze soluble factors in co-culture supernatants using bead arrays and proteomics (collab. DoC1,7). **A2:** To investigate effects of photopheresates on hepatocytes and tissue macrophages in human livers discarded from the transplant program during normothermic machine perfusion. Leveraging results from A1, read-outs will include biochemical and histological markers of liver injury and repair, as well as phenotypic and functional analyses of macrophages. Infusing photopheresates into some arterial territories, but not others, we can compare treated and untreated samples from one individual. Harvesting samples across a timeseries will allow us to examine response kinetics. To investigate changes in tissue-infiltrating macrophages, we will spike tracer dye-labelled monocytes into the perfusate to allow later recovery and separate analysis (collab. DoC1). **A3:** To investigate the impact of ECP on blood monocytes in (i) patients receiving ECP therapy for any cause, and (ii) wait-listed patients with ALF treated with ECP as a bridging therapy (collab. DoC9). Samples will be analyzed by flow cytometry and gene expression analysis, as well as being shared across the exTra consortium.

Expected Results: **R1:** We expect to show that photopheresates polarize monocyte-derived macrophages towards an anti-inflammatory state, which suppress co-cultured T cell proliferation and express tissue-reparative factors (eg. amphiregulin, VEGF, or HGF). We expect such macrophages will induce proliferation and differentiation of primary human hepatocytes in organoid cultures. **R2:** We expect to find changes in hepatocytes and blood monocyte-derived macrophages isolated from ECP-treated, perfused human liver similar to those observed *in vitro*. **R3:** We expect to find ECP-induced changes in blood monocytes consistent with development towards tissue-reparative macrophages. We hope to gain a clinical impression whether ECP has potential to prolong survival of critically unwell patients with acute liver failure.

Secondments: **(1)** 2 weeks to Mallinckrodt (M20) will provide the DoC with an insight into the economics of developing new therapies; **(2)** 2 weeks to Amedes (M26) to learn about the four key concepts of Quality Management; **(3)** 4 weeks to IGTP (M30) for technology transfer.

Enrolment in Doctoral degree(s): Medical University of Vienna (MUV)

Project-specific selection criteria: Candidates must show an interest in applied medical research leading to innovative therapies that improve patient care. Candidates must demonstrate a reasonable understanding of Immunology and Cell Biology. Candidates must have previous practical experience of laboratory research, including sterile cell culture techniques. Candidates must be familiar with data processing and basic statistics. Preferred candidates will have extensive experience of laboratory research, a deep understanding of Immunology, and/or an ability to code in R or Python.

Recommended reading: Hutchinson-JA & Benazzo-A. Extracorporeal Photopheresis Suppresses Transplant Fibrosis by Inducing Decorin Expression in Alveolar Macrophages. Transplantation, 2023; 107(5):1010-1012. https://doi.org/10.1097/tp.0000000000004536

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DoC9	Pharmacodynamic markers of ECP therapy in transplantation
Host Institution	Germans Trias i Pujol Research Institute (IGTP)
Primary Supervisor	MARTÍNEZ, Eva
Email address	emmartinez.germanstrias@gencat.cat
Planned duration	**36 months**
Subject Area	Molecular Immunology; Cellular Metabolism

Introduction: Being able to measure the pharmacodynamic effects of a drug in individual patients is a key to relating dose, drug exposure, mechanism of action and clinical efficacy. Presently, there is no consensus about the mechanism of action of ECP and, consequently, there are no defined biomarkers of pharmacodynamics effect. In our view, ECP is likely to have multiple effects on recipients' immune responses against transplanted organs - indeed, organ transplants may be injured by diverse mechanisms. Therefore, this project asks the question, what are the most important effects of ECP in solid organ transplantation and how can these be measured in a standardized way? As clinical immunologists, the investigators bring vital experience in immunological assay development and routine clinical diagnostics (Quirant-Sánchez B. *et al.* 2018. *CNS Neurosci Ther.*) as well as considerable experience of monitoring alloimmune responses in kidney transplant recipients (Iglesias-Escudero *et al.* 2020. Front. I*mmunol.)*. Building on existing methods and new pharmacodynamic markers proposed by other exTra participants, this project aims to define a consensus panel of assays to measure the therapeutic impact of ECP in transplant recipients.

Aims: **A1:** In collaboration with all exTra partners, we will compile a list of potential pharmacodynamic markers of ECP treatment and, as necessary, we will establish or adopt standardized, clinically applicable assays to measure these parameters in blood samples from patients (collab. DoC1-8). **A2:** To perform immune monitoring of serum and peripheral blood from ECP-treated patients to (i) corroborate existing phamacodynamic markers defined in A1, and (ii) identify new pharmacodynamics markers in a discovery-driven approach using spectral flow cytometry and/or proteomic profiling of plasma. **A3:** Building on our specialist expertise, we examine the relation of ECP therapy and myeloid derived suppressor cell (MDSC) and tolerogenic DC (TolDC) responses, including detailed in vitro characterization of these cells from ECP-treated patients using established methods (Mansilla et al. 2021. Cell Mol. Immunol; Morante-Palacios *et al.* 2021. *Trends Immunol.*). We will also investigate T cells (collab. DoC1), B cells (collab. DoC4), NK cells (*collab. DoC2*) and macrophages (collab. DoC8). **A4:** To create a database that relates pharmacodynamics markers, patient and photopheresate characteristics, and clinical outcomes (collab. DoC10).

Expected Results: **R1:** We expect to define an evidence-based list of candidate pharmacodynamic markers and to establish robust methods for measurement of these parameters. These standardized assays will be disseminated within the network. **R2:** We expect to collect immune monitoring results throughout the lifetime of the project, which will be analysed as training, validation and test batches as data are acquired. **R3:** We expect to record changes in MDSC and TolDC numbers, phenotype and function as a result of ECP therapy. **R4:** We expect our database will serve as a valuable resource for all exTra partners and the field of ECP research generally. Mining this dataset, we aim to identify the most informative pharmacodynamic markers of ECP in solid organ transplantation.

Secondments: **(1)** 2 weeks to Mallinckrodt (M22) will provide the DoC with an insight into the economics of developing new therapies; **(2)** 8 weeks to Aniling (M18) to learn the process from biomarker discovery to clinical diagnosis; 4 weeks to UHREG (M32) for technology transfer.

Enrolment in Doctoral degree(s): Faculty of Medicine, Autonomous University of Barcelona (UAB)

Project-specific selection criteria: We are looking for a highly motivated and engaged predoctoral researcher, preferably with an immunological background and lab experience in basic techniques (flow cytometry, cell culture). Candidates must be familiar with data processing and basic statistics. The selected candidate should have the ability to work independently and display problem solving skills.

Recommended reading: Not specified

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DoC10	Soluble mediators elicited by ECP that promote transplant tolerance
Host Institution	Univ. Hospital Erlangen (UKER)
Primary Supervisor	HACKSTEIN, Holger
Email address	Holger.Hackstein@uk-erlangen.de
Planned duration	**36 months**
Subject Area	Cellular and Molecular Immunology

Introduction: ECP is a successful therapy for heart and lung transplant rejection and other T-cell mediated pathologies. ECP is also used for treating cutaneous T cell lymphoma (CTCL) indicating that it elicits both immune-regulatory and immune-stimulatory responses. Despite these long-established clinical applications of ECP, little is known regarding its mode of action. Recently, we identified IL-1ß as a novel immune-stimulatory mediator triggered by ECP in mice and humans (Yakut *et al.* 2015. *J Immunol.*). Our group has established an experimental ECP procedure that allows us to assess release of ECP-triggered soluble mediators by professional antigen presenting cells (Hackstein *et al.* 2021. *Clin Exp Immunol.*). In this project, our system will be used to test the influence of soluble factors released by ECP upon FACS/MACS-purified stimulator and responder cell populations (Buchele *et al.* 2021. *Transfusion*). In particular, we aim to identify soluble factors released during ECP treatment that mediate the immune-regulatory effects of ECP, which might serve as novel biomarkers of potency or therapeutic targets.

Aims: **A1:** To compare supernatants of resting and activated PBMCs of healthy donors at different time points after experimental ECP treatment and then to analyze the secretome using proteomic, metabolomic and lipidomic approaches (collab. DoC1). We will monitor the expression kinetics of factors-of-interest in clinical ECP products. **A2:** To investigate the immunoregulatory capacity of ECP supernatants and factors-of-interest in mixed lymphocyte reactions (collab. DoC8). **A3:** To investigate the direct effect of ECP supernatants and factors-of-interest on different resting and activated cell populations, especially T cells (collab. DoC1), B cells (collab. DoC4), NK cells (collab. DoC2), MDSC (collab. DoC9) and DCs. **A4:** To validate major findings with clinical samples from transplant recipients under ECP-therapy by correlating the expression kinetics of factors-of-interest with clinical efficacy (collab. all DoC).

Expected Results: **R1:** We expect to identify known (eg. TGF-β and IL-1β) and unknown factors (possibly including cytokines, chemokines, secreted metabolites or lipid mediators) released from human PBMC after ECP treatment. **R2:** We expect that proliferation and activation of alloreactive T cells is suppressed by soluble factors present in ECP supernatants. **R3:** We will define which leucocyte populations respond to specific factors-of-interest and expect to discover the mechanisms by which these factors influence responder cells. **R4:** We expect that expression of soluble mediators identified experimentally will correlate with clinical efficacy of ECP in transplant recipients.

Secondments: **(1)** 2 weeks to Mallinckrodt (M22) will provide the DoC with an insight into the economics of developing new therapies; **(2)** 2 weeks to Beckman Coulter (M18) to gain an understanding of market potential assessment and of specific requirements for ISO9001- and IVDR-compliant flow cytometry assays with special emphasis on extracellular vesicle (EV) analysis; **(3)** 2 weeks to UHREG (M32) for method and data sharing.

Enrolment in Doctoral degree(s): Faculty of Medicine, University of Erlangen (FAU)

Project-specific selection criteria: Candidates must have previous research experience and must demonstrate a reasonable understanding of Immunology and Cell Biology. Candidates must be familiar with statistics, data analysis and presentation. Candidates must have the ability to work independently and must have a thorough and structured attitude. Candidates should be experienced in flow cytometry, cell culture and molecular biology.

Recommended reading:
Yakut E, et al. Extracorporeal photopheresis promotes IL-1beta production. J Immunol. 2015;194(6):2569-77. doi: 10.4049/jimmunol.1400694.
Buchele V, Hackstein H. A simplified extracorporeal photopheresis procedure based on single high-dose ultraviolet A light irradiation shows similar in vitro efficacy. Transfusion. 2021;61(3):883-93. doi: 10.1111/trf.16209.
Hackstein H, et al. CD11c(+) dendritic cells mediate antigen-specific suppression in extracorporeal photopheresis. Clin Exp Immunol. 2021;203(2):329-39. doi: 10.1111/cei.13539.

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DoC11	ECP preconditioning to reverse trained immunity in kidney transplantation
Host Institution	Instituto de Salud, Carlos III (ISCIII)
Primary Supervisor	OCHANDO, Jordi
Email address	jochando@isciii.es
Planned duration	**36 months**
Subject Area	Epigenetics

Introduction: This project builds on our previous work showing that trained immunity is induced by chronic kidney disease (CKD) and during renal replacement therapy with hemodialysis (HD). Consequently, the innate immune system, which initiates allorecognition and organ rejection, is dysregulated before transplantation and compromises long-term kidney transplant survival. We hypothesize that exposing trained monocytes (or their precursors in bone marrow) to apoptotic cell material through extracorporeal photopheresis (ECP) therapy will reverse trained immunity in our established mouse model of CKD and HD before kidney transplantation (KTx). Mechanistically, reversal of trained immunity could reflect an increased turnover of trained myeloid precursors, leading to their replacement, or direct epigenetic effects in trained cells. This project has two important perspectives – namely, (1) ECP-induced changes in trained immunity could be used to monitor clinical response to therapy, and (2) ECP might be a valuable preconditioning therapy in wait-listed patients on renal replacement therapy prior to transplantation.

Aims: **A1:** Leveraging experience within exTra of performing ECP in mice (collab. DoC2,5) we will use our established mouse kidney transplant model (diagram) to investigate whether ECP mitigates the detrimental effects of trained immunity. Specifically, we will perform graft survival experiments in mice preconditioned with (1) CKD +/- ECP (2) CKD + HD +/-ECP, or (3) CKD + HD + KTx +/- ECP. In addition, we will perform adoptive transfer experiments to demonstrate that trained monocytes are mediators of both the detrimental effects of trained immunity and therapeutic benefits of ECP in this model. **A2:** At intervals before and after transplantation, we will isolate myeloid precursors, monocytes, macrophages and DCs from bone marrow (BM), spleen, blood and the allograft to characterize their trained state using flow cytometry, functional assays and ChIP-seq. **A3:** We will collaborate with exTra partners to investigate trained immunity in human KTx (collab. DoC5) or BM-Tx (collab. DoC2,8) recipients undergoing ECP.

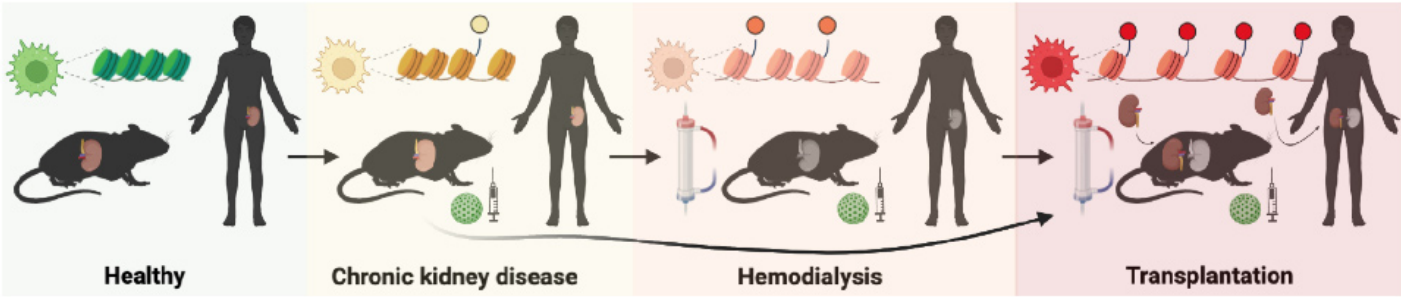

Diagram: During progression of CKD, naïve monocytes (green) are activated and display specific chromatin marks (yellow). Monocytes from CKD patients undergoing HD further increase their chromatin accessibility (orange). Memory-like inflammatory monocytes persist in kidney transplant recipients despite conventional immunosuppressive treatment (red).

Expected Results: **R1:** We expect to find that ECP therapy mitigates the effects of trained immunity induced by CKD and HD through monocyte-mediated mechanisms. **R2:** We expect that improvements in allograft survival in ECP-treated recipients will correlate with epigenetic and functional markers of trained immunity in monocytes. **R3:** We will exploit our results by developing tests for trained immunity in wait-listed dialysis patients or kidney transplant recipients undergoing ECP as a marker of clinical response to therapy.

Secondments: **(1)** 2 weeks to Mallinckrodt (M22) will provide the DoC with an insight into the economics of developing new therapies; **(2)** 2 weeks to Beckman Coulter (M18) to gain an understanding of market potential assessment and of specific requirements for ISO9001- and IVDR-compliant flow cytometry assays with special emphasis on extracellular vesicle (EV) analysis; **(3)** 2 weeks to IDIBAPS (M32) for method and data sharing.

Enrolment in Doctoral degree(s): UNED-IMIENS (at ISCIII)

Project-specific selection criteria: Candidates must provide evidence of previous expertise in epigenetics, which may include Chromatin ImmunoPrecipitation (ChIP,) Assay for Transposase-Accessible Chromatin (ATAC) or Cleavage Under Targets & Release Using Nucleases (CUT&RUN) assays. Candidates must be familiar with basic bioinformatic and biostatistic concepts.

Recommended reading: PMID: 30967658, PMID: 33293712 and PMID: 36253509

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