Specialized Hematology: An Ambition for HORIBA Medical

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With the increase of cancer occurrences, its management requires novel diagnostic tools, improved performance and adaptation to personalized medicine. Such technological development can only be successful by the convergence of expertise between the medical field and the experience of industry in translating innovation into products. In that spirit, HORIBA Medical teamed up with University Hospital Center (CHRU) of Montpellier. That collaboration is realized by the presence of a HORIBA laboratory within the hospital working closely with local teams. After a successful initial project on Minimal Residual Disease (MRD), the strategy was recently extended to other hemopathies aiming at the discovery of new oncological biomarkers that could advance earlier diagnosis as well as the constitution of an original bio-bank focused on hemopathies and in particular lymphoma and myeloma.

Introduction

The first benefit of our modern society is better health for people in general. However, with the increase of life expectancy we see also an increase in cancer. Among them, malignant hemopathies represent about 10% of the number of cancer cases but require 30-40% of oncology resources. Hematology, the science of blood, is at the forefront of this battle acting on several levels: diagnosis, treatment and follow-up. Furthermore, the earlier the detection the better the outcome and less aggressive the treatments required.

HORIBA group has a long history in fluid analysis and HORIBA Medical is one of the world's top-five leaders in blood analysis. With that history, it is natural to utilize that expertise to provide solutions to improve general practices in specialized hematology. This can be done by providing a range of analyzers that address some key points: automation (avoid the need of a specialist and therefore reduce the labor cost while increasing the throughput), innovative diagnostic algorithms for earlier detection of blood disorders and/or development of new markers, propose solution for unmet needs such as companion devices for the monitoring of specific treatments, increase sensitivity for better management of MRD, etc. MRD, etc.

The development of such tools requires the convergence of competences from different fields: medicine, biology, engineering, data management, computer sciences, etc. Furthermore, none of that can happened without access to fresh biological samples with complete medical background on the patient. It is then of paramount importance to have not only an easy access to these materials but also to the medical teams involved. This was achieved by setting up a privileged collaboration with Saint-Eloi Hospital in Montpellier and the installation within its premise of a HORIBA laboratory.

Partner's Description

CHRU of Montpellier

Montpellier, a dynamic city in the south of France, has a long medical history. Founded around a thousand years

ago, its medical school is the oldest medical faculty still active in the medical field today. At the beginning established to provide care to the pilgrims on their way to Rome, Jerusalem or Saint James of Campostela, it is now at the forefront of modern medical research and practices. CHRU of Montpellier, classified 5th of all CHRUs in France and first enterprise in the region, has a capacity of 3,000 beds and employs around 11,000 persons. Every year, there are more than 71,000 days of hospitalization, 500,000 consultations and 100,000 admissions in emergency room. The CHRU hosts 13 platforms of excellence distributed over 7 establishments. Its essential missions are centered on three activities: care, teaching and research but plays also a significant role in social issues and disease prevention areas.

Saint-Eloi hospital

Part of the CHRU, it is the oldest hospital in Montpellier built around a chapel named after the Minister of finance of King Dagobert (632-639). Although the Chapel and the old building of hospital-Dieu Saint-Eloi were preserved, a modern hospital of 521 beds was built around it combining progress with tradition. Saint-Eloi hosts now all the usual medical specialties including clinical and biological hematology.

Clinical hematology

The department of clinical hematology is one of the leaders in translational research in lymphoid hemopathies and coordinator or partner of more than 70 clinical trials in connection with pharmaceutical or bio-medical industrial partners. Furthermore, it is connected with other academic and medical groups in the region covering the entire south west of France from Toulouse to Nimes with strong support from the Languedoc –Roussillon regional administration but also nationally (Labex MAbImprove and Institut Carnot du Lymphome CALYM). Coordinated by Professor G. Cartron, the department is the regional reference center for both malignant and non-malignant hemopathies and deals every year with around 800 new cases and follow-up of 2000 patients. It is also the regional center for hematopoietic stem cell transplantation and performs annually 160 such transplants.

Biological hematology

The department of biological hematology, directed by Professor J.F. Schved, is responsible for all analysis on blood or from other hematopoietic organs. The activity of this technico-medical platform is divided along 5 axes: (1) cellular hematology of blood, bone marrow, lymph node or effusion fluids; (2) hemostasis investigations; (3) erythrocytic explorations; (4) genotypic molecular biology and (5) cytogenetics of malignant hemopathies.

The laboratory for Monitoring of innovative Therapies (STI, CHRU of Montpellier) headed by Professor B. Klein is dedicated to the biological follow-up of immunotherapy trials and the detection of rare circulating cancer cells in peripheral blood and bone marrow. This group is a reference center for the diagnosis of Multiple Myeloma (MM) and the post-treatment follow-up of MRD. MM is a hematological malignancy characterized by the accumulation of malignant plasma cells in the bone marrow. MM is the second most-common malignant hemopathy, accounting for 1% of all cancers. Despite the development of efficacious drugs, in particular proteasome inhibitors (bortezomib) and immunomodulators (lenalinomide) that have improved patients' survival, MM remains an incurable disease for a majority of patients. The tasks of STI laboratory are: (1) evaluation of residual or circulating tumor cells, of their proliferation rate and oxidative stress status, (2) characterization on how treatments affect the genetic diversity and instability using whole genome sequencing of purified tumor cells in order to further design tailor made treatments, (3) determination of gene expression risk scores prognostic for patients' survival using whole genome transcriptome profiling of tumor cells utilizing microarrays and RNA sequencing.

HORIBA medical laboratory at Saint-Eloi

The laboratory was recently established in 2012 in the historical building of Hospital Saint-Eloi, on the same floor of the laboratory of biological hematology and neighbor of the INSERM's group working on myeloma (STI) and across the street from the department of clinical hematology. (Figure 1) The close proximity favors the interactions and exchanges between the different partners helping in building dynamic and stable long run

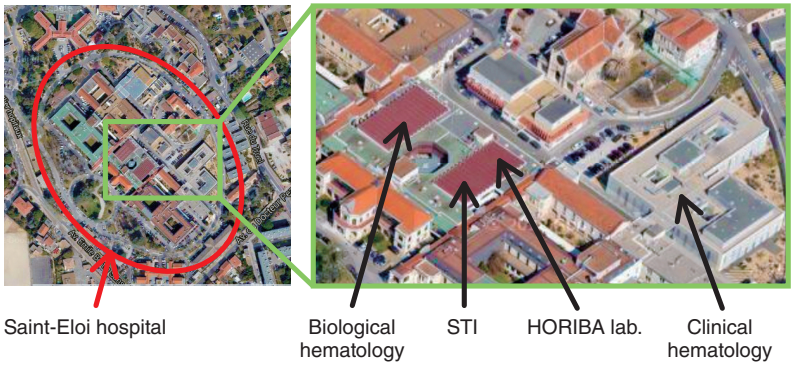

Figure 1 HORIBA Medical Laboratory at Saint-Eloi

collaborations. The laboratory was equipped to be able to process blood and bone marrow samples from patients affected by various hemopathies. On-site, we have HORIBA Medical's instruments already on the market (Pentra XLR 80, Pentra DX 120 Nexus) and one in development, as well as one from a competitor (ADVIA from Siemens).

Furthermore, a high range flow cytometer (LSRFortessa X20 from Becton Dickinson) was also added in order to look for innovative novel biomarkers. It is equipped with three lasers (Violet, Blue and Red) and an optical bench allowing for the detection of 14 fluorescent channels on top of the two morphological parameters (forward and side scatter) allowing a total of 16 parameters. For example, that high content capacity was put into use, with the development of a 16-parameters analysis panel for complete blood differential determination allowing the identification of the usual 5-diff counts (Lymphocyte, Monocyte, Neutrophils, Eosinophils and Basophils) but also the following sub-populations of leukocytes: T-cells (Th2 helper, T4, T8, senescent T-cells, gamma-delta), B-cells (B-mature and immature), NK-cells (NK-mature and immature), NKT-cells, monocytes (classical, non-classical, intermediary, pro-inflammatory) and different classes of immature cells (Immature granulocytes, various classes of blasts…). Furthermore, from our preliminary results, it seems possible to identify some immunophenotypic profil characteristic of some specific hemopathies (follicular lymphoma, CLL, Mantle lymphoma, lympho-proliferative disorder, large granular lymphocytic leukemia). That information could be used as orientation to direct toward more specific panels to be done such as those developed by the EuroFlow[1] (www.Euroflow.org) or Harmonemia European programs.[2] All these instruments are shared with the Clinical Quality department of HORIBA Medical for evaluation of the HORIBA automates in client-like conditions on real blood samples. All these pieces of equipment are connected to a server that manages the analyses to be done and that harvests all the corresponding data files for subsequent queries and analysis.

Sample processing

The laboratory of clinical hematology identifies hemopathies matching the following inclusion criteria: HIV negative, older than 18 years old, outpatient or hospitalized patient with suspicion of hemopathy justifying complementary exploration and to be registered in the national health care system. Once included, the patients sign an informed consent form and become part of a cohort that is managed with a protocol that has been submitted to the French health regulatory agency (ANSM/CPP) and complies with personal information protection agency (CNIL) regarding anonymity. The data are cross-related only with a unique identification number. Biological samples are then sent to biological hematology department for conventional analyses according to the physician prescription. Furthermore, some samples are processed for banking. Additional aliquots withdrawn after informed consents are collected from the patients and sent to the HORIBA laboratory in Saint-Eloi. Based on the pathology, various analyses are performed on the hematology analyzers and stored in a database. Information on the patients, including selected data from the electronic Case Report Form, as well as the biological hematology results can be accessed also to complete the medical profiles of analyzed samples.

Collaborative Projects

This platform within the hospital and the connections with the different departments of hematology allows HORIBA to have privileged access not only to valuable fresh biological samples with related medical information but also to the medical and biological expertize of leader research teams in the field of hematology and oncology. On the CHRU side, hospital laboratories can benefit from experience of HORIBA in translating innovative research into real world applications speeding up the access for the patients to the latest medical discoveries. Two projects have already started in partnership with these hospital laboratories.

Monitoring of MRD

The first project was a collaboration with the STI team of Professor B. Klein who developed a multiparameter immunophenotyping of multiple myeloma by flow cytometry. The methodology has already been previously reported in this journal (issue No. 39).[3] Briefly, MM is a neoplasm of plasma cells with accumulation of malignant cells in the bone marrow. The various treatments intend to eliminate selectively and completely these malignant cells while preserving the neighboring healthy cells. The elimination should be total to avoid rebound of the cancer from leftover or drug-resistant cancer cells. However, even in patients having well responded to treatment, some of the malignant cells can still be found in their body, which is called MRD. Flow cytometry has demonstrated to be a very sensitive technique to detect and monitor those residual cells as compared to conventional morphological techniques. Although newer technologies such as deep sequencing could have higher sensitivity[4] (but are more time consuming and more costly), flow cytometry is still a method of choice in most of the cases and easier to bring to full automation reducing the need

for skilled technician.

Few markers of malignancy could be followed and used to discriminate tumoral cells from the healthy ones within plasma cells. Using our high content flow cytometry, we are now exploring the redundancy on individual cells of these markers in single tube analysis with still the possibility to evaluate others yet to be discovered. Indeed, the samples from our original cohort will also be later used to do some molecular biology (genomics, proteomics) on the tumoral cells and hopefully allowing us through epidemiology studies to identify novel useful markers to be used in the diagnostic, prognostic, and/or monitoring of response to treatment. If found, we will then try to integrate these markers, probably in combination with others, in a sort of malignancy profile by design of a new generation of instruments in particular in the scope of companion diagnostic (a test specifically designed to monitor one particular treatment).

Although this particular project was focused on MRD of multiple myeloma, the general methodology (and experiences learnt in the process) will later be translated to other diseases for which there is a need to selectively distinguish a rare population of cells surrounded but a very large number of healthy cells to which they differ only by few markers. Indeed, apart from multiple myeloma,[3] MRD approach is already a key component in the management of Chronic Myeloid Leukemia(CML),[5] Acute Myeloid Leukemia (AML),[6, 7] Chronic Lymphocytic Leukemia (CLL),[8] Acute Lymphoblastic Leukemia (ALL),[9] lymphoid malignancies,[10] disease monitoring before and after stem cell transplantation[11] and during salvage therapy,[12] Burkitts's lymphoma,[13] anaplastic large-cell lymphoma[14] or childhood T-cell lymphoblastic lymphoma[15] to mention only a few. It is not limited to blood cancer but also applies to other cancers such as colorectal,[16] breast [17] or lung[18] cancers as well as in drug development (FDA requires the use of MRD as an endpoint for drug approval[19]). Currently, MRD testing is used to assess treatment efficiency, as prognostic tool for post-therapy relapse, in follow-up post-therapy to control the remission and eventually trigger early for pre-emptive treatment and when genetic methods are used for the analysis of genetic drift prior to relapse.[20] Mostly MRD and related MDD (Minimal Disseminated Disease) are monitored by polymerase chain reaction methods (sensitive but labor intensive) or multicolor flow cytometry (sensitive and more amenable to full automation). (Figure 2) However, new methods such as next generation sequencing are now being used.[21] Which method is the best is still a matter of debate.[22]

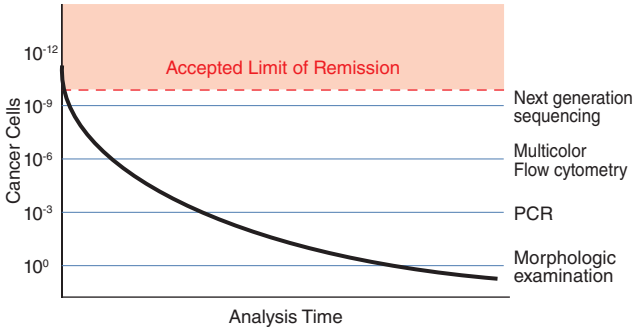

Figure 2 Schematic representation of relative temporal and quantitative limit of detection of technologies used in MRD monitoring

Although quantitative PCR shows high sensitivity in detecting MRD in patients with abnormal cytogenetics, rearrangements or mutations, suitable markers are still missing in 60% of the cases.[23] Flow cytometry technique while still very sensitive, doesn't present such issues and can be easily integrated in routine automates even if there are some limitations that will need to be addressed. The main limitation is the availability of specific and selective antibodies against the discriminating biomarkers. This is true in particular for newly described markers identified by epidemio-genetics investigations.

Other hemopathies

With this successful collaboration on the MRD of MM, we have started a larger project extended to other malignant hemopathies which will be in place between the partners of CHRU and HORIBA Medical. As regional center, patients with all the range of hemopathies come to the department of clinical hematology for consult. There is here a huge opportunity to constitute a unique bio-bank for the different types of blood cancer. Therefore, on top of the required analyses for the diagnostic testing, the blood and bone marrow samples collected in our cohort are processed to isolate cancerous cells (from blood, bone marrow or lymph nodes), their DNA, RNA, plasma, etc. All these biologics as well as their related epidemiological, clinical and biological information will be stored in the regional bio-bank hosted by the CCBH (center for hospital biological collections) that has already all the required logistic. These samples will be available for subsequent OMICS studies (genomics, proteomics, metabolomics, etc) to the scientific community (including HORIBA Medical and the close to 600 researchers working on cancer in Montpellier area). Currently, a research framework is being created in CHRU to support such projects. The first samples have been collected and used to setup the different steps in the collection, transfer, analysis and storage of the samples.

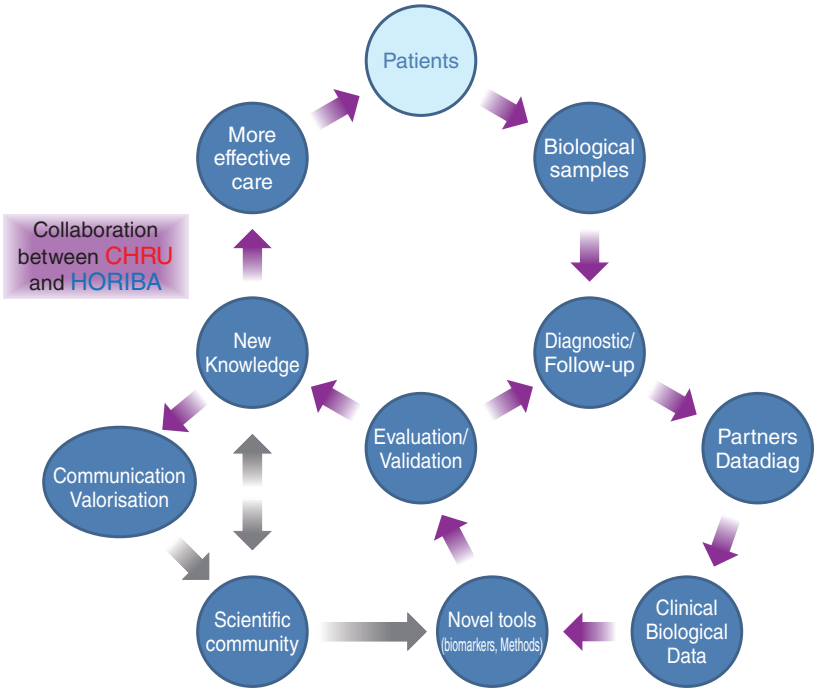

Figure 3 Collaboration between CHRU and HORIBA

Collected Data

This cohort provides a unique opportunity to collect data from known pathologies, with high quality references for abnormal cells thanks to 14-colors cytometry and clinical data. The samples being processed on several HORIBA instruments, and all measurement data being automatically archived in a database, it will be possible to use many of the emerging techniques of data mining, in order to find in the responses of each instrument, the best way to build robust algorithms and specific flags for the different pathologies. This will help the biologist for diagnosis, and therefore increase the quality of HORIBA instruments. Furthermore, it will be possible to analyze the contribution of each channel of each instrument to a given topic, and then to build an analyzer optimized for one or several pathologies.

Conclusions

Combining the medical and research expertize of the CHRU with the experience of HORIBA in terms of translating biological concepts into industrial processes to develop innovative instruments, we have great expectations that this project will deliver better performance, more robust, highly sensitive and specific tools for diagnostic testing, the therapeutic selection and the monitoring of patients suffering from hemopathies that will contribute to a higher standard of health care for cancer patients. (Figure 3)

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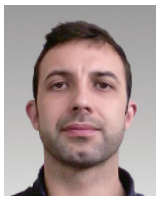

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