Team 7 (2006-2008) → *Team 4 (2008-2010)*:
Biology of dendritic cells and immunotherapeutic use in oncology (application to mesothelioma and AML)

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Résumé

La vaccination antitumorale implique l'activation de la réponse immunitaire. Contrairement à la chimiothérapie ou à l'immunothérapie adoptive impliquant l'utilisation d'anticorps ou de cellules T, la vaccination antitumorale n'a pas une activité antitumorale directe, mais son objectif est de déclencher une réponse immunitaire efficace et spécifique.

Les cellules dendritiques (DC) fournissent les outils efficaces pour induire une réponse immunitaire T « tumeur-spécifiques ». Cependant, malgré la potentialité de cette approche thérapeutique impliquant la vaccination de DC autologues chargées en antigènes de tumeur, les résultats actuels restent décevants. Ceci pourrait être attribué soit à la maturation non adaptée des DC lors de leur utilisation clinique (DC dites « exhausted » (épuisées) ou aux faibles propriétés immunogéniques des antigènes présentés par les DC au système immunitaire.

C'est pourquoi, l'objectif principal du notre recherche était d'étudier l'état de maturation DC dans le cadre de leur utilisation en approche thérapeutique, et de valider leur chargement en antigènes de tumeur par des cellules tumorales apoptotiques. Ces études biologiques et immunologiques ont été menées par notre équipe depuis le début des années 2000 et plus précisément dans le cadre de projet clinique avec la participation, depuis 2007, de la plate-forme de transfert clinique (PF DTC) que j'ai mise en place au sein du CIC Biothérapie (CHU de Nantes). Ces travaux ont vu leur implication concrète dans le traitement de la LAM et l'intégration en 2008 du Pr M. Mohty (Service hématologie et transfert clinique). En 2008 également, notre équipe a été renforcée sur le plan immunologique par l'arrivée du Dr. J.-F. Fonteneau (CR1 Inserm). Les travaux que nous avons développés sur les modalités d'induction d'apoptose « immunogène » ont été fortement renforcés en 2008 par l'arrivée du Dr. D. Pouliquen (expériences murines), et en 2009, du Dr. Ph. Blanquart (biologie moléculaire et physiologie cellulaire).

Abstract

Therapeutic cancer vaccines rely on the immune system to eliminate tumor cells. In contrast to chemotherapy or passive (adoptive) immunotherapies with antibodies or ex vivo-expanded T cells, therapeutic vaccines do not have a direct anti-tumor activity, but aim to reset patients' immune systems to achieve this goal.

Recent identification of effective ways of enhancing immunogenicity of tumor-associated antigens, including the use of dendritic cells (DC), provides effective tools to induce high numbers of circulating tumor-specific T cells. However, despite indications that some of the new cancer vaccines may be able to delay tumor recurrence or prolong the survival of cancer patients, their ability to induce cancer regression remains low. This could be attributed either to the non adapted maturation of the DC in the clinical use (non matured or over matured "exhausted" DC) or to the non immunogenic properties of the Tumor Associated Antigens (TAA) that were associated to the antigen presenting cells (APC).

Thus, the main objective of our researches was to investigate on the definition of the maturation state of the DC for clinical uses and the development of apoptotic process of tumor cells that rendered them immunogenic.

Biology of the DC and their implication in anticancer vaccines was initiated by the team before the years 2000 and largely reinforced with the help of the Platform of Clinical Transfer that I initiated in 2007 as a structure of CIC Biotherapy (Nantes Hospital) and recently with the integration in 2008 of Pr. Mohamad Mohty (Hematology and clinical transfer) and Dr. Jean-François Fonteneau (immunology). The induction of an immunogenic apoptotic process was investigated by the team as soon as the years 1992. However the investigations in the field of mesothelioma was strongly reinforced recently (2008) with the help of Dr. Pouliquen (murine experiments), and Dr. Blanquart (molecular biology and cell physiology).

Dendritic cells and their implication in antitumor treatments

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State of the Art

Anti-tumour immunotherapy using dendritic cells (DC) is a new therapeutic approach which was made possible by two key observations. Firstly, the identification of tumour antigens recognized by CD4+ and CD8+ T-cells which are able to mediate the rejection of tumours, and secondly, advances in the understanding of DC biology, including the development of techniques to culture large numbers of monocyte-derived DC. Together, these discoveries opened up the possibility of harnessing the unique T-cell stimulatory capacity of DC to augment immune responses against tumour antigens and thereby initiate tumour rejection by the immune system (1). In particular, DCs have the potential to overcome the tolerance to tumour antigens which is encountered in patients with many different type of cancer. Indeed, several studies have reported complete remissions in patients with metastatic cancers resistant to classical therapies (2). Although individuals with complete responses have up till now been rare, they have provided proof of principle that DC-based therapy can be clinically effective, even in patients with advanced disease. One of the most important challenges for trials is now to increase the efficacy of immunotherapy using DC in order to improve clinical responses.

In addition, the TAA loading of the DC is of crucial interest. Peptides, long peptides, proteins, DNA, RNA, cell extract, lysates or apoptotic cells are generally tested and compared (3). By tracing how anthracyclines and gamma-irradiation trigger immunogenic cell deaths, Zitvogel and Collaborators recently found that they were causally connected to the exposure of calreticulin (4) and HMGB1 (5) on the tumor cell surface, before apoptosis in the tumor cell itself occurred. Thus, several phagocytic signals ("danger signals") expressed on apoptotic cells mobilize complement proteins, which in turn can promote immune responses (4).

Investigations by the team

During the last ten years, we have shown that the maturation of monocyte-derived DC (Mo-DC) depends on the duration and the sequence of “danger” signals but also on the nature of dead cells that they phagocyte. The signals correspond either to inflammatory cytokines, to pathogenic agents, or also to molecules such as heat shock proteins (HSP), or CD40L. More recently, in collaboration with the team of Dr. I Anegon, Unite 643 INSERM, Nantes, and with the technical help of the "Development and Clinical Transfer Platform" that I initiated three years ago, we demonstrated that maturation of DC is much more efficient in terms of functional properties, when the maturation signals, "danger signal" or CD4+ T cell derived signal, are multiple and sequential (see publications below). In a recent in vitro study, we investigated the function of human Mo-DC matured sequentially by exposure to peripheral maturation stimuli followed 10hrs later by exposure to activated CD4+ T cells. We found that sequential maturation, compared to other maturation conditions, dramatically increased the maturation of human DC in terms of their phenotype and cytokine secretion. Furthermore, such sequential maturation led to activation of a stronger Th1 phenotype of the CD4+ T cell responses stimulated by these DC, with a limited expansion of CD4+CD25+FoxP3+ regulatory T cells and a better differentiation of anti-tumor CD8+ T cells with a long-term memory phenotype (*Simon T et al, submitted*). Finally, we developed a clinical investigation using autologous Mo-DC that phagocytose apoptotic blasts in the treatment of Acute myeloid leukemia. This clinical trial is in progress.

Collaborators:

- Dr. I. ANEGON, DR2, INSERM Unit 643, DC and tolerance (IDO, HO-1, Treg)
- Development and clinical Transfer platform, Clinical Investigation Center in Biotherapies, (Ms D. COULAIS)

Main publications of the team in the field:

→ Grégoire M. What's the place of immunotherapy in malignant mesothelioma treatment? *Cell Adhesion and Migration*, **4**, (1), 153-161, 2010.

→ Grégoire M. A near future for curative vaccine in mesothelioma *Expert Review of Respiratory Medicine* 4(3), 311-314, 2010.

→ Thomas Simon, J.-François Fonteneau, Marc Grégoire. Dendritic cell preparation for immunotherapeutic interventions. Immunotherapy 1: 2. 289-3020 2009.

→ Remy, S., Blancou, P., Tesson, L., Tardif, V., Brion, R. ,Royer, P. J., Motterlini, R., Foresti, R., Painchaut, Pogu, S., Gregoire, M., Bach, J. M., Anegon, I., Chauveau, C. Carbon monoxide inhibits TLR-induced dendritic cell immunogenicity, J Immunol., 182(4):1867-1884, 2009

→ Sapede C, Gauvrit A, Barbieux I, Padieu M, Cellerin L, Sagan C, Scherpereel A, Dabouis G, Grégoire M.Aberrant splicing and protease involvement in mesothelin release from epithelioid mesothelioma cells.Cancer Sci. Mar;99(3):590-4. 2008

→ P. Larrieu, V. Renaud, Y. Godet, F. Jotereau and J.F. Fonteneau. A HLA-Cw*0701 restricted Melan-A/MART1 epitope presented by melanoma tumor cells to CD8+ tumor infiltrating lymphocytes. Cancer Immunol Immunother. 57(5):745-52. 2008.

→ P. Larrieu, L.H. Ouisse, Y. Guilloux, F. Jotereau, and J.F. Fonteneau. A HLA-DQ5 restricted Melan-A/MART-1 epitope presented by melanoma tumor cells to CD4+ T lymphocytes. Cancer Immunol Immunother. 56(10):1565-75. 2007.

→ Pierre-Joseph Royer, Gwenola Bougras, Frederic Ebstein, Lucie Leveque, Severine Tanguy-Royer, Thomas Simon, Nadine Juge-Morineau, Patrice Chevallier, Jean-Luc Harousseau, Marc Gregoire (2008) Efficient monocyte-derived

dendritic cell generation in patients with acute myeloid leukemia after chemotherapy treatment: application to active immunotherapy. Exp Hematol 36: 3. 329-339 Mar 2008

→ **Clinical study :** Multicentric study, a phase I/II immune cell therapy based on autologous dendritic cell injection loaded with autologous apoptotic blasts from acute myeloid leukemia in 2nd recovery.

→ Royer Pierre-Joseph, Tanguy-Royer Severine, Ebstein Frederic, Sapede Carole, Simon Thomas, Barbieux Isabelle, Oger Romain, and Gregoire Marc. Culture medium and protein supplementation in the generation and maturation of dendritic cells. Scand. J. Imm. 63 (6), 401-409, 2006.

DNA methylation and histone deacetylation inhibitors

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State of the art:

Cancer cells contain multiple gene defects that lead to oncogene activation and the loss of tumor suppressor gene function. These defects in gene transcription are primarily mediated by epigenetic mechanism. The most widely cancer-related epigenetic modifications are DNA methylation and histone acetylation that clinicians try to revert using hypomethylating drugs and/or histone deacetylase inhibitors. Such drugs can restore expression of gene implicated in the cell cycle or gene with tumor suppressor functions that will limit or stop tumor growth. In addition, induction of tumor antigen expression was also observed in tumor cells treated with these drugs. Numerous preclinical and clinical studies have proved that hypomethylating drugs such as cytidine analogs 5-aza-2'-deoxycytidine (decitabine, 5-azaCdR), and histone deacetylase inhibitors (HDACis) such as valproic acid (depakin, VPA) and suberoylanilide hydroxamic acid (vorinostat, SAHA), have a potent anticancer activity and promising therapeutic potential (6,7).

Before 2006, we have demonstrated that specific treatment of colorectal cancer cells with hypomethylating agents such as sodium butyrate, induced apoptosis that acquired immunogenic properties (8). We demonstrated in vitro and in vivo that the phagocytose of such apoptotic tumor cells by antigen presenting cells (APC) induced an immune response that cured most of the tumor bearing rats **(9, 10)**. In order to reinforce our investigation, recent reports indicate that in contrast to prior belief, tumor cell apoptosis is not necessarily silent but can be immunogenic.

Investigations by the team

In a recent *in vitro* study, we investigated the toxicity of 5-azaCdR, VPA and SAHA, alone and in sequential combination on MPM tumor cell lines. We concomitantly assessed effects of the drugs on firstly the expression of New-York esophageal cancer (*NY-ESO-1*) and melanoma-associated antigen (*MAGE-A1* and *-A3*) in a panel of human MPM cell lines (over 25) that we have developed in the laboratory and secondly on the NY-ESO-1 specific T cell response. We demonstrated that combinations of 5-azaCdR/VPA or 5-azaCdR/SAHA, partly kill MPM cells and that expression of tumor antigens such as NY-ESO-1 is induced in remaining treated cells. We also demonstrate that 5-azaCdR/VPA treatment induces NY-ESO-1 expression in MPM cells and triggers their recognition and lysis by a NY-ESO-1 specific T cell clone. Furthermore, using a mouse model of mesothelioma adapted in our laboratory, we reported tumor regression associated with lymphocytes infiltrates after sequential 5-azaCdR and VPA treatment. Our results show that some chemotherapeutic drugs combinations, in addition to induce tumor MPM cell death, affect also their immunogenic status (*Leclercq S et al, under revision for publication in the European Respiratory Journal*).

We recently extended this work by studying effect of this treatment on expression of other tumor antigens, in particular those spontaneously expressed by MPM, such as MUC1. We observed a different outcome compared to testis specific antigens (NYESO1, MAGE-A1 and A-3). Indeed expression of MUC1 by MPM cells which is recognized by specific CD8+ T cells is downregulated by treatment, leading to a decrease of the MUC1 specific T cell response (*Roulois et al, submitted*).

Most mesothelioma commonly occurs in the pleural cavity in patients but distant recurrence in the abdomen is frequent after extrapleural pneumonectomy. For that reason and also because most rodents spontaneous mesothelioma are found in the abdominal cavity, experimental models of the most aggressive malignant mesothelioma have been based on injection of asbestos fibers in the peritoneal cavity of mice or rats. The tumoral cell lines obtained in these conditions present the same histological characteristics and the same gene alteration profiles than the most aggressive tumors observed in patients. Thus, we developed in vivo experimental models of aggressive malignant mesothelioma. Using these models and AK7 mouse model, we determined the optimal scheme of epigenetic treatment and concluded that the most significant reduction in tumor mass was obtained after multiple i.p. injections of 5-azadeoxycytidine (5-azadC) and suberoylanilide hydroxamic acid (SAHA). We also analyzed how the immune system reacts to the transplantation of mesothelioma tumor cells after immunization with AK7 cells treated in vitro with different therapeutic agents in the presence of BCG vaccine as adjuvant. Large conglomerates of immune cells were observed in the omentum, together with activated lymphocytes directed against residual AK7 cells in the interlobular connective tissue of the pancreas (*Guillot et al., submitted*).

However, HDACi are usually toxics, but less specific and present short half-life in plasma. In collaboration with Dr. Philippe Bertrand (Poitiers), we obtained new benzofuranone HDACi. To characterize these molecules, we developed a BRET (Bioluminescence Resonance Energy Transfer)-based assay to measure histone acetylation in living cells. Using this assay and a viability test, we determined the pharmacology of the HDACi to induce histone acetylation and inhibition of

cell growth of MPM and pulmonary ADCA from our biocollection. The results showed that one HDACi present interesting properties such as activity at nanomolar concentration and increase duration of histone acetylation. We extended the study to other cancer cell lines and observed an activity on all cell lines tested (pulmonary ADCA, MPM, colorectal ADCA and melanoma) (*Blanquart et al., submitted*).

Main publications of the team in the field:

→ Grigoriu, Chahine, Zerimech, Grégoire, Balduyck, Copin, Devos, Lassalle, Scherpereel (2009) Serum mesothelin has a higher diagnostic utility than hyaluronic acid in malignant mesothelioma. Clin Biochem **42 (10-11)**, 1046-1050, 2009.

→ Bogdan-Dragos Grigoriu, Arnaud Scherpereel, Patrick Devos, Bachar Chahine, Marc Letourneux, Pierre Lebailly, Marc Grégoire, Henri Porte, Marie-Christine Copin, Philippe Lassalle (2007) Utility of osteopontin and serum mesothelin in malignant pleural mesothelioma diagnosis and prognosis assessment. Clin Cancer Res 13: 10. 2928-2935 May 2007

→ Frédéric Ebstein, Carole Sapede, Pierre-Joseph Royer, Marie Marcq, Catherine Ligeza-Poisson, Isabelle Barbieux, Laurent Cellerin, Gérard Dabouis, Marc Grégoire. Cytotoxic T cell responses against mesothelioma by apoptotic cell-pulsed dendritic cells. Am J Respir Crit Care Med 169: 12. 1322-1330, 2004

→ Delphine Massé, Frédéric Ebstein, Gwenola Bougras, Jean Harb, Khaled Meflah, Marc Grégoire (2004) Increased expression of inducible HSP70 in apoptotic cells is correlated with their efficacy for antitumor vaccine therapy. Int J Cancer 111: 4. 575-583, 2004

Collaborators :

- SCHERPEREEL Arnaud, Service de Pneumologie et d'Oncologie Thoracique – Inserm U774, Lille
- Ph. BERTRAND, CNRS de Synthesis et Reactivity of natural substitute, UMR 6514, Univ. Poitiers
- Pierre-François CARTRON, Team 9 – U892, apoptotic process and resistance

Measles virus vaccine and cancer virotherapy

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State of the Art

Cancer virotherapy has recently emerged as an hopeful alternative therapeutic strategy in the aim of better responding to the diversity of cancerous pathologies that are resistant to conventional treatments by surgery, chemotherapy or radiotherapy. This novel approach is based on the specificity of certain "oncolytic" viruses for tumor cells. Such property is exhibited naturally by some RNA viruses including a live-attenuated vaccine strain of measles virus (MV).

The live, attenuated vaccine directed against measles disease is one of the most effective and safe human vaccines known to exist to date. It has been administered to hundreds of millions of children since the end of the 1960s. Attenuated vaccinal strains of MV are derived from the Edmonston strain, adapted to cell culture *in vitro* (11*)*. Several studies have shown that tumor cells derived from myelomas, gliomas, ovarian carcinomas or breast cancers are more susceptible to infection by the vaccine strains of MV than are cells from healthy tissues. In addition, in the *Mayo Clinic* (Rochester, Minnesota, USA), several clinical trials are currently underway, particularly concerning glioblastoma, multiple myelomas and ovarian cancers (12).

Previous investigations by the team

Oncolytic MV targets CD46 membrane complement regulatory molecule that is overexpressed in numerous cancers. In a study that we recently published (Gauvrit et al, 2008 referenced below), we showed that mesothelioma cell lines over-express the CD46 surface molecule. These cell lines are efficiently infected by our Schwarz strain of MV, and killed by apoptosis after forming syncitia, in contrast to normal, non-transformed cells that express a basal level of CD46 and that remain only slightly sensitive, or even insensitive, to infection. After its entry into the cytoplasm, MV induces the fusion of infected cells with neighboring cells, provoking the formation of multinuclear cells, namely syncitia, and leads later to the triggering of apoptosis in infected cells (13).

We recently demonstrated that MV exhibited oncolytic properties *in vivo* in nude mice against human mesothelioma, melanoma, colorectal and lung adenocarcinoma. Interestingly, we found that apoptosis triggering after MV infection is associated with immunogenic molecules production. These results led us to hypothesize a potential involvement of anti-tumor immune responses that would potentiate the direct cytotoxicity of measles virus. Indeed, we found that MV infection of tumor cells is associated with HSP70 synthesis, calreticulin translocation to cell surface and HMGB1 release *in vitro*. (*N. Boisgerault et al. in preparation, and oral presentation in international meetings*). These molecules are expected to play an essential role in the activation of the adaptive immune response by acting directly on dendritic cells. We recently observed that MV-infected tumor cells are effectively able to induce spontaneous maturation of both myeloid and plasmacytoid DCs in this immunogenic environment, and subsequently to prime a moderate antigen-specific T CD8 response against mesothelin. Moreover, when both myeloid and plasmacytoid DCs were cocultured together with apoptotic MV-infected tumor cells, we observed a potential synergy that would allow to initiate a strong anti-tumor response.

Main publications of the team in the field:

→ Anne Gauvrit, Samantha Brandler, Carole Sapede-Peroz, Nicolas Boisgerault, Frédéric Tangy, Marc Gregoire. Measles virus induces oncolysis of mesothelioma cells and allows dendritic cells to cross-prime tumor-specific CD8 response. Cancer Res 68: 12. 4882-4892 Jun 2008.

→ Nicolas Boisgerault, Frédéric Tangy,Marc Gregoire (2010). New perspectives in cancer virotherapy: bringing the immune system into play. Immunotherapy 2: 2. 185-99 Mar 2010.

Collaborators:

- Dr. Frédéric TANGY (Laboratoire de Génomique Virale et Vaccination, Institut Pasteur):
- Dr. François VALLETTE (Team 9 - U892): Apoptotic process and resistance

Dendritic cells iHDAC

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State of the Art

Allogeneic hematopoietic stem cell transplantation (allo-SCT) proved to be an effective therapy for a variety of life-threatening malignancies. However, allo-SCT is limited by the immunologic recognition and destruction of host tissues, termed graft-versus-host disease (GVHD). GVHD continues to be the major source of morbidity and mortality following allo-SCT (Appelbaum, Nature 2001). While there is no doubt that activation of donor T lymphocytes is the central event in alloreactivity, the role of antigen presenting cells (APCs), especially dendritic cells (DCs) is now well demonstrated as a factor of major importance in the hierarchy of the induction of allogeneic immune reactions (14). DCs are widely accepted as the most potent APCs capable of inducing protective adaptive immune responses in addition to tolerance to self-antigens. In response to a variety of microbial and endogenous stimuli, resting DCs in peripheral tissues undergo a complex maturation process that involves the regulation of genes controlling distinct DC functions, such as antigen capture and presentation, migration, co-stimulation, and production of T cell polarizing cytokines. The distinct functional properties of DCs also rely on the existence of several populations in humans and mice that differ in phenotype, localization, and response to the environmental stimuli. Indeed, the mainstay of immunosuppression has been the calcineurin antagonist cyclosporine A for more than 20 years. Its immunosuppressive effects can be unpredictable, and the combination of immunosuppressive antibodies with calcineurin antagonists is associated with an increased risk of post-transplantation lymphoproliferative disorders and opportunistic infections. Thus, there is a pressing need for selective, more specific, and relatively non-toxic immunosuppressive approaches.

Investigations by the team

Histone deacetylase (HDAC) inhibitors are antitumor agents that also have anti-inflammatory properties. However, the mechanisms of their immunomodulatory functions remain unknown. We investigated the mechanisms of action of an HDAC inhibitor, valproic acid (VPA), on Dendritic Cells (DC) phenotype and functions. Human peripheral blood monocytes obtained by elutriation were cultured with GM-CSF, IL-4 and 0,5 mM VPA for 6 days. Differentiated cells were assayed for their morphology, phenotype, cytokine secretion profile and allostimulatory capacities. First, we tested the effects of VPA on membrane molecule expression of DCs. By multiple staining, we observed, on mature DC, decrease of maturation and costimulation molecules expression (CD40, CD83 and CD86) with VPA treatment. A decrease of class II CMH molecules (HLA-DR) was also observed. Because VPA changed the phenotype of mature DCs, we asked whether it would similarly modulate the cytokine secretion profile. Secretion of cytokines was analyzed from culture medium with different ELISA measurement. Concerning inflammatory cytokines, we observed a reduction of IL-10, TNFα and IL-6 secretion. As well, for IL-12 family cytokines, we detected a diminution of IL-23 and IL-12 secretion. These results suggested an implication on the allostimulatory capacity of DCs. In order to confirm this hypothesis, allogeneic naive CD4+ T cells were co-cultured with DC previously treated or not. Cells were harvested after 7 days and re-activated with phorbol-12-myristate-13-acetate (PMA), ionomycin and brefeldin A for 5h. We observed a decrease of IFNg Th1 cytokine secretion and a light increase of IL-4 when CD4 T cells were co-cultured with VPA-treated DCs. VPA seems to induce modulation of DC maturation in vitro. In view of these results, we finally investigated the effect of VPA in vivo. We treated C57BL/6 mice with VPA for 1 week and then retrieved spleen and mesenteric lymph nodes. We demonstrated that VPA decreased the amount of mature DCs in vivo in spleen and mesenteric lymph nodes. Considering the central role of DCs in controlling immunity, our results suggest that valproic acid could have an interesting implication for management of autoimmune disease and Graft Versus Host Disease (GVHD).

Main publications of the team in the field:

→ Mohty M, et al. pDCs and cancer: a new role for an enigmatic cell. Trends in Immunol. 25: 397-398, 2004.
→ Mohty M, et al. Inflammatory cytokines and acute graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation. Blood. 2005 15; 106:4407-11.
→ Mohty M, et al. Inflammatory cytokines and dendritic cells in acute graft-versus-host disease after allogeneic stem cell transplantation. Cytok & Growth Fact Rev. 19:53-63, 2008.
→ Mohty M, et al. Association between BMI-1 expression, acute graft-versus-host disease, and outcome following allogeneic stem cell transplantation from HLA-identical siblings in chronic myeloid leukemia. Blood 2008; 112:2163-6.

Project feasibility: This research program relies on human material (peripheral blood and tissue samples collected retrospectively and prospectively) provided by the allo-SCT clinical program (>70 allo-SCT/year) directed by Pr M. Mohty at the CHU de Nantes.

Collaborators :

- INSERM U892, Nantes (D. Valmori)
- INSERM U645, Besançon (P. Saas and B. Gaugler)
- Pathology Lab, Nantes, (JF Mosnier)

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14. Vogelsang, et al. *Semin Oncol.*, **32**(3): 336-350, 2005.