MÉDECINE SORBONNE UNIVERSITÉ

Projet CIMI SARS-Cov2 – Sorbonne Université – Faculté de médecine

iCOVID

1. RÉSUMÉ DU PROJET

An exceptional effort to constitute prospective cohort with a biobank of blood samples from patients with COVID-19, admitted to the hospital wards of the Public Hospitals in Paris (Assistance Publique-Hôpitaux de Paris: AP-HP), will be undertaken as an emergency measure, with all required regulatory and ethical authorizations (the COVIDeF cohort).
The CIMI Research Centre will rely on this longitudinal cohort, also open to other researchers and clinicians as per the decision of its Scientific Council and kept at the Biological Resource Centre of Paris Hospitals, to conduct its own translational research program with financial support from Sorbonne Université.
Our goal is to determine immune pathways associated with clinical outcome in hospitalized patients that characterize the different COVID-19 disease evolution profiles. To achieve this goal, we will carry out case-control studies embedded in the COVIDeF cohort. Cases and controls will be defined primarily in terms of outcome (resolution or fatal clinical evolution), using an "immunomics" approach. The results from this basic research project will furthermore lead to the identification of biomarkers associated with disease severity and will make inroads to the development of new therapeutic targets.

2. DURÉE DU PROJET : 9 months

- First emergency results to be shared online with the medical and scientific community (first month)
- Analysis of first wave results (month 3)
- Extensive analysis (month 9)

3. CONTEXTE & JUSTIFICATIF DE LA RECHERCHE

Emergency actions in order to guide biotherapies. Coronavirus infections in the past have been characterized by the onset of a cytokine storm. The hallmark of a cytokine storm is an abnormal regulation and uncoordinated release of several pro-inflammatory cytokines at inappropriate time intervals during infection, such as interleukin (IL)-6, IL-β, Tumor necrosis factor (TNF-α) and Interferon (IFN)-α (*2*) resulting in an uncontrolled inflammatory response, acute respiratory distress syndrome, respiratory failure, shock, organ failure, and death (*1*). It is therefore reasonable to postulate that the inflammatory response measured both at cellular and molecular levels would represent a main prognostic signature for the disease.
The measurement of the above-mentioned pro-inflammatory cytokines may provide a rather accurate profile associated with the severity of the clinical condition of the patient (*3*). However, ELISAs and LUMINEX assays to detect cytokines in human sera have been hindered by low sensitivity and low specificity (*4*) and assays based on detecting biological activity are difficult to standardize. The new single-molecule array (Simoa) assay, also called digital ELISA, based on counting individual enzyme-labeled immunocomplexes of proteins captured on beads in single-molecule arrays, enables direct cytokine quantification at attomolar concentrations (*i.e.* fg/mL or $5x10^{-17}$ moles/mL), corresponding to 5,000-fold–increased sensitivity over ELISA (*5*–*7*).

Gaps in molecular and cellular signature of COVID-19 pathogenesis: Other immune correlates of COVID-19 severity are totally lacking and represent an unmet need in patient care. In fact, most studies that were conducted to evaluate potential cellular biomarkers specificity and sensitivity were done by comparing patients to healthy donors rather than to other patients with stratified severity, and by investigating one selective immune cell population at a time with limited number of targeted proteins. To reach this aim and avoid this limitation, we developed a multi-parametric immune profiling strategy to identify early **COVID severity-specific cytokines and leukocyte biomarkers**.
We hypothesize that **cytokine signatures, molecular and cellular biomarkers, and combinations thereof** would represent a biomarker of COVID-19-molecular related severity.

Cimi team fitness to respond to COVID-19 pandemic

1.1.1 Previous studies of Cimi-Paris on infectious diseases in epidemic and pandemic situations: Cimi-Paris has all the necessary expertise to conduct such a program in a state of emergency. In the recent past, several teams have successfully performed bioterrorism and smallpox (2003) (*10*, *11*), H1N1 (2009) (*11–13*) projects. During pandemic influenza A (H1N1), our groups demonstrated a pulmonary re-localization of Flu-specific T cells to the lung, despite severity, and showed that their peripheral blood recirculation correlated with the intensive care unit discharge (*14*).

1.1.2 Previous Cimi-Paris studies on immune resilience in acute medical events
The Coordinator has a long standing background of research on human anti-viral immunity (*29-30*) and played a pioneering role in the field of clinical digital ELISA translational research (*4*, *15, 27*) by demonstrating its superiority over bioassays and Quantiferon technology to quantify IFN-α and -γ, respectively. These studies highlight SIMOA potential to avoid false negatives and moreover to stratify patients according to clinical evolution.

The team of C. Combadiere recently used mass cytometry to phenotype whole blood of sepsis and of non-infectious inflammatory patients and identified novel, disease-specific cellular signatures (patent EP19305657.9). Using independent, unsupervised analysis strategies for high-dimensional data, we identified novel, early, and sepsis-specific cell subsets. These cell subsets were identified as immature neutrophils expressing IL-3 receptor (CD123) and/or PD-L1 (Combadiere C submitted).

The Vincent Vieillard/ Amelie Guihot team has a long lasting experience in monitoring NK and T cell responses to viruses.

The team of D. Sauce highlighted a profound cytokine storm occurring shortly post hip fracture (16). We postulate that it is also a persisting activation of innate immunity with resultant inflammation- and oxidative stress-induced collateral damages that is leading to COVID-19-associated death.

4. OBJECTIFS DU PROJET

The primary objective of the study is to **determine immune pathways associated with clinical outcome in hospitalized patients**, using results from isolated technologies (proteomics, transcriptomics and mass-spectro cytometry) or a combination of the latter.
In particular, biomarkers based on serum cytokine profiling are expected to be discovered during the first month of the study in order to efficiently stratify patients for severity at hospital admission and to guide biotherapies.

5. MÉTHODOLOGIE & MISE EN ŒUVRE

WP1 - Study design & biocollection: COVIDeF cohort.

Samples will be obtained from 120 patients divided in two groups: *i)* Patients admitted for surveillance remaining clinically stable at day 15 after admission, and *i)* patients admitted.
A multi-center COVIDeF "*Cohorte des patients infectés par le SARS-Cov-2 ou suspects de l'être (COVID-19 en Île-de-France) »* cohort program is currently implemented by the Assistance Publique-Hôpitaux de Paris (APHP). Coordinator : Pr Pierre Hausfater, Scientific coordinator : Pr Guy Gorochov, Methodologist : Pr Florence Tubach (CPP ethical application submitted 22/03/2020). The target population for this study is composed of adult, SARS-CoV-2-infected, patients who are hospitalized, in an APHP hospital, as well as patients suspected to be affected by COVID-19, but for whom the diagnosis has not been confirmed. Part of COVIDeF blood samples will be processed in the Department of Immunology (Pr. G. Gorochov) and banked (cryo-preserved PBMC, Paxgene and Serum) at the Centre de Ressources Biologiques of Pitié-Salpêtrière Hospital. Up to 2000 patients are planned to be included across the various CRBs.

As clinical evolution profiles materialize rapidly (days), 120 patients are expected to be selected from the large COVIDeF cohort after less than 2 weeks of banking in order to define two groups matched for age sex and co-morbidities: *i)* patients admitted for surveillance remaining clinically stable at day 15 after admission, and *ii)* patients admitted for surveillance with subsequent clinical deterioration requiring ventilation and/or leading to death. WP1 ***(Figure 1).***

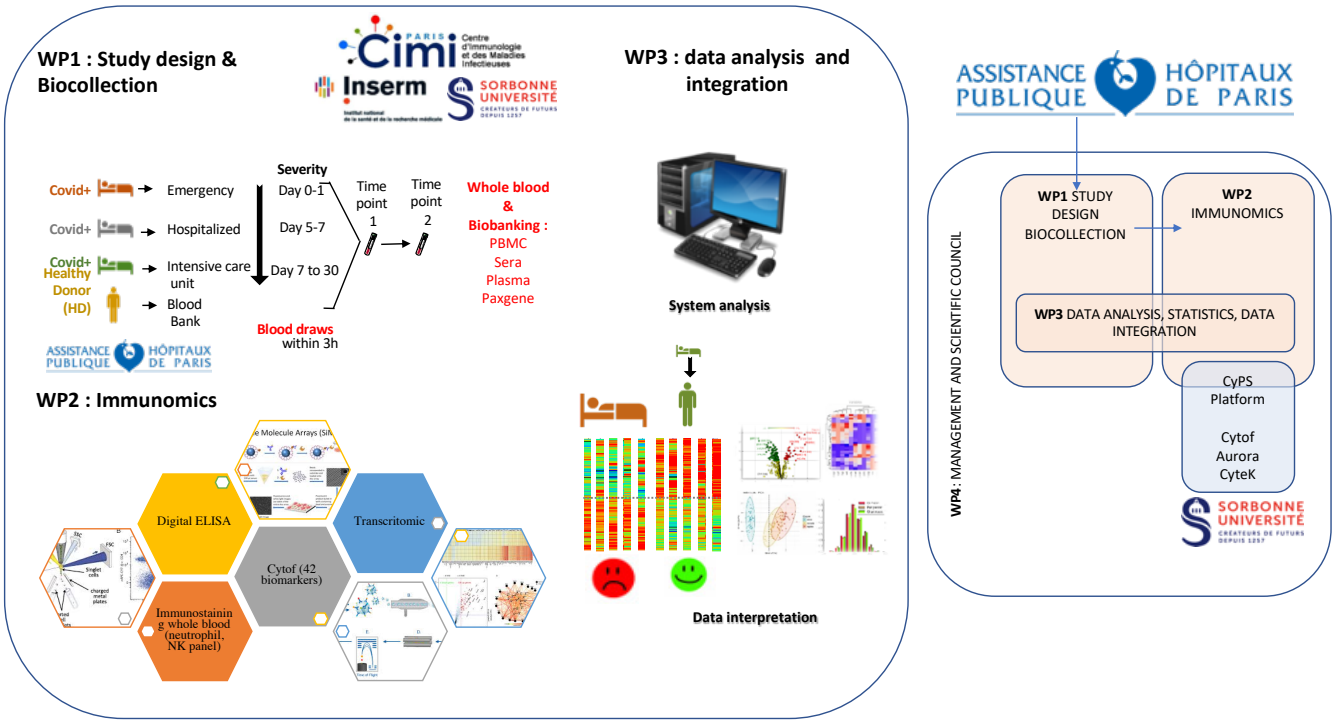

Figure 1: WP schema and consortium organization

WP2 - Immunomics approach *to determine molecular and cellular blood markers of severity*

The objective of WP2 *(Figure 1)* **is, in relation with WP3, to gather multidimensional data on both molecular and cellular innate immune networks in order to define the kinetic parameters that characterize the different disease COVID-19 evolution profiles, using a systems biology approach.**

Mass-spectro cytometry: Whole blood samples and PBMC will be analyzed at 4 time points (see above). This will enable us to get a deeper insight into the immune network modifications before and during COVID-19 complications. We already conducted a comprehensive analysis of whole blood circulating human immune cells. **A ready-to-go 42 panel biomarkers** will be used to identify over 20 immune cell populations and characterize their activation, polarization or differentiation status (CyPS

platform of Pitié-Salpêtrière, Catherine Blanc). Data wil be analysed using an unsupervised dimensional reduction algorithm (viSNE on Cytobank, hSNE on Cytosplore and FlowSom on R).

In parallel, functional assays will be performed to determine (i) the polyfuctional capacity of NK cells (cytotoxicity and inflammatory cytokine production) from infected patients in the presence of COVID-19-infected target cells and (ii) the function specific COVID-19 T cells by Elispot assay.

Digital Elisa (Simoa technology): Cytokines and chemokine concentrations, expressed in fg/mL, will be determined with Simoa, digital ELISA technology (Quanterix Simoa™ Lexington, MA, USA), using the HD-1 Analyzer (Quanterix™) (9). A cytokine profile will be determined by through multiparametric analysis of GM-CSF, IFN-a, IFNg, IL-1b, IL-2, IL-6, IL-8, IL-10, IL-15, IL-13, IL-17A, IP10, MCP1, TNF-a, IL-22 and IL-28A. Neopterin, a marker associated with clinical progression of patients infected with SARS *(17)* will also be measured.

Myeloid compartment modifications during SARS-CoV-2 infection. Different monocytes subpopulations such as patrolling, inflammatory or traditional monocytes are recruited from blood to the lung during pulmonary viral infection. Besides, polymorphonuclear neutrophils are essential to the ARDS (acute respiratory distress syndrome) immunopathology including mature and activated PMN. Therefore, such description of the myeloid compartment modification will be provided with the Mass spectrometry and with spectral flow cytomtry (Aurora® that will be available also on the CyPS platform of Pitié-Salpêtrière,(Catherine Blanc) in the context of the COVID sanitary emergency.

NK cell responses to SARS-COV2 : NK cells are crucial in the establishment of innate and specific immunity to viruses. The cytokine storm previously described is expected hamper the establishment of specific immunity to the virus and will be studied longitudinally.

Gene expression transcriptome: Holistic systems biology approaches provide a global picture of the response to infectious diseases and treatment, and thus identify immune correlates that can predict clinical outcomes. Transcriptomics will be applied to blood cells (whole blood or peripheral blood mononuclear cells [PBMC]). 2.5 ml wholeblood will be collected in PAXgene tubes (Qiagen) at inclusion. RNA will be amplified with the Ovation Pico WTA system version 2 (NuGEN) and the resulting cDNA hybridized on Illumina whole human genome oligo microarrays (HT12 version 4.0). Genome Studio (Illumina) will be used to analyze the data (WP3).

WP3 - Data analysis and integration *to help in decision-making*

WP3 is dedicated to data analysis, integration in order to help in decision-making for therapeutic approaches of COVID-19 patients ***(Figure 1).*** Due to multiple markers, test multiplicity must be considered. Because all clinical studies are primarily of exploratory nature, false discovery rate (FDR) methods are used for multiplicity adjustment. In addition, dimension reduction can facilitate the interpretation of the data. In first instance we shall use Principal Component Analysis (PCA) with a spearman correlation matrix.

The diagnostic performances of a short-listed biomarkers (blood gene expression, cytokines, cell populations) to predict clinical severity will then be investigated by analyzing receiver operating characteristics (ROC) curves, with very severe COVID-19 (defined as death and/or ventilation) as the gold standard for those analyses.

6. RÉSULTATS ATTENDUS

Our study will provide an accurate picture of the individual profiles associated with COVID-19 (resolutive of fatal evolution) and therefore the **biomarkers** needed to stratify patients at hospital entry in terms of

severity. It will also be particularly useful very rapidly (days) to inform on **therapeutic targets (anti-cytokines) to be used at the right time, in the right patient.**

7. ÉQUIPES IMPLIQUÉES

- **Équipe pilote : Guy Gorochov : PUPH, CIMI-Paris et Dpt. APHP d'immunologie.**
- **Équipes partenaires CIMI-Paris: Behazine Combadiere** (DR1), **Christophe Combadière** (DR1), **Delphine Sauce** (CRCN), **Vincent Vieillard (**DR2**) & Amélie Guihot** (MCU-PH).

AP-HP – Pitié-Salpêtrière Paris : Non-exhaustive list of main clinical collaborators
Emergency medicine Unit : Pr Pierre Hausfater
Clinical Research Unit: Pr Florence Tubach
Internal Medicine: Pr Zahir Amoura
Infectious disease unit : Pr Valérie Martinez
ICUs : Pr Martin Dres, Pr Thomas Similowski, Pr Alexandre Demoule, Pr Alain Combes, Pr Pascal Leprince, Pr Charles-Edouard Luyt.

Sorbonne-Université : Platform cytometry CyPS
Catherine Blanc (Operational Manager) and Aurélien Corneau (Engineer Mass-cytometry)

8. BUDGET

Consumables for Simoa are PHRC "Digital COVID"-funded (**135 k€**). Additional cost requested for *120 patients and 4 time-points* : **75 k€**; cytof kit and reagents, medium, flow cytometry: 32 k€ and transcriptomics : 15 k€ - Internal invoices platforms : 12 k€. Altrabio sub-contradting: data cleaning, normalization : 10 k€. - Sorbonne Université fees (8%) 6 k€.

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