INFRAFRONTIER
mouse disease models

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INFRAFRONTIER Research Infrastructure

INFRAFRONTIER2020 Project - Trans-national Access call
May 2019

Specialised phenotyping
Functional immune-phenotyping screen by mass cytometry

Call information and application form

Context and aims of the call

INFRAFRONTIER is the European Research Infrastructure for phenotyping and archiving of model mammalian genomes. The INFRAFRONTIER Research Infrastructure provides access to first-class tools and data for biomedical research, and thereby contributes to improving the understanding of gene function in human health and disease using the mouse model. The core services of INFRAFRONTIER comprise the systemic phenotyping of mouse mutants in the participating mouse clinics, and the archiving and distribution of mouse mutant lines by the European Mouse Mutant Archive (EMMA).

Main objective of this INFRAFRONTIER2020 Trans-national Access call is to facilitate access for the wider biomedical research community to the unique infrastructure and scientific expertise of the PHENOMIN-CIPHE mouse clinic, where mouse mutant lines can be analysed through specialised functional immune-phenotyping by mass cytometry. To characterize the functional impact, on the immune system, of the invalidation or the overexpression of a gene of interest, cellular immune-phenotype profiling is required. This primary immunophenotyping screen, essentially based on an

extracellular labelling, aims at quantifying the various cell populations present within a model by monitoring the expression of specific cell surface receptors in hematopoietic cells. This first approach describing cellular heterogeneity does not assess the effector functions of immune cells. To this end, a more functional secondary immune-phenotyping screen is required and offered in this call.

Access will be granted on the basis of scientific excellence and supports the development and in-depth characterisation of mouse models for investigating gene function and human pathophysiology. INFRAFRONTIER will provide open access to all characterised disease models and phenotyping data.

HORIZON 2020 The INFRAFRONTIER2020 project has received funding from the EU Research and Innovation programme Horizon 2020 (H2020-EU.1.4.1.1. Developing new world class research infrastructures)

Participating INFRAFRONTIER partner

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EXCELLENCE IN MOUSE PHENOGENOMICS

PHENOMIN-CIPHE / http://ciphe.marseille.inserm.fr/en/, http://www.phenomin.fr/

The **Centre d'Immunophenomics (CIPHE)** is a technological service provider unit that operates under the administrative authority of INSERM, CNRS and Aix-Marseille University and is dedicated to customized preclinical mouse model generation and high-content standardized multiparametric immunophenotyping analysis of the mouse and human immune systems under normal and pathological conditions (inflammation, infection, cancer). It provides academic and industrial partners with a unique integrated technological toolbox to comprehensively characterize the immune system of mouse models and of human samples of interest.

CIPHE participates as a technological platform to several research programs (ERC, ANR, INCA, ARSEP and Investissements d'Avenir program Labex) that focus on Immunology, Infection and Cancer. CIPHE is one of the founding members of PHENOMIN, the French infrastructure for Phenogenomics together with Institut Clinique de la Souris (Strasbourg) and TAAM (Orléans). Notably, CIPHE belongs to the Infrafrontier European Consortium and to the International Mouse Phenotyping Consortium (IMPC). CIPHE has been involved as a "technological core" service in several large-scale European projects (MUGEN, MASTERSWITCH and SYBILLA). At the regional

level, CIPHE benefits from its close collaboration with the Centre of Immunology Marseille-Luminy (CIML) and Infectiopole IHU Marseille. In addition, CIPHE is a founding member of MImABs, an industrial demonstrator funded by the program Investissement d'Avenir. CIPHE is also part of Marseille ImmunoPôle, a cluster that comprises Centre of Immunology (CIML), Centre of Cancer Research Marseille (CRCM), Cancer Institute Paoli-Calmettes (IPC), Aix-Marseille University (AMU), MImABs and the private company Innate Pharma. Marseille Immunopôle and is intended to accelerate discovery and development of new therapeutic targets in cancer and inflammatory diseases and to reinforce the global study of immune system confronted to infection risks.

Trans-national Access (TA) activity of the INFRAFRONTIER2020 project

Free of charge specialised immune phenotyping service

Access modalities:

- The EC Horizon2020 funded INFRAFRONTIER2020 project (2017 – 2020) supports eligible customers with a free-of-charge mouse model immune-phenotyping service implemented as a Trans-national Access activity supporting a total of 3 projects in this call.

- The access unit offered covers the specialised immune-phenotyping of a mouse mutant line by mass cytometry, and the preparation of a comprehensive phenotype analysis report.

- Details of the specific immune-phenotyping pipeline and service offer are described on page 6 of this application form.

- Support will be provided by the CIPHE mouse clinic to analyse and interpret the phenotyping data.

- A collaboration agreement will be established between applicants and the mouse clinics.

- Starting material for the phenotyping projects are 12 individual age-matched (8-10 weeks) mice split in 4 experimental groups (3 males and 3 females for both control and mutant mice) or alternatively 2 experimental groups (6 gender matched animals for both control and mutant mice for immuno-oncology pipelines). Acceptance of mutant mouse lines depends on health certificates. Mouse mutants from various sources (transgenes, knockout mice, mutants from mutagenesis screens like ENU) and of different genetic backgrounds can be accepted.

- The analysed mouse models and the generated phenotyping data will be made available to the scientific community. An optional grace period of up to 1 year for mouse resources and phenotype data may apply, with immediate release of mouse resources and data after expiry of the grace period. A phenotyping report with all phenotyping data will be prepared, and data will also be uploaded onto the INFRAFRONTIER portal at http://www.infrafrontier.eu. Mouse mutant lines will be deposited into the INFRAFRONTIER/EMMA repository for subsequent use by the scientific community, and will be distributed using the applicant's institutional MTAs.

- **Costs:** The access to the INFRAFRONTIER2020 phenotyping service is free of charge. However, the shipment cost of mouse mutant lines to the CIPHE mouse clinic must be borne by the applicants.

- **Eligibility:** The INFRAFRONTIER2020 Trans-national Access call is open and proposals can be submitted from applicants around the world.

- **Application:** Service requests for the INFRAFRONTIER2020 specialised phenotyping service can be made via this application form. Applications for the Trans-national Access activity must include a short description of the research plans for utilising the phenotyped mouse model that is being characterised by the INFRAFRONTIER2020 TA service.

- **Selection procedure:** Proposals from eligible customers for free of charge access to the INFRAFRONTIER2020 specialised phenotyping service will be subject to a review procedure. The review will be based on short descriptions of the projects involving the mouse mutants that will be phenotyped by the TA service. A mixed panel of members of INFRAFRONTIER and of an external Evaluation Committee will assess service requests supported by the TA activity. In addition to scientific merit of applicants, relevance and quality of preliminary data, soundness of the proposal and research plans, and the project objectives and prospects for exploitation of phenotype data will be assessed. In a further step experts of the mouse clinics will assess the technical feasibility of projects. The technical evaluation of projects may require the provision of additional data such as:

  -Information on the genetic modification of your mutant mouse line if applicable (e.g. affected gene, MGI ID of the gene, type of mutation, ES-cell line used, genetic background (e.g. number of backcross generations)
  -Description of DNA modification (vector, remaining non-recipient DNA, donor organism)
  -Mutant phenotype(s), special housing or care requirements
  -Current sanitary status
  -Intellectual property rights (who generated and who owns the mouse line)

  Applicants will be informed on the outcome of the evaluation within 4 weeks after the end of the call for which the TA application was submitted. All applications will be handled with strict confidentiality.

- **Acknowledgements:** Please do acknowledge any support under this scheme in all resulting publications with 'Part of this work has been funded by the European Union Research and Innovation programme Horizon 2020 (Grant Agreement Number 730879)'. The participating infrastructure, which provided the service, should be specifically mentioned in any publication resulting from the service.

Specialised phenotyping pipeline

PHENOMIN-CIPHE - http://ciphe.marseille.inserm.fr/en/

CIPHE can support three projects in this TA call

Secondary functional immune-phenotyping screen by mass cytometry

To characterize the functional impact, in the immune system, of the invalidation or the overexpression of a gene of interest cellular immune-phenotyping is required. This primary immunophenotyping screen, essentially based on an extracellular labeling, aims at quantifying the various cell populations present within a model by monitoring the expression of specific cell surface receptors in hematopoietic cells. This first approach describing cellular heterogeneity does not assess the effector functions of immune cells. To this end, a more functional secondary immune-phenotyping screen is required.

The effector function capacities of immune cells were traditionally assayed by extensive characterization of cytokine production, the detection of master gene regulator of transcriptional program and more recently intracellular signaling. We propose to investigate a set of these hallmarks (12 cytokines, 7 transcription factors) at the single cell level on 8 different lineages (CD4 and CD8 T cells, B cells, NK cells, DCs, macrophages, neutrophils and monocytes) by mass cytometry in one set of experiment on same mice.

The service offer entails the analysis of 12 individual age-matched (8-10 weeks) mice at basal stage or upon inflammatory conditions or tumor challenge. Animals will be split equally over two experimental groups CTRL (C57BL/6) and MUT (mutant). One tissue (spleen, lymph nodes or tumor) will be collected and immune cells extracted. CD45+ cells will be enriched using magnetic sorting if necessary. All work will be done according to CIPHE standardized procedures. Cytokine production expression will be evaluated upon brief *in vitro* restimulation (PMA/Ionomycin + Brefeldin A for 4 hours). Remaining cells will be banked for later analysis if necessary (secondary experiments not funded through this call).

Application Form - INFRAFRONTIER2020 specialised phenotyping
Rolling deadline - collecting applications at the 1st of every month
The evaluation process will start directly after the deadline for applications has passed

Contact details of applicant

First name	
Family name	
Email	
Phone	
Fax	
Institution	
Address	
Town	
Postcode	
Country	
Link to lab website	
Link to publication list	

The following data is required by the EC for statistical purposes
Applications can only be considered if all data are provided

Gender	
Birth year	
Nationality	
Researcher status (e.g. Prof, Postdoc)	
Scientific background	

☐ **I have read, understood and agree to the INFRAFRONTIER data privacy policy**

Description of proposed project

Please describe briefly the proposed project involving the mouse mutant line to be phenotyped. This proposal will be the foundation for the evaluation of your project. Informal enquiries prior to proposal submission are welcome via proposals@infrafrontier.eu

Gene of interest	

Please, do not extend beyond the provided space (max 2 pages including references)

Send your proposal to proposals@infrafrontier.eu