

**WORKSHOP**  
**« Haematopoiesis and its Disorders.**  
**Modelling, Experimental and Clinical Approaches »**  
**March 20-21, 2008, in Paris**

**Comité d'Organisation / Organizing Committee**

Mostafa Adimy (INRIA Futurs Bordeaux), Jean Clairambault (INRIA Rocquencourt), Fabien Crauste (CNRS Université Lyon 1), Jean-Pierre Marie (Hôtel Dieu Paris)

**Jeudi 20 mars 2008 / Thursday, March 20th 2008**

Maison de la Chimie (SFH Congress), 28 rue Saint-Dominique, VIIth arrondissement

9h	Mackey Michael C. (McGill University, Montreal)
10h	<i>A 30-year retrospective on continuous mathematical models for haematopoiesis, with confrontation to data</i>
10h30	Pujo-Menjouet Laurent (Institut Camille Jordan, University Lyon 1)
10h30	<i>Multi-Agent System Approach for Hematopoiesis Modelling</i>
10h30	Coffee
11h	break
11h	Kitano Hiroaki (Systems Biology Institute, Tokyo)
12h	<i>A robustness-based approach to systems-oriented drug design</i>
12h	Lunch
14h	Plenary Conference of the SFH Congress
14h45	
14h45	Coffee
15h	break
15h	Pacheco Jorge (ATP Group, Lisbon)
16h	<i>Estimating the number of haematopoietic stem cells and maturation compartments in mammalian species</i>
16h	Génieys Stéphane (Institut Camille Jordan, University Lyon 1)
16h30	<i>Evolutionary branching processes and cellular differentiation</i>
16h30	Clairambault Jean (INRIA Rocquencourt)
17h	<i>Optimising cancer chronotherapeutics under the constraint of preserving healthy tissues from unwanted toxicity</i>
17h	Discussions
18h	

**Vendredi 21 mars 2008 / Friday, March 21st 2008**

Hôtel-Dieu (amphithéâtre de Lapersonne), 1 place du Parvis Notre-Dame, IV<sup>th</sup> arrondissement

9h 10h	Mahon François-Xavier (INSERM U879, Université Bordeaux 2) <i>Mécanisme moléculaire de la résistance aux inhibiteurs de Tyrosine kinase</i>
10h 10h30	Coffee break
10h30 11h30	Rousselot Philippe (Hôpital Mignot, Versailles) <i>Resistances to treatments in CML and clinical strategies used to circumvent them.</i>
11h30 12h	Gandrillon Olivier (CGMC, University Lyon 1) <i>Adding self-renewal in committed erythroid progenitors improves the biological relevance of a mathematical model of erythropoiesis</i>
12h 14h	Lunch
14h 15h	Legrand Ollivier (Hôtel-Dieu, Paris) <i>Intérêt des protéines ABC dans la chimiorésistance des leucémies aiguës myéloblastiques</i>
15h 15h30	Coffee break
15h30 16h30	Kim Peter (Supelec) <i>Dynamics of Leukemia, Gleevec, and the Immune Response</i>
16h30 17h	Bernard Samuel (Institut Camille Jordan, University Lyon 1) <i>Cyclic neutropenia and chronic myeloid leukemia: closely linked dynamical diseases</i>
17h 17h30	Peet Matthew (INRIA Roquencourt) <i>Model Analysis of Chronic Myelogenous Leukemia with Treatment Options using Structural Methods and Computation: Sensitivity to Nonlinearity and Delay</i>
17h30 18h	Discussions

## Résumés / Abstracts

Jeudi 20 mars / Thursday, March 20th

Michael C. MACKEY (McGill University, Montreal)

*A 30-year retrospective on continuous mathematical models for haematopoiesis, with confrontation to data*

In this talk I will review the evolution of mathematical models for the regulation of hematopoiesis, and how they have shaped our understanding of the origin of periodic hematological diseases and been shaped by the accumulation of clinical and laboratory data from these diseases. The emphasis will be on the dynamics of model construction and the crucial interactions between experimentalists, clinicians, and mathematicians.

Laurent PUJO-MENJOUET (Institut Camille Jordan, University Lyon 1)

*Multi-Agent System Approach for Hematopoiesis Modelling*

In this work, we introduce the last version of a new software created to study hematopoiesis at the cell population level with the individually based approach. It can be used as an interface between theoretical works on population dynamics and experimental observations. We show that this software can be useful to study some features of normal hematopoiesis as well as some blood diseases such as myelogenous leukemia. It is also possible to simulate cell communication and the formation of the cell colonies in the bone marrow.

Hiroaki KITANO (Systems Biology Institute, Tokyo)

*A robustness-based approach to systems-oriented drug design*

Many potential drugs that specifically target a particular protein considered to underlie a given disease have been found to be less effective than hoped, or to cause significant side effects. The intrinsic robustness of living systems against various perturbations is a key factor that prevents such compounds from being successful. By studying complex network systems and reformulating control and communication theories that are well established in engineering, a theoretical foundation for a systems-oriented approach to more effectively control the robustness of living systems, particularly at the cellular level, could be developed. Here, I use examples that are based on existing drugs to illustrate the concept of robustness, and then discuss how a greater consideration of the importance of robustness could influence the design of new drugs that will be intended to control complex systems.

Jorge PACHECO (ATP Group, Lisbon)

*Estimating the number of haematopoietic stem cells and maturation compartments in mammalian species*

Hematopoiesis appeared once during evolution. Among mammals, the diversity in size and function required suitable adaptations. In this work, I utilize allometric principles to determine the size of the active stem cell pool across mammals[1]. For Humans, allometry combined with available data is also used to predict how the active stem cell pool changes during ontogenic growth[2]. In face of the results above, I will bridge the gap between circulating blood cells and their root, stem-cells, by developing a hierarchical compartmental model of hematopoiesis, based on the principle of stationary cell flow conservation[3]. Finally, I show that it is possible to express both the number and rate with which hematopoietic stem cells replicate as well as total marrow output across all mammals as a function of adult mass. This unified view, compatible

with existing data, suggests that there was no need for major adaptations in the architecture of hematopoiesis across mammals[4].

[1] David Dingli, J. M. Pacheco, Allometric scaling of the hematopoietic stem cell pool across mammals, PLoS ONE 1 (2006) e2.

[2] David Dingli, J. M. Pacheco, Ontogenic growth of the haematopoietic stem cell pool in humans, Proceedings Royal Society B274 (2007) 2479.

[3] David Dingli, Arne Traulsen, J. M. Pacheco, Compartmental Architecture and Dynamics of Hematopoiesis, PLoS ONE 2(4) (2007) e345.

[4] David Dingli, Arne Traulsen, J. M. Pacheco, Dynamics of hematopoiesis across mammals (to be published).

Stéphane GENIEYS (Institut Camille Jordan, University Lyon 1)

*Branchement évolutif et différenciation cellulaire*

*Evolutionary branching processes and cellular differentiation*

L'idée d'évolution darwinienne dans les populations cellulaires a été introduite, par exemple, par Jean-Jacques Kupiec dans [1,2].

De récents développements mathématiques permettent de comprendre comment ce type d'évolution peut pousser une population initialement monomorphique à se subdiviser en deux sous-populations de morphologies bien distinctes [3].

La mise en évidence de ces effets de population nous semble susceptible d'éclairer d'un jour nouveau la question du contrôle de la différenciation cellulaire, question bien évidemment centrale de l'hématopoïèse normale et leucémique.

[1] J.J. Kupiec, "A Darwinian theory for the origin of cellular differentiation" Molecular and General Genetics, 1997 ; vol. 255 ; pp. 201-208.

[2] J.J. Kupiec, P. Sonigo, "Ni Dieu ni gène. Pour une autre théorie de l'hérédité", Paris, Seuil, 2000.

[3] S. Génieys, V. Volpert, P. Auger, "Adaptive dynamics: modelling Darwin's divergence principle", Comptes Rendus Academie Sciences Biologies, 2006 ; vol. 329 ; pp. 876-876.

Jean CLAIRAMBAULT (INRIA Roquencourt)

*Optimising cancer chronotherapeutics under the constraint of preserving healthy tissues from unwanted toxicity*

The chronotherapy concept takes advantage of the circadian rhythm of cells physiology in maximising a treatment efficacy under the constraint of limiting its toxicity side effects on healthy organs. The object of the first part of the talk is to investigate mathematically and numerically optimal strategies in cancer chronotherapy.

To this end a simple mathematical model will be presented, describing the time evolution of efficacy and toxicity of an oxaliplatin anti-tumour treatment. The results of an optimal control technique used to obtain the best drug infusion law will be discussed.

In a second part will be presented recent developments involving a physiologically structured model of the cell division cycle. It is designed to describe the complex mechanisms of its control by cytotoxic/cytostatic drugs acting in synergy on proliferating cell populations, again in the perspective of drug delivery optimisation.

Vendredi 21 mars / Friday, March 21st

François-Xavier MAHON (INSERM U879, University Bordeaux 2)

*Mécanisme moléculaire de la résistance aux inhibiteurs de Tyrosine kinase*

La leucémie myéloïde chronique (LMC) est une maladie modèle pour l'oncogenèse ou la leucémogénèse. Il s'agit d'une hémopathie maligne ou syndrome myéloprolifératif qui a la particularité d'être caractérisé par un marqueur appelé chromosome Philadelphie. Ce dernier a un équivalent moléculaire, le gène BCR-ABL qui code pour une oncoprotéine à forte activité tyrosine kinase. Depuis plusieurs années, il existe une thérapeutique ciblée spécifique de cette leucémie, appelée imatinib, inhibiteur de tyrosine kinase BCR-ABL. Le recul des premiers essais cliniques est maintenant supérieur à la durée de phase chronique de la maladie avant l'apparition de ce nouveau médicament. Ceci permet de démontrer l'efficacité remarquable de l'imatinib dans la LMC. Cependant, un patient sur dix est considéré résistant à ce traitement. Les mécanismes moléculaires de résistance à l'imatinib sont multiples et peuvent être résumés de la façon suivante :

1) La cible moléculaire se modifie quantitativement (mutation du domaine tyrosine kinase de BCR-ABL) ou qualitativement (amplification génique). En effet, une trentaine de mutations localisées dans le domaine tyrosine kinase de BCR-ABL ont été décrites et rapportées chez des patients résistants. Leur pertinence fonctionnelle est variable mais elles illustrent l'instabilité génique de cette maladie. Il existe aussi parfois une amplification du gène BCR-ABL lui-même qui conduit à une hyperproduction de la protéine cible comme cela a été démontré dans des lignées cellulaires.

2) Le médicament n'atteint pas suffisamment la cible (concentration intra cellulaire insuffisante, pompe extracellulaire, métabolisme, pharmacocinétique...).

3) La cellule se transforme et acquiert d'autres anomalies. D'autres tyrosines kinases peuvent coopérer avec BCR-ABL ou prendre son relais telles que les src kinases.

Si l'imatinib, inhibiteur de première génération demeure le traitement de référence en première intention de la LMC de novo, les inhibiteurs de deuxième génération tels que le dasatinib ou le nilotinib permettent de rattraper les résistances dans un peu plus de 2/3 des cas.

Nous discuterons les différents mécanismes de résistances à l'imatinib et aux autres inhibiteurs de tyrosine kinase.

Philippe ROUSSELOT (Hôpital Mignot, Versailles)

*Resistances to treatments in CML and clinical strategies used to circumvent them.*

Clinical resistance to treatment in chronic myelogenous leukaemia (CML) could be separated in two categories: primary resistance, ie failure to achieve an adequate response and secondary resistance, ie losing a response. The occurrence of primary resistance depends on the response criteria. On the other hand, secondary resistance is less dependent on the response criteria.

Response criteria in CML have been established in parallel with the progress of therapy. Under treatment with hydroxyurea, only complete haematological responses (CHR) could be achieved with a moderate impact on survival. With interferon therapy, cytogenetic responses have been observed for the first time outside the context of bone marrow transplantation. Achievement of a cytogenetic response and more precisely of a complete cytogenetic response (CCR, no Ph+ metaphases) had a major impact on survival. In the area of imatinib and other tyrosine kinase inhibitors (TKI), the value of CCR remains strong. An additional level of response has been defined for patients in CCR, the molecular response evaluated by the quantification of the BCR-ABL signal detected by PCR. A BCR-ABL/ABL ratio of 0.01% defined the major molecular response and correlates with the absence of progression.

Primary resistance to imatinib is the failure to achieve response criteria defined as time dependent end points: CHR after 6 months of therapy, major cytogenetic response after 12 months of therapy, CCR after 18 month of therapy. Slowly responding patients are considered as resistant, even if they would have achieved an adequate level of disease reduction later. A study presented in the ASH meeting in 2007 indicates that the prognostic of late responders is comparable to the prognosis of early responders but that the probability to achieve a response is decreasing over time. No mechanism of resistance could be identified in the majority of primary resistant patients.

Secondary resistance indicate the progression of the disease despite the continuation of TKI therapy. The majority of patients develop identified mechanism of resistance with a high incidence of BCR-ABL TK domain mutations.

Strategies to circumvent resistance are different in primary or secondary resistant patients. In primary resistant patients the main concern is to provide an adequate exposure to the drug. This can be achieved by increasing the dosage of the TKI, empirically or after monitoring the plasmatic dosage and by increasing the drug access to the target kinase by optimizing cellular intake or decreasing the cellular efflux via MDR. This latter strategy has been validated in vitro but is not currently used in vivo. A second approach is to combine the TKI with another non targeted active agent such as interferon, Ara-C or homoharringtonine. Ultimately, the switch to a second generation TKI is more and more frequently recommended. In secondary resistant patients, the current strategy is to switch to a second generation TKI. These inhibitors (dasatinib, nilotinib) have a greater potency than imatinib and are less susceptible to the mutation of BCR-ABL compared to imatinib. However, in the case of the T315I mutation, none of these drugs is effective. Each of these inhibitors display also is his own spectrum of resistant mutations. Inhibitors specifically designed to the mutated T315I BCR-ABL are in development and combination strategies between inhibitors are already investigated.

An alternative approach is to reduce the probability of the resistant event without waiting to the occurrence of resistance. Optimization of TKI therapy based on a prospective monitoring of the plasmatic level of the drugs will be tested in a prospective clinical trial, both for imatinib and dasatinib front line.

Olivier GANDRILLON (CGMC, University Lyon 1)

*Adding self-renewal in committed erythroid progenitors improves the biological relevance of a mathematical model of erythropoiesis*

We propose a new mathematical model of erythropoiesis that takes a positive feedback of erythrocytes on progenitor apoptosis into account, and incorporates a negative feedback of erythrocytes on progenitor self-renewal. The resulting model is a system of age-structured equations that reduces to a system of delay differential equations where the delays account for progenitor compartment duration and cell cycle length. We compare this model with experimental data on an induced-anemia in mice that exhibits damped oscillations of the hematocrit before it returns to equilibrium. When we assume no self-renewal of progenitors, we obtain an inaccurate fitting of the model with experimental data. Adding self-renewal in the progenitor compartment gives better approximations, with the main features of experimental data correctly fitted. Our results indicate the importance of progenitor self-renewal in the modelling of erythropoiesis. Moreover, the model makes testable predictions on the lifespan of erythrocytes confronted to a severe anemia, as well as for the progenitors behavior.

Ollivier LEGRAND (Service d'hématologie, Hôtel-Dieu, Paris)

*Intérêt des protéines ABC dans la chimiorésistance des leucémies aiguës myéloblastiques*

La chimiorésistance dans les LAM est un réel problème, puisque 50% à 80 % de ces patients rechutent suivant les tranches d'âge. Un des premiers mécanismes de résistance aux chimiothérapies décrits dans cette pathologie a été celui médié par la P-glycoprotéine (ABCB1/MDR1) une ABC (ATP Binding Cassette) protéine. Depuis lors d'autres protéines ABC ont été impliquées (MRP1/ABCC1, BCRP/ABCG2, MRP3/ABCC3...). Différents travaux ont démontré leur implication dans la chimiorésistance, expériences de transfection de leur cDNA dans des lignées cellulaires sensibles les rendant résistantes à un large spectre de chimiothérapies, corrélations entre expression, fonctionnalité de ces protéines et pronostic de cette hémopathie. A terme des essais cliniques randomisant chimiothérapie en présence ou non de modulateur de ces protéines ont donné des résultats contradictoires. Plusieurs explications ont été avancées. En particulier la sélection des malades qui n'exprimait pas toutes les protéines ciblées par les modulateurs, la trop grande toxicité de certains modulateurs, l'interaction des modulateurs avec la pharmacocinétique des chimiothérapies, l'implication d'autres protéines ABC, l'intervention d'autres mécanismes de résistance aux chimiothérapies. Ainsi de nouvelles voies de recherche se sont ouvertes pour améliorer l'efficacité de la modulation des protéines ABC. Ces différents thèmes seront développés durant la présentation.

Peter KIM (Supelec)

*Dynamics of Leukemia, Gleevec, and the Immune Response*

Various models exist for the interaction between the molecular-targeted drug, Gleevec, and chronic myelogenous leukemia (CML). However, the role of the host immune response during Gleevec treatment remains unclear. Based on experimental data from the Lee Lab at Stanford Medical School, we hypothesize that Gleevec gives rise to a brief anti-leukemia immune response as patients enter remission. From this hypothesis, we propose that a combination of cancer vaccination and Gleevec can boost the existing host immune response and lead to a sustained remission or a potential cure.

To mathematically analyze this hypothesis, we take a particular model by Michor et al. and extend it by incorporating an anti-leukemia immune response. We show that properly timed vaccines can optimally sustain the host immune response to potentially eradicate residual leukemia cells for a durable cure.

Our model of immune dynamics also includes a time delay that corresponds to the duration of T cell division. We analyze the stability of the linearized system with respect to the delay value, and conclude that the system is highly stable. On the other hand, without intervention,

the rate of convergence to the stable fixed point is extremely slow.

As a further extension, we consider an alternative Gleevec model by Roeder et al. that is related, but structurally different from the model of Michor et al. We describe the fundamental differences between the two models and explain how we intend to incorporate the immune response into the agent-based formulation of Roeder et al.

Samuel BERNARD (Institut Camille Jordan, University Lyon 1)

*Cyclic neutropenia and chronic myeloid leukemia: closely linked dynamical diseases*

Cyclic neutropenia (CN) and chronic myeloid leukemia (CML) are two hematological diseases in which white blood cell production is deregulated. In CN, the mutation of the neutrophil elastase causes white blood cell precursors to die by apoptosis, while in CML, the fusion gene *bcr-abl* on the abnormal Philadelphia chromosome causes an increase in white blood cell production. I present evidence for a close link between cyclic neutropenia and chronic myeloid leukemia from a dynamical disease viewpoint. Despite having different genetic origins, the two diseases share strikingly similar features and epidemiological studies have pointed out increased risk of development of leukemia in patients with different forms of congenital neutropenia. A simple blood cell production model is used to show that: (1) CN and CML may have high rates of apoptosis of white blood cell precursors, (2) for CN, this translates into an increased probability of oncogenic mutation in one of the hematopoietic stem cells (HSCs), and (3) leukemic cell proliferation is still under tight physiological control. I conclude that treatment design for both CN and CML should take into account the impact on the dynamics of the HSCs or early precursors. For CN, treatments that decrease HSC proliferation might attenuate the risks of developing leukemia, while in CML treatments that target the mostly quiescent HSCs might help slowing down the development of resistance to anti-cancer drugs. Dynamical models of hematopoiesis may help predicting counter-intuitive effect of CN and CML therapy.

Matthew PEET (INRIA Roquencourt)

*Model Analysis of Chronic Myelogenous Leukemia with Treatment Options using Structural Methods and Computation: Sensitivity to Nonlinearity and Delay*

This talk focuses on the analysis of delay-differential models of Leukemia and T-cell dynamics during Gleevec treatment and during stem cell transplantation for chronic myelogenous leukemia. The analysis shows that both models admit three fixed points. The second fixed point corresponds to an equilibrium solution in which the leukemia population is kept below the cytogenetic remission level. In this presentation, we consider the robustness of the models to nonlinearity and delay. In the first part of the analysis, we consider a delayed linearization of the model about the second equilibrium. By using the geometric structure in the frequency domain representation, we give an estimate of the range of delays for which the models remain stable. In the second part of the analysis, we use recently introduced computational methods based on Lyapunov analysis to verify the frequency domain results. We then use a related computational approach to give estimates of the domain of attraction of the nonlinear model. Our results indicate that the Gleevec treatment model is significantly more robust than the stem cell transplantation model.