Faculté de Pharmacie
Aix*Marseille Université

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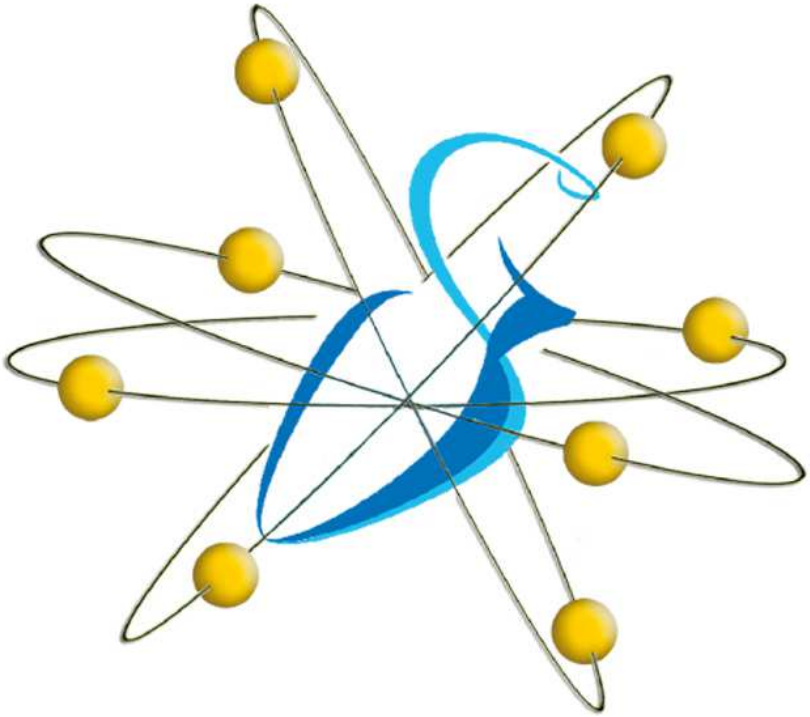

13ème Journée de la Recherche de la Faculté de Pharmacie

Instituts thématiques Inserm
Institut national de la santé et de la recherche médicale

cnrs dépasser les frontières

Jeudi 8 Octobre 2015

Aix-Marseille Université - Faculté de Pharmacie - 27 bd Jean Moulin – CS 30064 - 13385 Marseille cedex 05 - France
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JOURNÉE DE LA RECHERCHE
8 octobre 2015

Cher(e)s Ami(es),

Nous vous adressons ci-après le programme prévisionnel de la Journée de la Recherche qui se déroulera le **Jeudi 8 Octobre 2015** à la Faculté de Pharmacie.

Nous vous rappelons que cette journée a pour objectifs :

- de sensibiliser les étudiants à la recherche,
- de renforcer la communication et les synergies scientifiques autour des axes forts de la Faculté de Pharmacie.

Nous avons choisi de faire figurer au programme de cette journée :

- une présentation des Masters affiliés à la Faculté,
- une présentation des thématiques de recherche et stages proposés par la Faculté
- une session poster/discussion

Nous vous remercions pour votre participation active à la préparation de cette journée et vous incitons également à bien œuvrer à la diffusion des informations relatives à cette manifestation. Nous vous attendons nombreux à cette journée importante pour notre Faculté.

Bien amicalement,

Jean-**P**aul **BORG**
Vice-Doyen Chargé de la Recherche

Françoise **DIGNAT-GEORGE**
Doyen

Programme

14h00 – 14h20 (Amphi 3) – Jean-Paul Borg

- Présentation générale de la Recherche à la Faculté *(axes/thématiques, bilan de la production scientifique récente)*
- Présentation des Masters affiliés à la Faculté

14h20 – 15h40 (Amphi 3)

Présentation des thématiques de recherche et équipes de la Faculté (10 minutes/thématique)

- Chimie *(UMR CNRS 7273 – N. Prima)*
- Toxicologie et Santé Environnementale *(IMBE – PH. Villard)*
- Transporteurs membranaires, Chimiorésistance et Drug-design *(UMR MD1 - J.M. Bolla)*
- Maladies infectieuses et tropicales *(URMITE UM 63 CNRS 7278 IRD 198 INSERM U1905 - P. Colson)*
- Infections parasitaires *(UMR-MD3 - N. Azas)*
- Endothélium, pathologies vasculaires et cibles thérapeutiques *(UMR INSERM 1076 – M. Blot-Chabaud et S. Burtey)*
- Centre de Recherche en Oncologie biologique et Oncopharmacologie *(CRO2 – H. Kovacic et M. Carré)*
- Centre de Recherche en Cancérologie de Marseille *(CRCM – JP. Borg)*

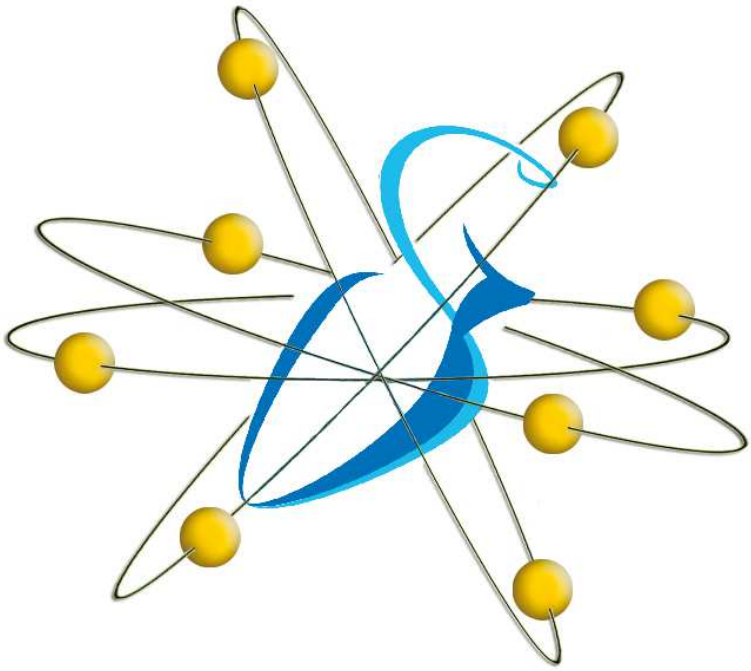
15h40 – 16h25 **(Amphi 3)**

3 présentations de projets de thèse par les étudiants

- Tacy Santana Machado *(UMR_S 1076)* : Les transporteurs de toxines urémiques au cours de l'insuffisance rénale chronique
- Clémence Tabélé *(UMR CNRS 7273)* : New antileishmanial agents : Synthesis, evaluation and structure-activity relationship study hydroxyamidine derivatives
- Marie Petit *(CRO2 UMR_S 911)* : Modeling the effects of EB1 and vincristine action on microtubule dynamic instability in glioblastoma cells: An explanation for EB1 sensitization to microtubule targeting drugs

16h25 - 18h00 (Grand Hall)

Discussion devant les posters autour d'un gouter



UMR INSERM 1076

Aix-Marseille Université

Vascular Research Center of Marseille

CD146 deficiency leads to accelerated atherosclerosis in mice

Muriel G. Blin[1], Samantha Fernandez[2], Benjamin Guillet[1,2,3], Karim Fallague[1], Stéphane Robert[1], Richard Bachelier[1], Christophe Heymes[4], Nathalie Bardin[1,5], Marcel Blot-Chabaud[1], Françoise Dignat-George[1,5] and Aurélie S. Leroyer[1].

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Abstract

Rationale. The progression of atherosclerosis is based on the continued recruitment of macrophages in the vessel wall. The previously described role of CD146 in leukocyte infiltration *in vitro* suggests a role for this endothelial junction molecule in the early stages of atherogenesis. However, its involvement in atherosclerotic plaques formation has never been investigated.

Objective. We evaluated the role of CD146 in atherogenesis.

Methods and Results. CD146 -/-/ApoE -/- and ApoE -/- mice were fed a Western diet for 24 weeks and were monitored for aortic wall thickness using high frequency ultrasound. The arterial wall thickness was significantly higher in CD146 deficient mice. We evidenced a significant increase of atheroma in both total aortic lesion area and aortic sinus of CD146 deficient mice. We observed a maladaptive immune response in double KO mice since the circulating neutrophils were significantly increased whereas circulating T lymphocytes were decreased. We detected a significant increase in neutrophils recruitment to the atherosclerotic plaques of the double KO mice. In addition, CD146 deficient plaques contained more macrophages, less smooth muscle cells, demonstrating that these atherosclerotic lesions were more inflammatory. Consistent with the higher recruitment of inflammatory cells to the atheroma, we demonstrated that RANTES was upregulated in CD146 deficient atherosclerotic arteries, and that its plasmatic level was significantly increased.

Conclusions. Our data indicate that CD146 deficiency is associated with the upregulation of RANTES and increased inflammation of atheroma, which could influence the atherosclerotic plaque fate. Thus, CD146 should be considered as a potential target for atherosclerosis treatment.

Generation of soluble CD146 by shedding of membrane CD146 involves a matrix metalloprotease-dependent process in endothelial progenitor cells.

Marie Nollet, Alexandrine Bertaud-Foucault, Jimmy Stalin, Alexandre Muller, Richard Bachelier, Françoise Dignat-George, Marcel Blot-Chabaud

Aix-Marseille University ; Vascular Research Center of Marseille, Inserm, UMR-S 1076, 13385 Marseille, France

CD146 is a junctional adhesion molecule which exists as three forms, 2 membranes forms (short (shCD146) and long (lg CD146)) and a soluble form (CD146s). It was first discovered in human melanoma cells where its expression correlates with a poor prognostic. Since this initial discovery, it has been described in many cancer cells (kidney, pancreas, breast, ovary, …).

Our team has demonstrated that it is also expressed throughout the vascular and tumor endothelium. It is involved in various functions such as vascular growth, angiogenesis and control of the permeability and cohesion of the endothelial monolayer. We have first shown that CD146s is generated by ectodomain shedding of the membrane CD146 by a matrix metalloprotease-dependent (MMP) process in endothelial progenitor cells.

We have therefore studied which MMP is involved in this shedding. Experiments with MMP specific inhibitors allowed to target some MMPs. Through experiments of transfections with siRNA, we could show the implication of ADAM 10 and Tace (ADAM 17) in the shedding of both CD146 isoforms. Techniques of co-immunoprecipitation, immunofluorescence and silencing/overexpression of CD146 isoforms in cells with do not express CD146 are now used to determine which MMP specifically cleaves each isoform.

Involvement of the K- Cadherin in the interactions of platelets with colorectal cancer cells

Léa Plantureux[1], Lydie Crescence[1], Soraya Mezouar[1], Françoise Dignat-George[1,2], Laurence Panicot-Dubois[1], Christophe Dubois[1]

[1] *Aix Marseille Université, INSERM UMR-S1076, Marseille, France.*
[2] *Laboratoire d'Hématologie, Centre Hospitalo-Universaire Conception, Marseille, France*

Purpose: The relationship between thrombosis and cancer is well established. Platelets are able to interact with cancer cells and participate to the development of the tumor and the formation of metastasis. To date, Tumor Cell Induced Platelets Aggregation (TCIPA) constitutes the main cellular consequence described following the interactions of platelets and cancer cells. Here, we investigated the capacity of colorectal cancer cells to interact independently of TCIPA with platelets via the interactions of K-cadherin.

Material and Methods: To investigate the capacity of cancer cells to interact independently of TCIPA with platelets, we determined the expression of cadherin at the surface of platelets and colorectal cell lines. Aggregation assays were performed on isolated platelets and on plasma rich platelets. The interactions between platelets and cancer cells and the involvement of K-cadherin were studied in static and in dynamic conditions. Last, a murine orthotopic syngenic model of colorectal cancer was set-up to study the K-cadherin dependent interactions of platelets and cancer cells.

Results: We show that the colorectal cancer cell lines HT29 (human) and CT26 (mouse) expressed the K-cadherin and did not induced TCIPA. The interaction between platelets and the cancer cells is significantly diminished using a blocking antibody against K-cadherin. Whereas the k-cadherin dependent binding of platelets to cancer cells did not affect the proliferation of the tumor, it induces the binding of cancer cells to the endothelium, mainly via the transfer of the beta3 subunit from platelets to cancer cells. *In vivo*, platelets are present in the microenvironment of the primary tumor in area in which the k-cadherin is overexpressed.

Conclusion: Our results indicate that platelets could interact with cancer cells, independently of the TCIPA, via the expression of k-cadherin. This interaction is involved in the adhesion of cancer cells to the vessel wall.

Les transporteurs de toxines urémiques au cours de l'insuffisance rénale chronique

Tacy Santana Machado, Claire Cerini, Nathalie Mc Kay, Stéphane Burtey

UMR_S 1076, Endothélium, Pathologies Vasculaires et Cibles Thérapeutiques

Dysfonction Endothéliale et Insuffisance Rénale chronique

Introduction L'insuffisance rénale chronique (IRC) est liée à la diminution irréversible du nombre de néphrons, conduisant à un état où les reins n'assurent plus leur fonction d'épuration. L'IRC entraine une intoxication de l'organisme par accumulation de nombreux solutés appelés toxines urémiques. Les maladies cardio-vasculaires sont la cause majeure de morbi-mortalité chez les patients IRC. Deux toxines du groupe des indoles, l'indoxyl sulfate et l'acide indole acétique, induisent une dysfonction endothéliale. Notre objectif est de comprendre comment les indoles, hydrophiles, rentrent dans la cellule endothéliale et s'ils modulent l'expression des transporteurs membranaires.

Résultats Nous avons réalisé une cartographie par PCR *array* des transporteurs présents dans les cellules endothéliales. 50 transporteurs au moins sont exprimés dans la CE. L'utilisation d'inhibiteurs ne nous a pas encore permis d'identifier un transporteur d'indoles. Il existe probablement plusieurs vecteurs de l'entrée des toxines urémiques. Nous avons pu grâce à des cultures cellulaires réalisées en milieu sans sodium montrer qu'au moins un des transporteurs est un transporteur sodium dépendant.

Parmi les 29 transporteurs dont l'expression est modulée par l'indoxyl sulfate, nous avons choisi la P-glycoprotéine (P-gp, *ABCB1*), un transporteur de nombreuses molécules toxiques. Nous avons trouvé une augmentation de sa transcription dans des cellules hépatiques (HepG2). Nous avons mesuré l'activité d'efflux de P-gp en utilisant la rhodamine 123. L'augmentation de l'activité de P-gp induite par les indoles a été associée à une réduction significative de l'accumulation intracellulaire de rhodamine 123, qui a été restaurée à des niveaux de contrôle par un inhibiteur de P-gp, le vérapamil.

Perspectives Nous prévoyons de déterminer si cette augmentation de l'activité de P-gp peut entrainer un changement de la biodisponibilité de certains médicaments. Nous envisageons également d'utiliser des ARN interférents afin d'identifier les transporteurs des toxines urémiques dans les cellules endothéliales. L'identification de la voie d'entrée permettra d'envisager une nouvelle approche thérapeutique pour limiter l'endothéliotoxicité des indoles au cours de l'IRC.

Adaptive self-assembling dendrimers for theranostics : a proof of concept on angiogenesis

Philippe GARRIGUE[a*], Anaïs MOYON[a], Xiaoxuan Liu[b], Cheng Liu[b], Palma Rocchi[c], Ling PENG[b], David TAIEB[d], Benjamin GUILLET[a]

a VRCM Inserm UMR_S1076, CERIMED, Aix-Marseille Université, Marseille – FRANCE.

b Centre Interdisciplinaire de Nanoscience de Marseille (CINaM), UMR 7325, Luminy, Aix-Marseille Université, Marseille – FRANCE.

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d CERIMED, Inserm UMR_S1068, Aix-Marseille Université, Marseille – FRANCE.

Nanoparticle-based drug delivery aims at specifically delivering therapeutic and/or diagnostic agents to the right site at the right time, in order to improve the outcome while reducing adverse effects. Many validated or experimental therapeutic drugs simulate or inhibit angiogenesis in cardiovascular or oncology applications: as companion tools, agents enabling non-invasive angiogenesis evaluation are an actual necessity. In this work, we introduced a proof of concept of adaptive self-assembling amphiphilic dendrimersomes for angiogenesis imaging on a rodent hind limb ischemia model.

A bifunctional $\alpha_v\beta_3$-integrin-targeting dendrimersome was designed, synthesized and radiolabeled with indium-111 chloride. The dendrimersome resulted from the self-assembly of 2 different dendrimer conjugates: one for imaging ([^{111}In]In-NOTA conjugate, 92 MBq/100µg) and one for specific targeting (C18-PEG-RGD conjugate) or aspecific targeting (C18-PEG conjugate), mixed under a 3:1 ratio, with sonication. Previous studies ensured dendrimersome stability for more than 4 days in serum. 7 days after hind limb ischemia induction on Balb/c mice, preliminary biodistribution studies were carried out by µSPECT/CT imaging and a blood kinetic was performed: both early dynamic planar acquisitions (T+0 to T+20' post-IV injection of 5-10 MBq, 7.5s frames) and late multi-pinhole SPECT/CT (T+120', D+1 and D+2 or D+3 post-IV) were performed using a Bioscan NanoSPECT/CTplus camera. Autoradiography was also carried out on 3 other mice right after the imaging on the day of the injection. Animal experiments were carried out in accordance to Helsinki Declaration and underwent the local preclinical ethics committee agreement.

We reached a radiolabeling yield >95%. The dendrimersomes were mainly eliminated through the urinary system at an early stage, with a biological half-life of 24 minutes. Late T+120' and D+1 SPECT/CT imaging showed significant ($P<0.05$) higher uptake on the ischemic site between the untargeted ^{111}In-dendrimersomes (i/c 1.40±1.0% at the day of the injection, 2.59±0.07% one day after) and the ^{111}In-RGD-dendrimersomes (i/c 6.29±0.6 % at the day of the injection, 5.25 ±0.6% one day after) (n=3/group). Autoradiography analysis of gastrocnemius sections showed a significant uptake of the ^{111}In-RGD-dendrimersomes in the ischemic hind limb (85573 ±8778 DLU/mm^2, n=3) compared to the contralateral hind limb (11462 ±931 DLU/mm^2, n=3) ($P< 0,01$).

Preliminary results with these radiolabelled nanovectors showed great promises in specific targeting to the site of interest. Additionally, dendrimers have numerous side advantages: high drug payload, stable formulation and controllable structure. Therefore, is our results are further confirmed on preclinical tumor models, these dendrimer-based nanocarriers could constitute novel and effective means for the delivery of radiopharmaceuticals and other biomedical imaging agents or therapeutic medicines, with obvious outlooks in theranostics.

Keywords : angiogenesis, RGD, radiolabeling, ischemia, SPECT, dendrimer.

SIRT deficiency in endothelial progenitor cells drives prosenescent microparticules release through MKK6 upregulation.

Anne-Line Chateau, Stéphanie Simoncini, Nais Baschet, Stéphane Robert, Isabelle Ligi, Richard Bachelier, Catherine Yzydorczyk, Laurence Louis, Umberto Simeoni, Frédérique Magdinier, Françoise Dignat-George, Florence Sabatier

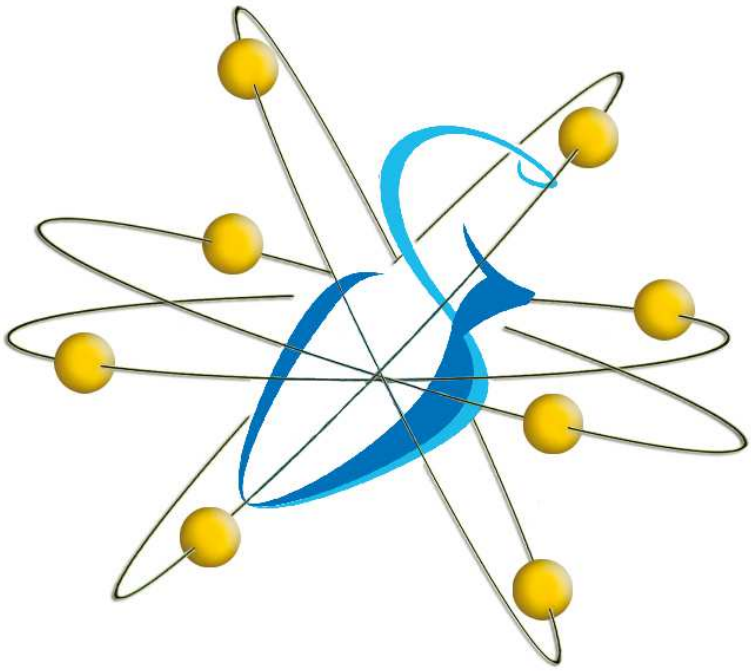
Single speciality keyword : Endothelium

Single abstract keyword : Microparticles.

We previously reported that cord blood derived-endothelial progenitor cells from preterm neonates display accelerated senescence linked to SIRT1 deficiency. Various studies indicate a role of senescence associated secretory phenotype (SASP), in particular soluble factors as IL-6, on endothelial dysfunction. However, the biogenesis and role of microparticles (MP) from senescent endothelial cells is not clearly established. We hypothesized that endothelial senescence could lead to a SASP, and mainly the biogenesis of pro-senescent MP, which could represent a mechanistic link between prematurity and early programming of cardiovascular risk at adulthood.

We investigated here the relationship between SIRT1-deficiency and the biogenesis of MP in endothelial colony forming cells from 25 preterm (PT-ECFC) compared with 18 term neonates (CT-ECFC). In PT-ECFC, we observed a significant increase in IL-6 production and endothelial MP release compared to CT-ECFC. MP release was dependent on SIRT1 deficiency and positively correlated with senescence. Gene expression profiling was conducted to investigate the molecular pathway that link SIRT1 defect and MP production and revealed an increased expression of MKK-6 in PT-ECFC. MKK6 overexpression in PT-ECFC was shown to drive p38 pathway activation and MP release by pro-senescent ECFC. Overexpression of SIRT1 or chemical-induction by resveratrol treatment of PT-ECFC reversed MKK6-p38 pathway activation and MP release. Finally, we showed that PT-ECFC derived MP exerted a paracrine pro-senescent effect on naive mature endothelial cells.

This is the first report demonstrating a mechanistic link between SIRT1 silencing and pro-senescent MP release. Deciphering the role of MP in the SASP will improve our understanding of how early endothelial senescence impacts on vascular homeostasis and cardiovascular risk of adults born preterm or with a low birth.



UMR CNRS 7273

Aix-Marseille Université

Institut de Chimie Radicalaire
Laboratoire de Pharmaco-Chimie Radicalaire (LPCR)

4,5-DIMETHOXYBENZENE DERIVATIVES: SYNTHESIS, ANTI-RHINOVIRAL EVALUATION, *IN SILICO* OPTIMIZATION.

Laurène Da Costa,[a] Manon Roche,[a] Johan Neyts,[b] Pieter Leyssen,[b]** Thierry Terme,[a] Romano Silvestri,[c]*** and Patrice Vanelle.[a]*

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[b] *Laboratory of Virology and Experimental Chemotherapy, Rega Institute for Medicinal Research, KU Leuven, Belgium.*
[c] *Department of drug Chemistry and Technologies, Sapienza University, Rome, Italy.*

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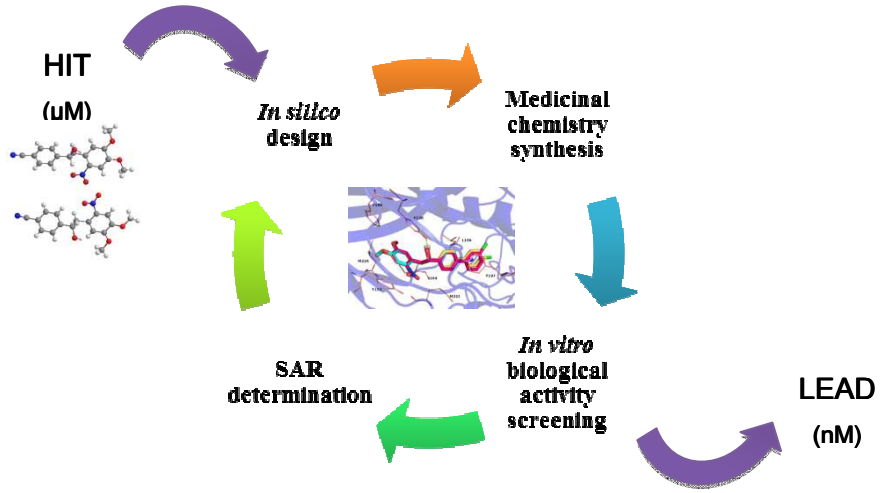
Once considered a cause of relatively benign upper respiratory illnesses, human rhinovirus (hRV) are also now linked to severe respiratory tract infections. This observation has led to a renewed interest in anti-rhinoviral molecules.

Our research team has recently identified a novel chemical scaffold of inhibitors of rhinovirus replication in a virus-cell-based cytopathic effect (CPE) reduction assay. In this context, we explored their structure–activity relationships using an original synthesis method, *i.e.* through one organic agent Tetrakis(DimethylAmino)Ethylene (TDAE). Among the 1,2-diarylethanols developed, 4-[2-(4,5-dimethoxy-2-nitrophenyl)-1-hydroxyethyl]benzonitrile has shown an interesting biological profile on HeLa cells (EC_{50} of 2.2 ± 0.4 µM) with the same action mechanism as the capsid-binder Pleconaril (EC_{50} of 0.3 ± 0.1 µM).

According these preliminary results, we oriented our study to a hit-to-lead process optimization based on Quantitative Structure Activity Relationship (QSAR) and computational approach.



We report herein chemical synthesis and first biological results of this optimization strategy.

[1] M. Roche, C. Lacroix, O. Khoumeri, D. Franco, J. Neyts, T. Terme, P. Leyssen, P. Vanelle, *Eur. J. Med. Chem.*, **2014**, 76, 445-459.
[2] C. Lacroix, J. Querol-Audí, M. Roche, D. Franco, M. Froeyen, P. Guerra, T. Terme, P. Vanelle, N. Verdaguer, J. Neyts, P. Leyssen, *J. Antimicrob. Chem.*, **2014**, 69, 2723-2732.

NOVEL ANTILEISHMANIAL LEAD COMPOUND IN 3-NITROIMIDAZO[1,2-*a*]PYRIDINE SERIES

Cyril Fersing[a], Louise Basmaciyan[b], Anita Cohen[b], Caroline Castera-Ducros[a], Nicolas Primas[a], Sébastien Hutter[b], Maxime D. Crozet[a], Michèle Laget[b], Pierre Verhaeghe[c], Pascal Rathelot[a], Patrice Vanelle[a], Nadine Azas[b].

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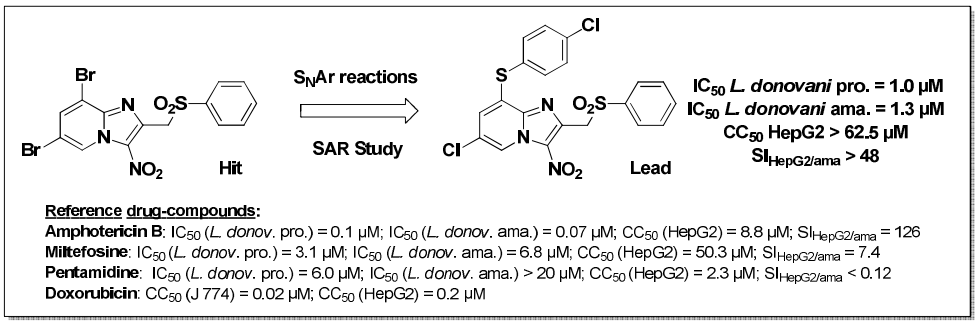
Looking for original heterocyclic molecules presenting antiparasitic activity, our research team develops a new program focusing on nitroaromatic compounds with antileishmanial activity.[1-3] Thus, in 2013, we reported the identification of a promising antileishmanial pharmacophore centered on the 3-nitroimidazo[1,2-*a*]pyridine scaffold.[4]

We present herein the antileishmanial pharmacomodulation study that we conducted at positions 6 and 8 of the imidazo[1,2-*a*]pyridine ring, by introducing new halogen atoms or by using nucleophilic aromatic substitution reactions. A series of twenty five original derivatives was then synthesized and highlighted a lead compound, bearing a *p*-chlorophenylsulfide substituent at position 8 and a chlorine atom at position 6. This lead compound displays very good *in vitro* activity on both the promastigote and amastigote stages of the parasite (IC_{50} = 1-1.3 µM) in comparison with Amphotericin B (the most active antileishmanial drug on the market) and Miltefosine (the only orally available antileishmanial drug on the market). Moreover, the lead compound did not show any cytotoxicity on the human HepG2 cell line (CC_{50} > 62.5 µM).



Reference drug-compounds:
Amphotericin B: IC_{50} (*L. donov*. pro.) = 0.1 µM; IC_{50} (*L. donov*. ama.) = 0.07 µM; CC_{50} (HepG2) = 8.8 µM; $SI_{HepG2/ama}$ = 126
Miltefosine: IC_{50} (*L. donov*. pro.) = 3.1 µM; IC_{50} (*L. donov*. ama.) = 6.8 µM; CC_{50} (HepG2) = 50.3 µM; $SI_{HepG2/ama}$ = 7.4
Pentamidine: IC_{50} (*L. donov*. pro.) = 6.0 µM; IC_{50} (*L. donov*. ama.) > 20 µM; CC_{50} (HepG2) = 2.3 µM; $SI_{HepG2/ama}$ < 0.12
Doxorubicin: CC_{50} (J 774) = 0.02 µM; CC_{50} (HepG2) = 0.2 µM

The research for the mechanism of action of the lead molecule, the evaluation of its activity toward other protozoa (to assess its selectivity) and the determination of its physicochemical properties and *in vitro* pharmacokinetic parameters are under progress.

Références:

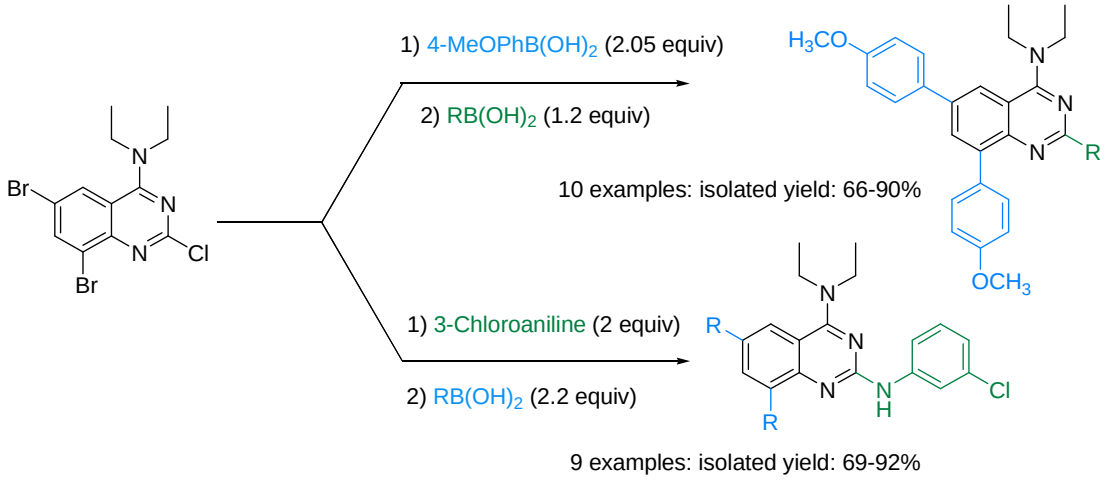
[1] L. Paloque, P. Verhaeghe, M. Casanova, C. Castera-Ducros A. Dumètre, L. Mbatchi, S. Hutter, M. Kraiem-M'rabet, M. Laget, V. Remusat, S. Rault, P. Rathelot, N. Azas, P. Vanelle, *Eur. J. Med. Chem.* 2012, 54, 75–86.
[2] C. Kieffer, A. Cohen, P. Verhaeghe, S. Hutter, C. Castera-Ducros, M. Laget, V. Remusat, M. Kraiem M'Rabet, S. Rault, P. Rathelot, N. Azas, P. Vanelle, *Eur. J. Med. Chem*. 2015, 92, 282-294.
[3] C. Kieffer, A. Cohen, P. Verhaeghe, L. Paloque, S. Hutter, C. Castera-Ducros, M. Laget, S. Rault, A. Valentin, P. Rathelot, N. Azas, P. Vanelle, *Bioorg. Med. Chem*. 2015, 23, 2377-2386.
4 C. Castera-Ducros, L. Paloque, P. Verhaeghe, M. Casanova, C. Cantelli, S. Hutter, F. Tanguy, M. Laget, V. Remusat, A. Cohen, M. D. Crozet, P. Rathelot, N. Azas, P. Vanelle, Bioorg. Med. Chem. 2013, 21, 7155–7164.

Green and efficient synthesis of 2,6,8-trisubstituted 4-aminoquinazolines

Youssef Kabri, Maxime D. Crozet, Thierry Terme, Patrice Vanelle*

Aix-Marseille Université, Institut de Chimie Radicalaire ICR, UMR CNRS 7273, Laboratoire de Pharmaco-Chimie Radicalaire, Faculté de Pharmacie, 27 Boulevard Jean Moulin – CS 30064, 13385 Marseille Cedex 05, France - Fax: +33(4)91794677. E-mail: patrice.vanelle@univ-amu.fr

The palladium catalyzed Suzuki-Miyaura reaction is one of the most highly regarded processes in synthetic chemistry and a widely studied cross-coupling reaction for carbon-carbon bond formation.[1] On the other hand, organic reactions using water[2] as a cheap, non-toxic, non-volatile solvent, together with multistep sequences carried out in a single flask, such as tandem, cascade or sequential reactions, have received considerable attention in organic chemistry.[3] In this context and in connection with our research program on the design and synthesis of original molecules with pharmacological properties,[4] the S_NAr reaction was combined with the Suzuki-Miyaura cross-coupling reaction to perform one-pot sequential polyfunctionalization of the quinazoline ring.



This approach affords rapid and efficient access to 2,6,8-trisubstituted 4-aminoquinazoline derivatives in high yields using an one-pot chemoselective methodology via consecutive tri-Suzuki-Miyaura or SNAr/bis-Suzuki-Miyaura cross-coupling reactions in water under microwave irradiation. In addition, this environmentally friendly procedure tolerates a wide range of boronic acids and represents a promising green route for the synthesis of these important pharmaceutical heterocyclic compounds.

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RAPID AND EFFICIENT SYNTHESIS OF SUBSTITUTED - PYRIDO[4,3-*B*] QUINOXALIN-1(2*H*)-ONE DERIVATIVES

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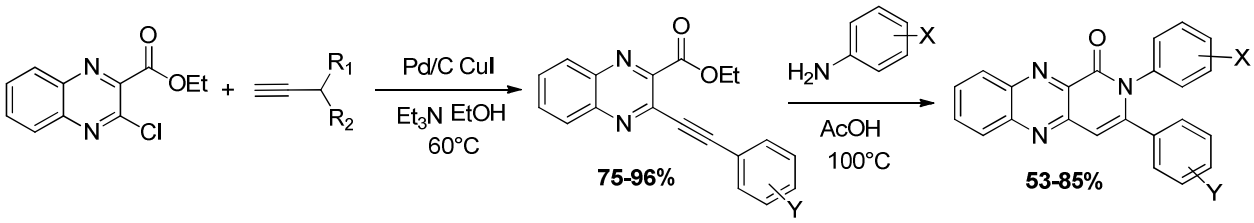
The quinoxaline derivatives show very interesting biological properties,[1] such as antibacterial,[2] antiviral,[3] anticancer,[4] antifungal, antihelmintic, antileishmanial,[5] anti-HIV,[5] insecticidal, anti–inflammatory activities[6] and their interest in medicinal chemistry is far from coming to an end. Many drug candidates bearing quinoxaline core structures are in clinical trials in antiviral, anticancer, antibacterial,[2] and CNS (central nervous system) therapeutic areas. Among them, the XK469 ((±)-2-[4-(7-chloro-2-quinoxaliny)oxy]phenoxy propionic acid) was known as antineoplastic quinoxaline topoisomerase II inhibitor and possesses antitumor activity especially against murine and human solid tumors.[7]

On the other hand, the pyridin-2(1*H*)-one derivatives exhibited interesting biological activity as anticancer agents.[8] In spite of the great interest that could represent combined structures presenting the quinoxaline and the pyridin-2(1*H*)-one nucleus, few synthesis of pyrido[4,3-b]quinoxalin-1(2*H*)-one derivatives have been reported.

We report herein an original and efficient synthesis of new substituted pyrido[4,3-*b*]quinoxalin-1(2*H*)-one derivatives by the Sonogashira reaction in the first step followed by cyclization reaction with different anilines.



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NEW ANTILEISHMANIAL AGENTS: SYNTHESIS, EVALUATION AND STRUCTURE-ACTIVITY RELATIONSHIPS STUDY OF HYDROXYAMIDINE DERIVATIVES

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The preparation of hydroxyamidines as antileishmanial agents is described [1-2] by cyclization of alkenes with *β*-ketosulfones using an oxidative free-radical mechanism mediated by manganese(III) acetate (Figure 1). The biological assessment of hydroxyamidines series highlighted a hit. Further pharmacomodulations on amidoximes showed that R^1 moiety must contain a -CH_2- group in *α*-position of the dihydrofurane to provide antileishmanial activity [3]. In order to synthesize such new derivatives, we developed an original cross-coupling reaction between 2-methyl-2-propen-1-ol and various boronic acids (Figure 2) to obtain methylallyl derivatives [4,5]. Then corresponding hydroxyamidines were obtained and evaluated on *L. amazonensis* promastigote and amastigote (Cf Figure 3): antileishmanial activity is enhanced by *ortho*- or *meta*- substitution of the benzyl, -NO_2 or –CF_3 groups and *ortho*- or *meta*- polysubstitution of benzyl group.

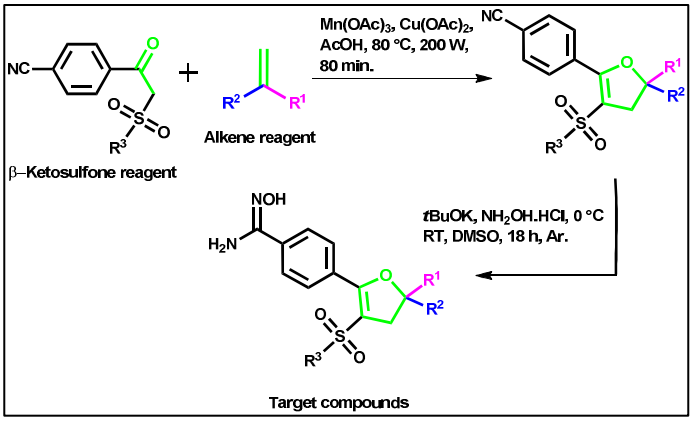

Figure 1: Synthesis of hydroxyamidines

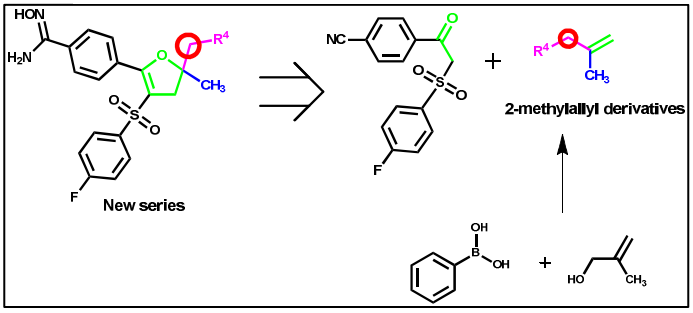

Figure 2: Pharmacomodulation

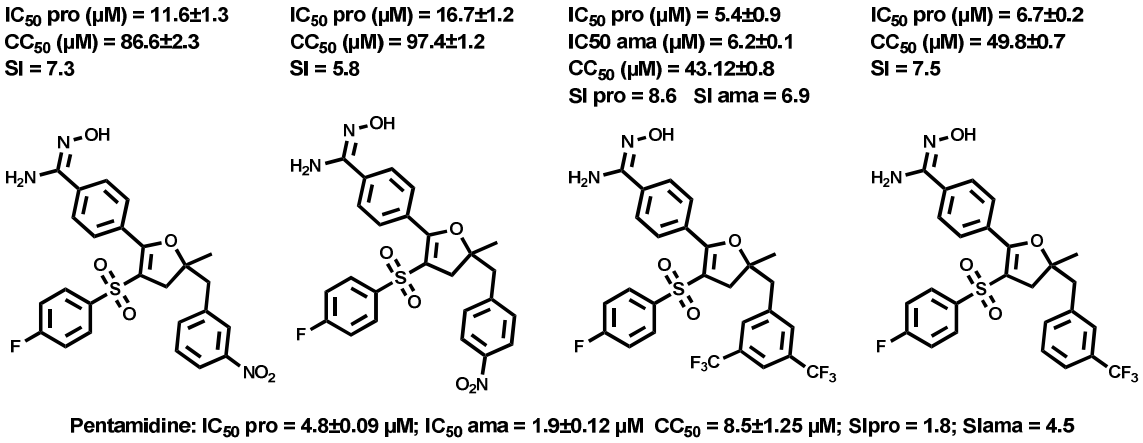

Figure 3: Biological evaluation of hydroxyamidines.

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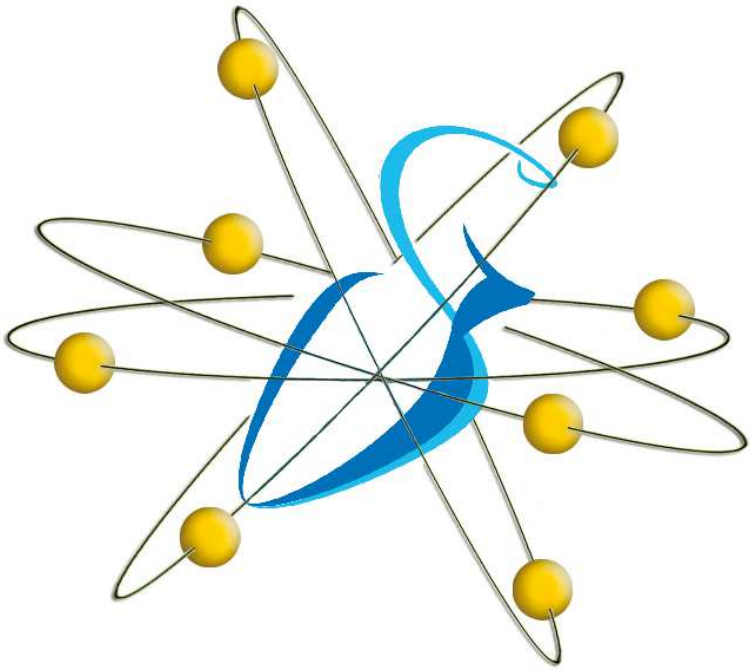
Prototypes	**IC_{50} / µM / 72hours** *L. amazonensis*	**$SI=LD_{50}/IC_{50}$ intracellular amastigote** ***L. amazonensis**
CLEM6086	**6.2±0.05**	**6.9**



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Centre de Recherche en Oncologie Biologique et Oncophamacologie (CRO2)

ACDC: Antibody nano-Conjugate Designed for Cancer: Application to breast cancer

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Today, breast cancer is the most common cancer among women in France. One of its standard treatments is to combine of biotherapy with chemotherapy. The association of Herceptin® (trastuzumab) with Taxotere® is rapidly becoming a reference treatment for patients suffering from a hormono-dependant breast cancer. However, this addition presents several limits e.g., significant impact but still limited in terms of survival and toxicities, that can be reduced with the use of nanovectors.

This project aims at developing an innovative entity combining stealth liposomal docetaxel presenting trastuzumab on its external surface. This new formulation is expected to exhibit a higher efficacy while reducing treatment-related toxicities because of its higher specificity towards malignant cells.

Here, our goal is to encapsulate docetaxel in a stealth single-unilamellar-vesicle (SUV) liposome of 150-200 nm in diameter, to anchor trastuzumab on the external surface through cholesterol or PEG linking, so as to yield a new entity called antibody-nano conjugate (ANC). This ANC is expected to exhibit a more specific distribution and delivery towards HER2+ breast cancer cells through both enhanced permeability retention (EPR) effect and active HER2 targeting through trastuzumab. Consequently, higher efficacy and better tolerance should ultimately be achieved in patients with breast cancer.

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Modeling the effects of EB1 and vincristine action on microtubule dynamic instability in glioblastoma cells: An explanation for EB1 sensitization to microtubule targeting drugs

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We recently demonstrated that EB1 overexpression represents: 1) a poor prognostic marker in glioblastoma patients and 2) a predictive marker for *vinca* alkaloid anticancer action (Berges at. al, oncotarget, 2014). However, the molecular mechanism of such sensitization remains poorly understood. The design of a pertinent mathematical/computational model of microtubule dynamic instability in the presence of EB1 is crucial for a better understanding of its role EB1 in cancer progression and of the pharmacological action of microtubule-targeting drugs. For this purpose, we propose a new stochastic model that accounts for the growth, shortening, catastrophe and rescue processes of steady state microtubules assembled in presence of various concentrations EB proteins and/or microtubule-targeting drugs. This model allowing us to formulate mechanistic hypothesis as numerical simulations, which are subsequently compared to the biological results to determine whether theses hypothesis are compatibles with reality. We have studied the role of EB1 overexpression on microtubule dynamic instability parameters in various glioblastoma cells overexpressing EB1 and the effects of a large range of vincristine concentrations on microtubule dynamic instability by using Plustiptracker, a Matlab software. Our results suggest in one hand that EB1 induce a continuing growth and decreased MT catastrophes in glioblastoma cells; and on the other hand that EB1 overexpression in cells potentiates vincristine-induced catastrophes as recently demonstrated *in vitro* for EB3 (Mohan at. al, 2014). However, our biological results also highlight nonlinear effects of vincristine on microtubule dynamics according to drug concentration as previously suggested (Pasquier at. al, 2005; Pourroy at. al, 2006). These results are difficult to understand without mathematical modeling. The description and simulations of the mathematical model, and the mechanistic hypothesis explaining theses biological results will be presented.

"This work has been carried out thanks to the support of the A*MIDEX project (n° ANR-11-IDEX-0001-02) funded by the « Investissements d'Avenir » French Government program, managed by the French National Research Agency (ANR)".

Pharmacogenetics of gemcitabine in pancreatic Adenocarcinoma : Where did we go wrong?

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There is a rising concern about how to limit toxicities and improve treatment outcome upon gemcitabine (GEM)-based treatment since evidence has been made that cytidine deaminase (CDA) is the major enzyme responsible for its inactivation into dFdU. CDA is subject to a wide inter-individual variability in terms of activity, due to genetic variations, leading to decreased or increased activity. We propose to study the effect of CDA phenotype and genotype on incidence of early severe hematologic toxicities upon GEM. We also conducted a pharmacokinetic (PK) study of GEM and dFdU.

120 patients with pancreatic ADK were enrolled in a prospective clinical trial FFCD-1004, all receiving GEM in monotherapy (1000mg/m², 30-minutes IV infusion). Blood samples were collected prior to treatment to evaluate CDA status by a double approach: a phenotypic and a genotyping tests. PK study was conducted with a LSS: on 1rst course, samples were withdrawn prior administration, at the end of infusion, and 90 min and 120 after the end of infusion. GEM and dFdU levels were measured by UPLC-MSMS on plasma fraction after development and validation of the analytical method according to FDA guidelines. GEM and dFdU kinetics were analyzed using non-linear mixed effect modeling software Monolix 4.3.2®.

109 patients were analyzable for CDA activity, with a mean age of 66 years. 40 patients displayed early severe toxicities, including 26 hematological toxicities and 20 other toxicities. 39 kinetics could be collected. CDA activity range from 0.86 to 8.56 U/mg. Mean and median CDA activity were respectively 2.6 and 2.2 U/mg, which is not different from our previous study. There was not difference in CDA activity between patients with early severe hematological toxicities versus no toxicities. For rs2072671 SNP, 38 patients were wild-type, 41 heterozygous and 14 were homozygous variant (92 samples). Patients with a C/C genotype were not at higher risk of early severe toxicity. PK of GEM and dFdU was best described by a two-compartment model, with a first order transfer rate. BSA was covariate of dFdU volume distribution whereas serum creatinine levels were associated with the clearance of gemcitabine. Here, CDA has no influence on PK parameters, as it was expected.

This multicenter prospective clinical trial doesn't confirm predictive role of CDA status in occurrence of early severe toxicities in patients with pancreatic adenocarcinoma treated with GEM only. Genotypic data could neither explain discrepancies in toxicity. Furthermore, we designed a sparse data pharmacokinetic study allowing to estimate population and individual parameters of GEM from few samples. We succeeded in estimating population and individual PK parameters of gemcitabine and its inactive metabolite.

A smoothing effect has been observed in the inter-individual variability of CDA activity compared to what was observed in retrospective studies. Selection in patients must introduce a bias, especially by selecting pancreatic ADK patients. Predictive role of CDA in occurrence of toxicity should be investigated in a larger cohort including various cancers.

Relationships between SLC polymorphisms and anemia in HIV-HCV co-infected patients treated with either Peg-interferon(IFN)-ribavirin bitherapy or direct antiviral agents-based triple therapy

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Background

Single nucleotide polymorphisms (SNPs) on the concentrative nucleoside transporter (CNT) gene SLC28A3 and the equilibrative nucleoside transporter (ENT) gene SLC29A1 gene have been described to be associated with ribavirin (RBV) induced anemia[1]. The impact of SLC SNPs in HIV-HCV co-infected patients treated with RBV-Peg-INF bitherapy or direct antiviral agents (DAAs)-based regimen is unclear and has been poorly explored[2,3]. In addition, first generation DAAs, such as boceprevir (BOC) and telaprevir (TLV) are also frequently associated with the occurrence of severe anemia. Our goal was to determine the relationships between these SNP and RBV-induced anemia both in co-infected patients treated by a RBV-based therapy with or without combination to hematotoxic DAAs such as BOC and TLV.

Material & Method

Patients from the ANRS BocepreVIH/TelapreVIH phase 2 studies and patients from a local study were genotyped on the rs10868138 & rs5350726 SNPs of the SLC28A3 gene and on the rs760370 SNP of the SLC29A1 gene using Taq-Man RT PCR. The relationship between these SNPs and hemoglobin (Hb) decline was assessed at week 4 (W4) and week 8 (W8) as well as their impact on EPO use and RBV dose reduction.

Results

A total of 200 patients were genotyped for all SLC SNPs (63 from BocepreVIH group, 69 from TelapreVIH group and 68 from bitherapy group). In patients treated with RBV-Peg-IFN bitherapy, the two SNPs of the SLC28A3 gene were associated with lower Hb decline only at W4 (1.1 vs. 2.3g/dL, p=0.037) and the homozygous variant of the SLC29A1 SNP was associated with higher Hb decline at W8 only (2,3 vs. 3,6 g/dL, p=0.027). In patients treated with a DAA-based regimen, no association was found between SLC variants and anemia. Moreover, no impact on RBV dose reduction or EPO use was observed for all SLC SNPs both in patients treated with bitherapy or tritherapy regimens.

Discussion and conclusion

In patients treated with RBV-Peg-IFN bitherapy, SLC28A3 polymorphism had a protective effect on RBV-induced anemia whereas SLC29A1 polymorphism was associated with higher risk of anemia as previously described in the literature1,2. However, when hematotoxic DAAs such as BOC and TLV were combined with RBV, these polymorphisms were no longer associated to anemia. Cumulative hematotoxicity of DAAs might overcome the effects of SLC SNPs on anemia.

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Metronomic scheduling: novel strategy to manage intratumor heterogeneity and control cell resistance to chemotherapy?

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To date, the emergence of drug resistance is never dealt with until it occurs, andconstitutesagenuinedailychallengeforpatientsandoncologists.Ourresearchfitswiththefieldof clonalheterogeneity,whichhasemergedasakeymechanismthatunderliesresistanceinclinicaloncol ogy.Acomplexpatternoflowfrequencymutationsinoncogenesexistsatdiagnosisandinfluencetreat mentresponse.Being able to decipher the relationship between sensitive and resistant cancer cell subpopulations, and characterize the impact of intratumor heterogeneity on treatment efficacy, is essential to understand tumor biology and design new therapeutic strategies to overcome cancer resistance.

We developed 2D co-culture models to analyze over time the expansion of stably fluorescent A549 cells and A549 EpoB40 cells, which are lung carcinoma sensitive and resistant cells to various chemotherapy agents respectively. We also developed spheroidco-culturemodelsthatrecapitulatethe3Dorganizationofamicrotumor.We showed that an unexpected suppressive effect on the resistant cell growth was exerted by the sensitive cells, which consequently constitute the predominant subpopulation in the absence of any chemotherapeutic treatment. By using cell-free supernatants and Transwell co-culture systems, this inhibition was characterized as independent of cell-to-cell interactions. We are currently analyzing the molecular mechanisms responsible for this competition between clones.

In addition, we showed that a treatment schedule that drastically reduced the number of sensitive cells (cytotoxic dose, once a week) irremediably resulted in the selection of the resistant subpopulation in the different co-culture models. Conversely, a metronomic treatment schedule (frequent administration of pro-tracted low doses of drugs) was an effective strategy both to suppress sensitive cell growth and to prevent selection of resistant cells by preserving intratumor heterogeneity. Our results thus support the fact that daily low doses of drugs would lead to better long-term results than high cytotoxic doses, to overcome problems of drug resistant cell selection that underlies many cases of cancer recurrence. The mathematical modeling of cell expansion and response to treatment is in progress to further define situations of tumor heterogeneity where resistant cells could be controlled by the metronomic-based treatment schedules, to achieve a stable or reduced global tumor population.

Pharmacocinétique de population (PK-POP) de la Rilpivirine (RPV) chez l'adulte infecté par le virus de l'immunodéficience humaine de type 1 (VIH-1)

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La rilpivirine est un antirétroviral (ARV) appartenant à la classe des inhibiteurs non nucléosidiques de la transcriptase inverse (INNTI), utilisée en première ligne comme 3ème agent dans la trithérapie ARV. La RPV présente des propriétés pharmacocinétiques qui peuvent être à l'origine de variabilité significative pouvant affecter son efficacité et/ou son profil de tolérance. De plus les concentrations plasmatiques de RPV sont fortement corrélées à la réponse virologique.

Notre étude a pour but d'optimiser et d'individualiser le traitement ARV en identifiant et en expliquant les sources de variabilité pharmacocinétique par l'approche de pharmacocinétique de population.

Il s'agit d'une étude multicentrique réalisée dans les services de maladies infectieuses (Hôpital Bichat-Claude Bernard, Paris ; Hôpital de la Conception, Marseille ; Hôpital Nord, Marseille ; Hôpital Sainte-Marguerite, Marseille), regroupant 307 patients infectés par le VIH-1, traités par l'association fixe RPV (25 mg), TDF (245 mg) et FTC (200 mg) en une prise par jour. Le suivi est réalisé sur un an.

Les dosages plasmatiques sont effectués dans les laboratoires de Pharmacocinétique et de Toxicologie de l'hôpital de la Timone à Marseille et de l'hôpital Bichat-Claude Bernard à Paris. Ils sont réalisés par chromatographie liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS) avec une limite de quantification à 10 ng/ml. Les dosages sont effectués à l'état d'équilibre soit au minimum un mois après la mise sous traitement. Les études PK-POP et PK-PD ont été réalisées à l'aide de deux logiciels, non-linéaire à effet mixte: NONMEM et MONOLIX.

L'analyse PK-POP met en évidence une forte variabilité interindividuelle du volume de distribution (Vd) comme précédemment décrit dans la littérature. Les résultats de l'étude des covariables ont montré une tendance entre BMI (Body Mass Index)/VdRPV avec une corrélation positive, VdRPV augmentant quand le BMI est élevé. Or la rilpivirine est un dérivé diarylpyrimidine fortement liposoluble (logP=4.86) avec une demi vie longue (45h), présentant de ce fait une forte affinité pour les tissus adipeux. Cependant les limites du modèle PK n'ont pas permis de valider les résultats de façon significative.

Il serait souhaitable de poursuivre l'analyse à l'aide de modèles PK plus robustes afin de confirmer les premières tendances observées dans l'objectif d'optimiser le traitement ARV à marge thérapeutique étroite et d'évoluer vers l'ère de la médecine personnalisée.

4-Indolylcoumarin Exhibits Potent Activity Against Renal Carcinoma Cells without Affecting Hematopoietic System

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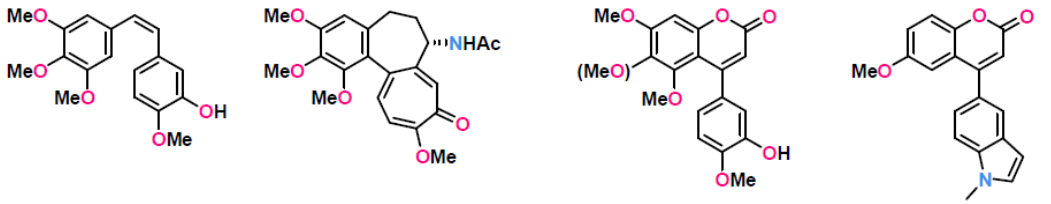
Abstract:
Microtubules are a highly-validated target in cancer therapy, explaining the abundance of efforts to develop novel agents devoted to this dynamic field. In the current search for microtubule binding agent, enhanced tumor specificity, reduced neurotoxicity and insensitivity to chemoresistance mechanisms are the main objectives. In this context, arylcoumarins, a novel class of CA-4 derivatives modified at ethylene bridge have been recognized as interesting candidates for a preclinical development. In the past recent years, the indol moiety was proved to be a good surrogate for the 3-hydroxy-4-methoxyphenyl ring of arylcoumarin and new indolyl derivatives were identified as potent antiproliferative agent in nanomolar range. We therefore decided to investigate the biological efficiency of the 6-methoxy-4-(*N*-methyl-indol-5-yl)-coumarin toward renal carcinoma cell lines.

The present work describes the anticancer activity of a new indolylcoumarin named COUFIN and more specifically, its efficiency against clear cell renal carcinoma (CCRC). COUFIN inhibited microtubule formation and bound on tubulin to or near the colchicine site. In vitro, COUFIN showed potent anticancer activity on renal carcinoma cells (RCC) both in monolayer (2D culture) (IC50 of 88±8 nM) and multicellular tumor spheroid (3D culture) (IC50 of 180±20 nM). The drug blocked cell cycle transition at G2/M phase, induced a subsequent apoptotic process but did not modulate clonal growth of CFU-GM. On the other hand, the coumarin derivative decreased the activity of P-gp and BCRP but was not substrate for these ABC pumps. In vivo, the indolylcoumarin increased the survival rate after 3 weeks of treatment. Based on the present study, it is suggested that COUFIN could be a promising chemotherapeutic agent for treating renal carcinoma.

Keywords: 4-indolylcoumarin, renal carcinoma, 2D and 3D cultures, apoptosis, P-gp and BCRP, colony forming unit-granulocyte/macrophage



Combrétastatine A-4 | Colchicine | Arylcoumarin | Indolylcoumarin

Synthesis and Biological Evaluation of Furanoallocolchicinoids

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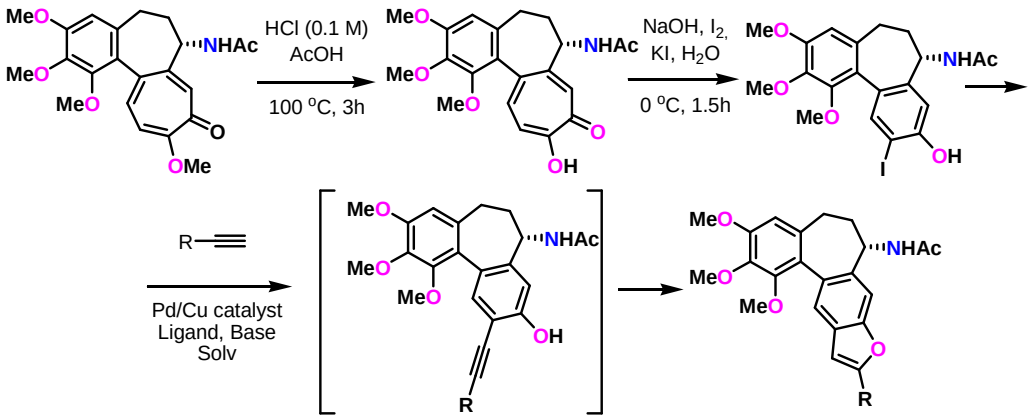
Abstract: The search for selective inhibitors of tubulin assembly or disassembly has led to the development of some of the most useful antitumor drugs currently in clinical use. These are the naturally occurring Vinca alkaloids as well as the taxoids, which are widely used for the treatment of breast, ovarian, and nonsmall cell lung carcinomas. However, these structurally rather complex molecules share several drawbacks as they are difficult to synthesize and prone to the development of resistance. Therefore, the development of novel small-molecule antimitotics with low-toxicity remains a clinically relevant challenge.

We have devised and synthesized a series of furan-derived allocolchicinoids as a novel class of analogues of the natural product colchicine. The compounds were efficiently obtained in three preparative steps starting from commercially available colchicine under conservation of

the absolute configuration at C-7. Compounds containing a hydroxyl group in the pseudobenzylic position of the furan side chain, exhibited high cytotoxicity toward epithelial and lymphoid cell lines in the nanomolar range. In comparison with colchicine and combretastatin A-4, allocolchicinoid proved to be a more effective tubulin assembly inhibitor, acting in a substoichiometric mode. Biochemical assays and in vitro experiments clearly demonstrated these compounds to effectively inhibit both tubulin polymerization and proliferation of endothelial cells as a consequence of two major effects i) disruption of the interphase and ii) induction of cell cycle arrest, both as a direct consequence of tubulin binding. The in vivo experiments also demonstrated that endothelial cells treated with compounds quickly lose adhesion and become “leaky”. In contrast, BBB endothelial cells become more tightly packed, which possibly leads to an insufficient supply to the brain of nutrients and oxygen. Still, treatment of mice with compounds resulted in the inhibition of tumor growth.

Keywords: furanoallocochicine, cytotoxicity, tubuline assembly, cell cycle, apoptosis, tumor growth



Effects of Nox1 over-activation in cultured hippocampal neurons

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Reactive oxygen species (ROS) and NADPH oxidases have been involved in Alzheimer and Parkinson diseases, vascular dementia and traumatic brain injury. Low ROS production is known to participate to signal transduction while excess of ROS production is toxic and represents a hallmark of cell inflammation. Many chemotherapies particularly those against cancer induce a ROS production leading to the efficacy of the therapy. We previously showed that the NADPH homologue Nox1 activity improves oxaliplatin efficiency in metastatic colorectal cancer cells. Nox1 is activated by its association with Noxo1 and Noxa1 regulatory proteins. Here, we show that overexpression of a Noxo1 mutant leads to an increase of ROS production and mortality of metastatic colorectal cancer cells compared to the wild-type. In order to address the neuronal effects of the Noxo1 mutant expression, we transfected mature cultured hippocampal neurons with the wild-type or mutant Noxo1 construct. Both wild type and mutant Noxo1 are targeted to a somatodendritic compartment and to the plasma membrane of a cell body. In contrast to wild type, Noxo1 mutant is not localized in dendritic spines. Thus, mutant noxo1 did not alter its interaction with nox1 and noxa1 regulatory proteins at the plasma membrane of a cell body.

Role of the protein Tau in glioblastoma progression

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In collaboration with:

-Aurélie Tchoghandjian, Carole Colin, Dominique Figarella. *Team 4, CRO2, UMR INSERM U911, AMU.*

-Marlène Martinho, Valérie Belle. *BIP, UMR CNRS 7281, AMU.*

Microtubule (MT) associated protein Tau is a MT stabilizing protein known to be implicated in neurite outgrowth and cell polarity. Besides, its expression seems to be correlated with resistance to MT-targeting therapies and metastatic potential in many cancer such as breast cancer. We aimed to study the implication of Tau in glioblastoma cancer invasion. We stably down regulated Tau expression in the glioblastoma cell line U87 by a shRNA approach and we studied cell migration and invasion. Random motility on fibronectin matrix (cell speed and total distance) was reduced by 40% in cells downregulating tau (shTau cells) compared to vector control transfected cells (shctrl cells). Cell transmigration (Boyden chambers) was also significantly reduced by 80%. As a complementary approach, we studied cell evasion from spheroids on fibronectin matrix. Cell evasion was significantly reduced in shTau cells compared to shctrl cells at two days of evasion. More interestingly, we noticed that after one day shTau cells presented a complete disassembly of the spheroid compared to shctrl cells showing cell evasion from a still intact spheroid. A similar behaviour was confirmed in experiments of 3D invasion through collagen matrix, suggesting strongly an involvement of Tau in cell adhesion and cell-cell interaction. To explain the migratory defects of shTau cells we are also currently studying the MT dynamics and the cross-talk of actin cytoskeleton and MT network in shTau cells compared to shctrl cells. Our preliminary results indicate a role for Tau in glioblastoma cell invasion and deserve further investigation in vitro and in vivo.

La régulation de Nox1 par les calpaïnes : un nouvel outil pour prédire la résistance des cellules tumorales à l'oxaliplatine ?

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Equipe 2, CRO2, UMR INSERM U911, Aix Marseille University

Le cancer colorectal est devenu un cancer majeur en raison de sa fréquence et de son taux de mortalité. Les stades avancés sont traités avec différents protocoles de chimiothérapie contenant notamment de l'oxaliplatine. Cependant le développement de résistances à ces molécules entraine l'échec de ces traitements, ce qui explique les faibles taux de survie observés pour les cancers métastatiques. Il est donc crucial de comprendre le mécanisme de résistance à l'oxaliplatine et d'identifier les acteurs impliqués. Des travaux du laboratoire ont montré que l'efficacité de l'oxaliplatine dans les cellules tumorales colorectales est dépendante des ROS produits par la NADPH oxydase Nox1 et que cette dernière est régulée négativement par les calpaïnes. Ces protéases ont été récemment identifiées comme des actrices de la résistance à l'irinotécan, autre molécule utilisée pour traiter le cancer colorectal. Nous nous sommes donc intéressés aux rôles joués par les calpaïnes et Nox1 dans le développement de la résistance à l'oxaliplatine. Dans un premier temps, nous avons sélectionné des cellules tumorales colorectales résistantes à l'oxaliplatine. L'étude de ces cellules nous a montré que le développement de la résistance s'accompagne d'augmentations significatives de la production de ROS dépendante de Nox1 et de l'activité des calpaïnes-1 et -2. Cette dernière n'est pas due à une augmentation d'expression, il s'agit d'une activation de ces protéases par l'augmentation de calcium intracellulaire. L'inhibition de Nox1 et des deux calpaïnes n'entraine pas de sensibilisation à l'oxaliplatine des cellules résistantes, mais induit au contraire une plus forte résistance. Les calpaïnes et Nox1 sont donc nécessaires aux faibles effets cytotoxiques de l'oxaliplatine sur ces cellules. Nous avons ensuite étudié les régulations existantes entre Nox1 et les calpaïnes dans les cellules résistantes. Nos résultats montrent que comme dans les cellules sensibles les calpaïnes sont en amont de Nox1. Cependant cette régulation est inversée dans les cellules résistantes puisque les calpaïnes régulent positivement, via les PKCs, l'activité de Nox1 et donc la production de ROS. Le développement de la résistance induit donc une inversion de la régulation. Nous avons également réalisé un screening de différentes molécules de chimiothérapie sur les cellules résistantes à l'oxaliplatine. Nous avons ainsi pu voir que différentes molécules, telle la gemcitabine, resensibilisaient nos cellules résistantes. Enfin nous avons commencé une confirmation de ces données sur des modèles 3D (sphéroïdes) plus proches de l'environnement tumoral. En conclusion, nos données indiquent que, contrairement à ce qui est observé pour l'irinotécan, les calpaïnes ne sont pas responsables de la résistance à l'oxaliplatine mais qu'au contraire elles participent aux faibles effets cytotoxiques induits par cette molécule. Nos données sont très intéressantes car elles mettent en évidence une inversion du type de régulation de Nox1 par les calpaïnes, régulation négative dans les cellules sensibles et positive dans les cellules résistantes. Bien que des études complémentaires soient nécessaires, la régulation de Nox1 par les calpaïnes pourrait donc servir de marqueur prédictif de l'efficacité de l'oxaliplatine.

Plateau Microcalorimétrie Timone : applications de l'ITC et la DSC à la cancérologie

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La microcalorimétrie regroupe un ensemble de techniques qui permettent la caractérisation des propriétés thermodynamiques de stabilité et d'interactions des macromolécules biologiques. Elle est utilisée depuis longtemps en Cancérologie pour décrire les mécanismes d'actions au niveau moléculaire, qu'il s'agisse de protéines ou de médicaments et autres petites molécules.

L'ITC (Isothermal Titration Calorimetry) permet de caractériser une interaction moléculaire par mesure de la variation de chaleur associée à la formation du complexe. Elle permet de déterminer en une seule expérience la stœchiométrie (n), la constante d'affinité et donc la variation d'énergie libre (ΔG) d'une interaction, mais aussi la variation d'enthalpie (ΔH), et d'en déduire la variation d'entropie (ΔS). La connaissance de tous les paramètres thermodynamiques donne des indications sur la nature de l'interaction (ionique, hydrophobe, …) et peut donc aussi être utiliser pour des questions de relation structure activité.

La DSC (Differential Scanning Calorimetry) mesure la variation de la capacité calorifique d'une macromolécule lors de sa dénaturation par chauffage. Elle permet d'évaluer la variation d'enthalpie (ΔH), la température de dénaturation (Tm) et la variation de capacité calorifique (ΔCp) associées à la dénaturation d'un domaine de la macromolécule. Cette technique est principalement utilisée pour caractériser la stabilité d'une molécule, ses différents domaines, et mettre en évidence des interactions avec des ligands.

A travers deux exemples pris dans nos travaux récents ou en cours nous illustrerons les possibilités de ces deux techniques dans la recherche en Cancérologie. Ainsi, nous présenterons comment l'ITC nous a permis de proposer un nouveau mécanisme moléculaire pour des agents anticancéreux (Taxanes, *Vinca* alcaloïdes) pourtant connus depuis longtemps, ainsi qu'une explication moléculaire à la résistance à ces agents anti-cancéreux. Enfin nous présenterons une nouvelle application de la DSC qui permet l'utilisation des profils de dénaturations des biofluides (serum, plasma, LCR) à visée diagnostique voire pronostique.

Le Plateau Microcalorimétrie Timone, hébergé par la Faculté de Pharmacie et animé par des membres de l'équipe 2 du CRO2, compte deux microcalorimètres dédiés aux mesures d'interaction entre biomolécules (VP-ITC et ITC200) et un microcalorimétre dédié aux mesures de stabilité thermique (VP-DSC) qui ont maintenant vocation à être ouverts au plus grand nombre.

Rapid identification of anti-angiogenic peptides isolated from venom fraction library screening

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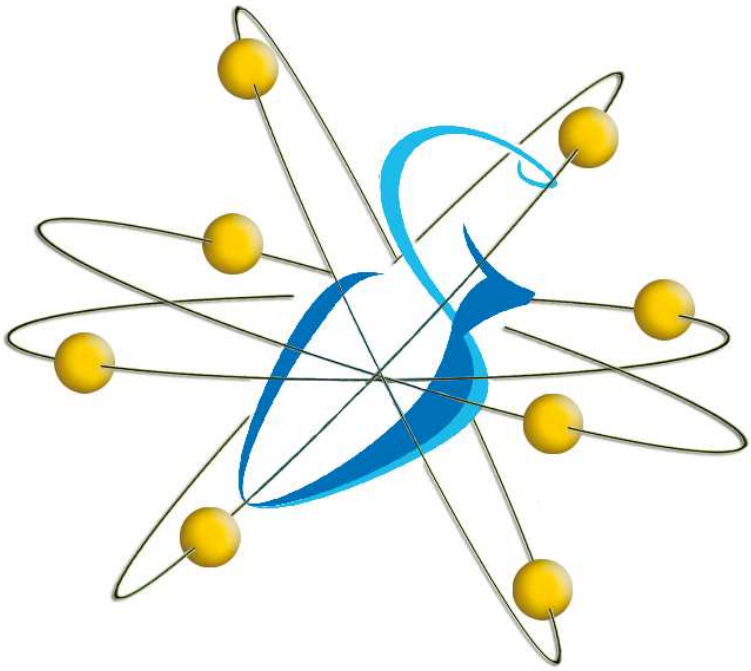
The venoms of scorpions, snakes, spiders and amphibians provide peptide toxins highly potent and highly selective of various targets with many possible pharmacological activities. For example: i) selective blockade of Kv1.3 channels in lymphocytes by charybdotoxin, *Tityus cambridgei* toxin, margatoxin and other Kv channel blockers might have therapeutic utility in the treatment of some neurological diseases, ii) Ancrod and batroxobin, thrombin-like enzymes, respectively purified from *Calloselasma rhodostoma* and *Bothrops moojeni* may be effective in ischaemic brain damage, iii) chlorotoxin from *Leiurus quinquestriatus* and snake venom disintegrin contorstatin exert antitumor activity *in vivo* and *in vitro*, iv) *Agkistrodon* antithrombogenase and *Naja naja* venom factor were found to be effective against arthritis, v) many snake venoms components are used in the diagnosis and treatment of haemostatic disorders.

Accelerating the discovery of venom bioactive molecules by pharmaceutical research, involves a facilitated access to them, but also guaranties on their origin, on the ability to durably resupply further quantities of constant quality, and capacity to help in the hit characterization process.

Starting from our long time know-how in catching, farming and milking of over 200 venomous species, and production of high quality venoms, we developed Kitoxan® a library of venom fractions available in sets of well plates. Crude venoms containing 500 to 1000 components are cleared off molecules over 10 kDa and split into 20 fractions, on the basis of HPLC chromatograms. Each well is then expected to contain only 5 to 10 peptides making the identification of the detected active peptide easier. After assessment of a hit pharmacological activity, we propose i) the hit purification, identification and characterization, ii) peptide synthesis and iii) structure-activity relationship with synthetic analogs to map and enhance the anti-angiogenic activity.

To validate this concept, we evaluated different venom fractions for their ability to inhibit the angiogenesis, a phenomenon highly involved in tumorigenesis.



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RECHERCHE D'INHIBITEURS DE L'INTERACTION ERBIN / ERBB2 DANS LE CANCER DU SEIN

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Les interactions protéine-protéine représentent une classe émergente de cibles thérapeutiques aux caractéristiques bien spécifiques. Pour étendre la chimie médicinale traditionnelle à cette nouvelle classe de cibles, plusieurs chimiothèques dédiées ont été développées pour faciliter l'identification d'inhibiteurs.

Nous avons utilisé ces outils pour tenter de moduler une interaction protéine-protéine impliquée dans le cancer du sein HER2+ : l'interaction entre le récepteur à activité tyrosine kinase HER2 (Human Epidermal Growth Factor Receptor 2 ou ERBB2) et la protéine à domaine PDZ ERBIN. Cette protéine a été identifiée comme partenaire d'ERBB2, leur interaction conduisant à la stabilisation de ce dernier à la membrane basolatérale des cellules épithéliales. ERBIN est donc impliquée dans la tumorigenèse et la prolifération des cellules tumorales HER2+.

Un essai d'HTRF (Homogeneous Time-Resolved Fluorescence) a été mis au point pour mesurer la capacité de petites molécules chimiques de différentes chimiothèques à inhiber l'interaction entre les deux partenaires. Deux criblages par HTRF ont permis la sélection de 7 composés d'une banque de fragments dédiée aux protéines à domaine PDZ fournie par ENAMINE et de 3 composés d'une banque de 89 composés sélectionnés *in silico* à partir de la banque2P2I$_{REF}$ dédiée aux interactions protéine-protéine (140 000 composés).

Pour valider les composés sélectionnés, nous mettons au point des essais orthogonaux utilisant la technique de DSF (Differential Scanning Fluorimetry) et la technique d'ELISA. Pour réaliser l'essai de DSF, nous avons produit une forme clivable de GST-ERBIN. Les composés présentant des profils d'inhibition prometteurs dans toutes les approches de validation seront testés *in cellulo* pour évaluation de leur activité biologique.

Cibler l'interaction ERBIN/ERBB2 est une stratégie innovante et prometteuse qui pourrait avoir une application dans le traitement des cancers du sein HER2+, qui représentent 25 % des cancers du sein.

Tao Y., Shen C.*, Luo S., Traoré W., Marchetto M., Santoni M.J., Xu L., Wu B., Shi C., Mei J., Bates R., Liu X., Zhao K., Xiong W.-C., Borg J.-P.*, and Mei L.* A role of Erbin in ErbB2-dependent breast tumor growth. (2014) ***PNAS,*** 111: E4429-38. *co-corresponding authors.

Mei L. and Borg J.-P. ERBB2 oncogenicity: ERBIN helps to perform the job. ***Molecular and Cellular Oncology***, 2015 Aug 21;2:3.

Role of Lano in Basal Breast Cancer

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The loss of polarity is an early morphological event observed during tumorogenesis of epithelial cells. It partially results, in expression abnormalities, in protein involved in the initiation and maintenance of cellular architecture, including members of the LAP family (LRR and PDZ) which my laboratory participated in cloning and characterization. This family of scaffold-protein conserved during evolution, is composed of four members in vertebrates: Scrib, Erbin, Densin-180 and Lano. LAP proteins have a ubiquitous expression and localize in the basolateral domain of epithelial cells. Among the members of the LAP family, Lano is less well characterized. It shares more than 60% homology with Scrib. Recent studies, including those of my laboratory, have shown the effect of deregulated expression of Erbin or Scrib on the incidence of breast cancer and in particular on basal breast cancers (BBC). Breast cancers are classified in different molecular subtypes: Luminal A and B, Basal, HER2, Normal-Like. The BBC represents approximately 15% of the breast cancers. They are very aggressive and the therapeutic strategies are limited. A contribution of Lano in hepatocellular carcinoma has recently been described. In contrast a role of Lano in breast cancer is unknown.

In order to investigate the role of Lano in breast cancer, first we used an in silico approach. Indeed, a correlation analysis between the level of expression of the LRRC1 transcript encoding for Lano and metastasis-free survival was achieved. More than 2000 patient samples including different molecular subtypes of breast cancer have been analysed. These results showed that low transcript level of LRRC1 was associated with a good prognosis in the BBC, in contrast to all other subtypes. Then, we investigated the function of Lano in vitro, using different models of breast cancer cell lines in which expression of Lano was either silenced by the sh-RNA technique or over-expressed. These cells lines were submitted to functional tests. The loss of the Lano expression affects migration and proliferative capabilities, as well as ALDH1 activity, a marker of stem cells. At last, these Lano-depleted cells were studied by in vivo approach. Xenografts of these cells in immunodeficient mice showed an effect on tumor growth and metastasis. Together, these results suggest that Lano has a role in fundamental cellular processes altered during mammary carcinogenesis.

LKB1 associated with Stradα or Stradβ regulates distinct cellular functions

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Genetic alterations leading to LKB1 expression loss has been defined responsible of the Peutz-Jeghers Syndrome (PJS) an autosomal dominant inherited disorder characterized by melanocytic macules of the lips and multiple gastrointestinal hamartomatous polyps. Patients affected by this syndrome have a short life span, around 45 years, mainly due to their elevated risk of developing malignant tumors, including breast and gastrointestinal cancers. Outside PJS context, Lkb1 expression loss is also frequently found in several cancer types such pancreas or lung carcinomas. So, LKB1 has been classified as a tumor suppressor. However, mechanism(s) by which LKB1 exerts its tumor suppressor property remains elusive even though there is no doubt of the involvement of its enzymatic activity. LKB1 is a serine threonine kinase and a better understanding of the regulation of its kinase activity and beyond cell signalization regulated by it should unravel LKB1 tumor suppressor activity mechanism.

To be active Lkb1 needs to be associated with scaffolding protein Mo25 and a pseudokinase Strad either α or β. Poorly studied to date, difference between Lkb1/Stradα and LKB1/Stradβ complex is often overlook and largely unknown. My work focuses on this latter point and shows, that although less represented compared to Stradα, LKB1/Stradβ complex has specific functions. Indeed, LKB1/Stradβ complex appears to regulate canonical Wnt pathway whereas Lkb1/Stradα does not. This functional specificity appears correlated to a specific cellular subdomain localization of the LKB1/Stradβ complex.

Importance of the canonical Wnt pathway deregulation during tumors progression being well established, my results suggest that LKB1 tumor suppressor activity require its association with Stradβ.

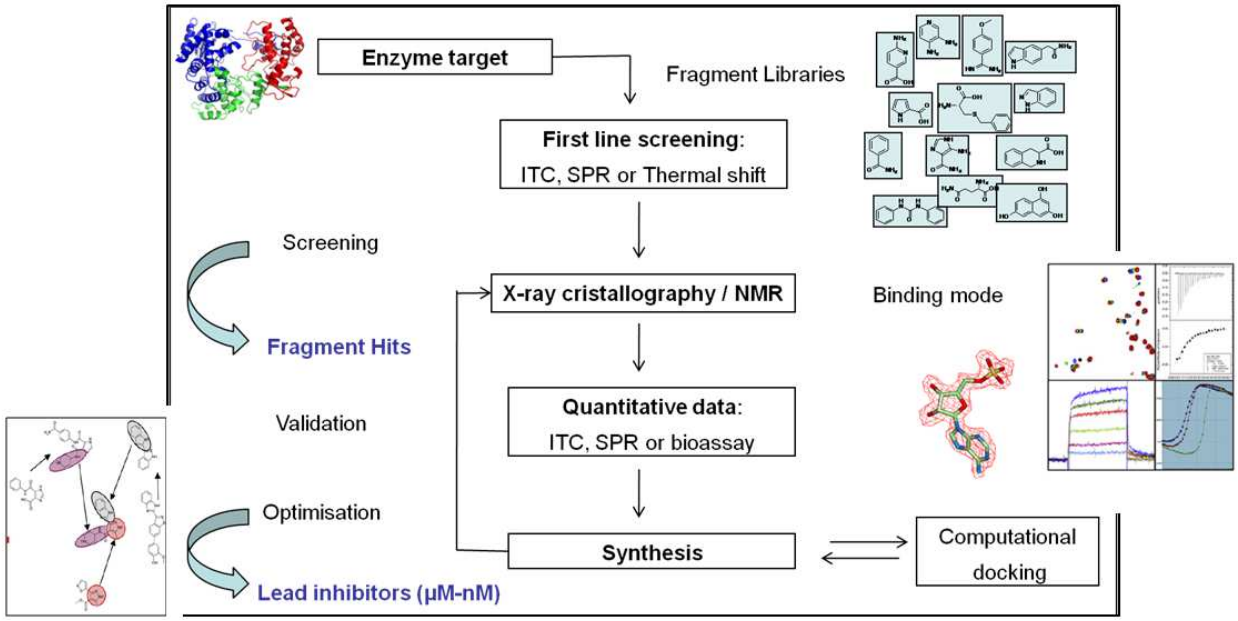
Identification et optimisation d'inhibiteurs par une approche de « Fragment-based drug discovery » : application au domaine méthyltransférase NS5 du virus de la Dengue

Fatiha Benmansour, Bruno Coutard, Etienne Decroly et Karine Barral

Depuis quelques années, le virus de la Dengue est en pleine progression, posant un sérieux problème de santé publique à l'échelle mondiale. A ce jour, il n'existe ni vaccin, ni traitement spécifique antiviral. Un des moyens de combattre cette infection par thérapie antivirale est d'en inhiber les protéines virales portant les activités enzymatiques essentielles à la réplication du virus. Les méthyltransférases virales, enzymes de coiffe (ou capping), ont récemment été décrites comme essentielles à la maturation de l'ARN viral et donc à la réplication du virus.

L'objectif de ce projet de recherche est de développer une stratégie de découverte de candidat-médicaments par « Fragment-based drug discovery » (FBDD) appliquée à l'identification et l'optimisation d'inhibiteurs du domaine méthyltransferase NS5 du virus de la Dengue. Cette approche de FBDD consiste à étudier l'interaction de molécules de petite taille (ou fragments) sur une cible biologique grâce à des méthodes de détection biophysiques telles que la cristallographie par rayon X, la RMN, la résonance plasmonique (SPR), la microcalorimétrie différentielle (ITC) et le Thermal-shift assay.

Les données structurales obtenues sur l'interaction entre le fragment et sa cible permettent d'une part l'identification des sites et du mode de fixation des fragments, et d'autre part une optimisation rapide et efficace en puissants inhibiteurs.



Stratégie de FBDD

Caractérisation d'une nouvelle isoforme de vangl2, une proteine de la polarité surexprimée dans les cancers du sein

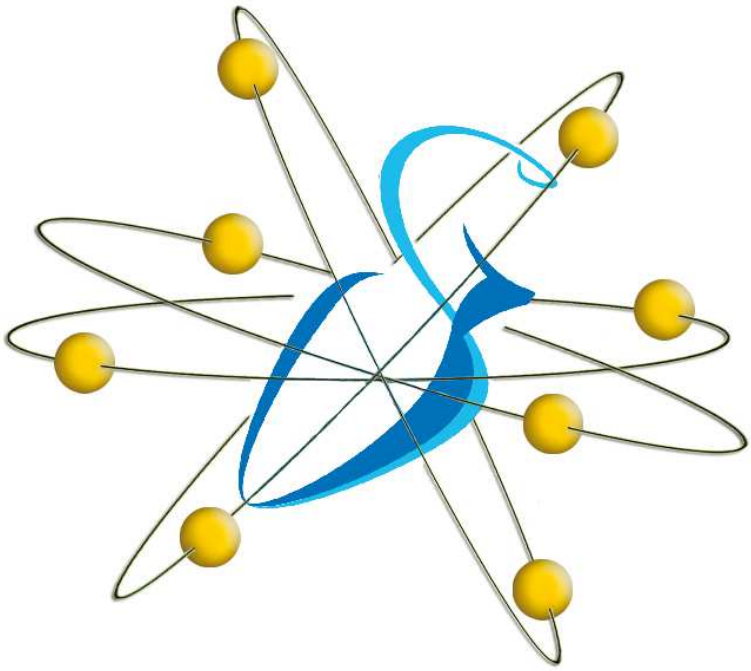
Walton Alexandra, Bailly Éric, Guenneau-Puvirajesinghe Tania, Marchetto Sylvie, Borg Jean-Paul

Le cancer du sein basal est un sous-type très agressif. Malgré les nouvelles approches thérapeutiques, les patientes atteintes de ce type de cancer du sein ne peuvent bénéficier de traitements hormonaux ou de thérapies ciblées et sont traitées par de la chimiothérapie standard. Il y a une nécessité d'identification de nouvelles cibles thérapeutiques pour ce sous-type de cancer.

VANGL2 est un récepteur impliqué dans la polarité planaire. La dérégulation de son expression a été décrite dans certains cancers solides et hémopathies malignes. Dans les cancers du sein de type basal, VANGL2 est souvent surexprimé et corréle à un mauvais pronostic. Son mécanisme d'action moléculaire, son mode de régulation et son ligand restent pour l'instant inconnus. Cependant, dans des études récentes, une nouvelle isoforme de VANGL2 a été identifiée. Cette dernière est produite grâce à la présence d'un site d'initiation de la traduction non conventionnel en amont de la méthionine entraînant la production d'une isoforme de VANGL2 plus longue du côté N-terminal. Cette extension N-terminale est absente chez la drosophile et conservée au cours de l'évolution des vertébrés indiquant une probable importance fonctionnelle. Il existe de nombreuses protéines qui présentent des isoformes plus longues ou plus courtes dues à une initiation alternative de la traduction. Pour certaines, les recherches ont mené à des explications fonctionnelles et à d'éventuelles implications en pathologie.

Nous avons poursuivi la caractérisation de cette isoforme longue de VANGL2 (VANGL2-Long) en mettant à profit des anticorps tout juste générés au laboratoire.

Nous avons dans un premier temps cherché à s'assurer de la spécificité de ces nouveaux outils immunologiques. Deuxièmement, nous avons utilisé ces anticorps pour en savoir plus sur les interactions protéiques de VANGL2-Long grâce à des expériences de co-immunoprécipitation et pour déterminer la localisation sub-cellulaire de cette isoforme. Pour cela, il a fallu générer des cellules IMCD3 déficientes pour VANGL2 grâce à la technologie Crispr/Cas9. Ces cellules ont servi de contrôle négatif pour les expériences d'immunofluorescence. Cette isoforme longue de VANGL2 étant maintenant mieux caractérisée biochimiquement, la suite du projet consistera à déterminer sa fonction.



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Aix-Marseille Université

Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale (IMBE)

ETUDE COMPARÉE DE LA TOXICITÉ DU NICKEL, DU CADMIUM ET DE L'ALUMINIUM AU NIVEAU DE LA BIOÉNERGÉTIQUE CELLULAIRE.

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Keywords: microcalorimétrie, bioénergétique cellulaire, métaux, respiration cellulaire

Les métaux sont présents de façon naturelle dans l'environnement en faibles concentrations. Certains, indispensables au bon déroulement des fonctions biologiques, constituent les oligo-éléments. Toutefois selon leur nature et/ou leur concentration ils peuvent se révéler toxiques. L'augmentation de leurs concentrations est directement liée aux activités anthropiques et à leurs propriétés de bioaccumulation. A l'heure actuelle les intoxications aigües aux métaux sont en fortes diminutions, en revanche la toxicité chronique liée à l'exposition environnementale est un problème de santé publique(1,2). Dans ce domaine les effets du cadmium, du nickel et de l'aluminium ne sont pas encore clairement définis(3). Ce travail a pour but l'étude de l'effet de ces trois métaux au niveau de la bioénergétique cellulaire. Ainsi, leur impact sur la thermogénèse et la respiration cellulaire est déterminé sur des fibroblastes cutanés humains en culture respectivement par microcalorimétrie isotherme et oxymétrie. En effet, la thermogénèse résulte essentiellement du catabolisme du glucose, source d'énergie pour la cellule, et la respiration est le fidèle reflet de la fonction mitochondriale, usine énergétique de la cellule, dont l'activité principale est la synthèse d'ATP.

Les résultats montrent que le cadmium (0 à 1.5 mM) diminue la thermogénèse et la consommation en oxygène de façon dose dépendante alors que l'aluminium (0 à 3.87 µM) exerce seulement un effet inhibiteur sur la consommation en oxygène et aucun effet n'est observé pour le nickel. Après 24 heures d'incubation à 40 µM, le cadmium a un effet létal sur les cellules alors que le nickel et l'aluminium induisent une augmentation de la thermogénèse et de la consommation en oxygène. Ces résultats indiquent que le cadmium, le nickel et l'aluminium ont des effets différents sur la bioénergétique ce qui suggère un mécanisme d'action propre à chacun de ces métaux possiblement associé à leur temps d'exposition. Il sera donc intéressant d'approcher les mécanismes d'action impliqués pour expliquer ces perturbations.

(1) http://www.senat.fr/rap/l00-261/l00-2611.pdf: RAPPORT sur LES EFFETS DES MÉTAUX LOURDS SUR L'ENVIRONNEMENT ET LA SANTÉ, par M. Gérard MIQUEL, Sénateur

(2) Willhite, Calvin C. « Total allowable concentrations of monomeric inorganic aluminum and hydrated aluminum silicates in drinking water ». Critical reviews in toxicology (05.2012) 358 - 442.

(3) Koedrith, P. « Toxicogenomic approaches for understanding molecular mechanisms of heavy metal mutagenicity and carcinogenicity ». International journal of hygiene and environmental health (08.2013) 587 – 598.

Effects of exposure to PCB-DL (the PCB 118) and to PCB non DL (the PCB 153) on adipogenesis and on expression of genes involved in the establishment of a pro-inflammatory state in vitro

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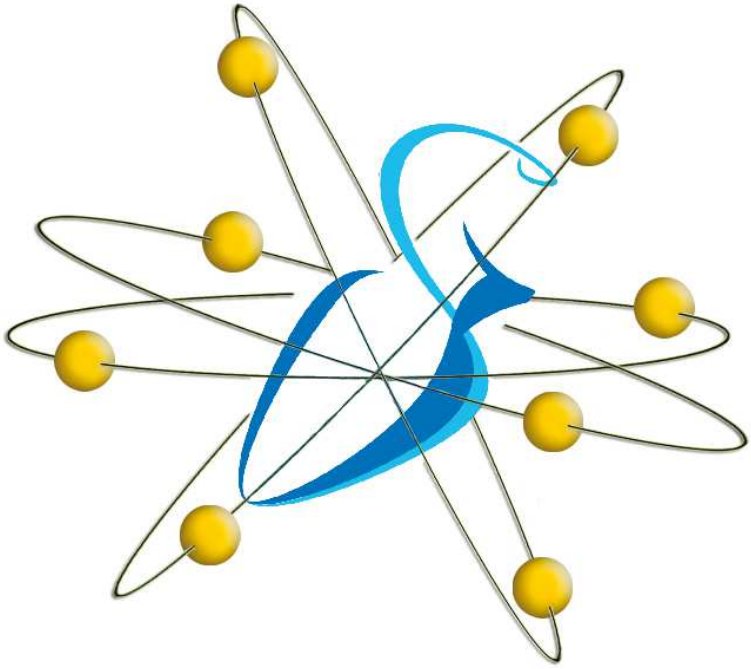
Obesity may lead to metabolic syndrome and then to type 2 diabetes, which is characterized by insulin resistance. This disease is associated with the expansion of adipose tissue, inflammation and cardiovascular diseases. Expansion of adipose tissue seems to play an essential role as a source and a site of inflammation, thereby contributing to the release of pro-inflammatory cytokines. Exposure to Polychlorinated biphenyls (PCBs) was associated with an increased risk of type 2 diabetes. PCBs accumulate in the adipose tissue because of their lipophilicity. This accumulation of PCBs increases with obesity.

The objective of our study was to investigate *in vitro* the effects of the exposure to PCB118 and PCB 153 on adipogenesis, on the expression of genes involved in the adipogenesis (PPARγ, Glut 1, FAS, ATGL) using the preadipocyte 3T3-L1 murine model.

The results of Oil Red O staining (ORO) that quantify the accumulation of lipid droplets in the cytoplasm, show that exposure to PCBs studied change slightly adipocyte differentiation. The data obtained by qRT-PCR showed that exposure to PCBs is accompanied by a moderate modification of some target gene expression, such as the decrease of FAS, Glut1 and the increase of ATGL, which are correlated with the reduction of the formation of lipid droplet. PCBs 118 and 153 seem to have different effects on PPARγ expression. PCB 118 (DL-PCBs) tends to slightly induce the expression of PPARγ while PCB 153 tends to reduce it in a dose-dependent manner.

In conclusion, exposure to PCBs modifies only mildly the adipocyte differentiation *in vitro*.



UMR-MD1

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Transporteurs Membranaires-Chimiorésistance

Studying the florfenicol's Potentialisation by polyamino-isoprenic derivatives in the treatment of *Bordetella bronchiseptica* porcine Pulmonary infections

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Multidrug resistant bacteria is a worldwide concern that arose from both, the misuse Of antibiotics in human and animals and from the release of antibiotics in the environment, Thus, French government has established a national plan to control antibiotics consumption and consequently reduce the risk of resistance. Livestock Industries are primarily responsible for antibiotic release in the environment.
Thus, new strategies to decrease their antibiotic consumption while preserving the animal health and the profitability, are needed.

One of the significant problems affecting the swine industry are respiratory diseases, often resulting from the infection by several bacterial species. *Bordetella bronchiseptica* is a Gramnegative coccobacillus involved in this disease, which has a high level of infectivity partly due to its mobility, allowing spreading of the bacteria to the entire respiratory tract. The aim of this study was to find a molecule able to decrease the consumption of florfenicol, the antibiotic generally used to treat the *B. bronchiseptica* infections. A compound was identified by screening our chemical library, and we showed that less than 1 mg/L of this compound was sufficient to decrease 50 % of the amount of florfenicol used to kill the bacteria. We thus demonstrated that the compound promotes the antibiotic action by a permeation activity on the outer membrane together with affecting the motility machineries by collapsing the proton gradient motive force.

The use of such a molecule may allow to reduce both, the doses of antibiotics used, and the infectivity of the bacteria by inhibiting virulence factors.

EFFLUX PUMP: AN ATTRACTIVE TARGET TO TACKLE BACTERIAL RESISTANCE

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The emergence of multidrug-resistant (MDR) pathogens is a worldwide health problem. In Gram-negative bacteria, transporters belonging to the resistance-nodulation-cell division (RND) superfamily play an essential role in MDR phenotype. Indeed, efflux pumps (EP) extrude out of the bacterial cell a wide range of substrates, especially different families of antibiotics that contribute more and more to the treatment failure of infectious diseases.

In this context, a new strategy to overcome antimicrobial resistance is to find the way to block the active drug efflux using “escort molecules” of usual antibiotics, to improve and restore their efficacy. [1]

The screening of various chemical libraries on Enterobacteriaceae allowed selecting BG1190 as hit compound (figure 1) to design and synthesize quinolone antibiotic chemosensitizers based on the quinazolinone scaffold capable to target AcrAB-TolC EP (figure 2). The challenge is to identify pharmacophores that bind AcrB moiety of this transporter and to determine their role in the pump activity. Molecular modeling and QSAR studies guide the pharmacomodulations and the rational synthesis of new blockers of antibiotic efflux.

Project funding was supported by grant ANR-11-BS07-019-01 “IBEF” from Agence Nationale de la Recherche (ANR, France)

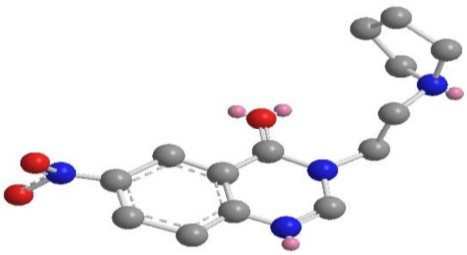


Figure 1: Quinazolinone Hit compound

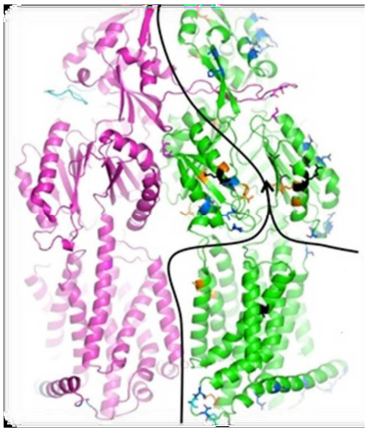


Figure 2. AcrB model on Enterobacter aerogenes

References:

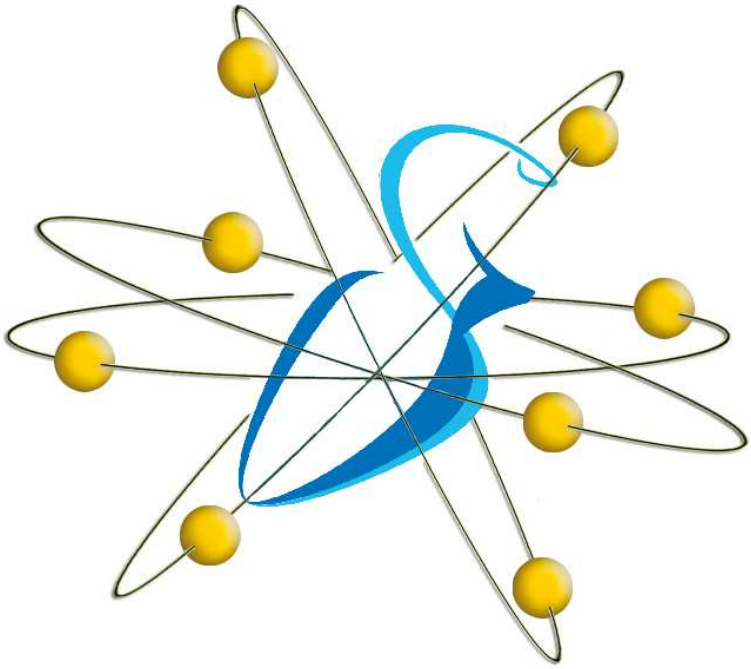
[1] J. -M. Bolla, S. Alibert-Franco, J. Handzlik, J. Chevalier, A. Mahamoud, G. Boyer, K. Kiec-Kononowicz, J. - M. Pagès, FEBS letters 2011, 585, 1682-169

Regulation of porin expression and impact on antibiotic resistance

Sushovan Dam, Muriel Masi and Jean-Marie Pagès
UMR-MD1, Aix-Marseille Université, Marseille, France

Infections caused by multidrug resistant bacteria are a major concern worldwide. In clinically important enterobacteria, such as *Escherichia coli* and *Enterobacter aerogenes*, the major mechanisms of resistance to β-lactam antibiotics involve (i) enzymatic degradation due to overexpressed β-lactamases and (ii) a decrease in outer membrane permeability. In this regard, alteration of outer membrane porins, conferred by downregulation of porin synthesis and/or porin modifications, restricts the access of antibiotics to their intracellular targets. In order to design new drugs with enhanced translocation property across the cell membrane, it is important to decipher the molecular mechanisms underlying regulation of porins. In *E. coli*, the two general porins OmpF and OmpC have been shown to be posttranscriptionally regulated by small RNAs (sRNAs) *micF* and *micC*, respectively. In this work, we outline results showcasing *micC* induction mechanism(s) — including environmental conditions and regulatory pathways — as well as the effect of *micC* on porin expression in *E. coli*. First, we used transcriptional *lacZ* fusion assays to screen through numerous stress conditions and genetic backgrounds. Then, the porin expression profile was evaluated in carefully chosen growth conditions and mutants by Western blot analysis. Another aspect of our study is elucidating the common regulatory mechanism between *micC* induction and the adjacent porin gene *ompN*. Although OmpN is a quiescent porin, we hypothesize that it may play a key role in bacterial adaptation to stress.

Keywords: Gram-negative bacteria, multidrug resistance, porins, sRNAs.



UMR-MD3

Aix-Marseille Université

Infections Parasitaires, Transmission, Physiopathologie et Thérapeutique

Studies on natural bio-insecticides from Cambodian plant biodiversity to control malaria and dengue vectors

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The main objective of the present work is to enhance Southeast Asian plant biodiversity, particularly from Cambodia, for the evaluation of insecticide or insect repellent properties of plant extracts to be used in vector control programs, respectful of the environment. Ethnobotanical surveys conducted in medicinal plant shops in different markets and in the National Center of Traditional Medicine of Phnom Penh enabled the selection of plants used in traditional medicine for their insecticidal/ repellent activity. This study was completed by literature search obtained from Cambodian books published by the National Center of Traditional Medicine. All in all, 73 plants were mentioned for their insecticidal/ repellent activity and five plants were selected for the evaluation of insecticidal properties. After collecting the plants, extracts were prepared following a standardized methodology. A study of the behavioral responses of *Aedes aegypti* and *Anopheles minimus* at three concentrations of plant extracts were performed using an excito-repellency test system[1,2]. Results showed that *Strophanthus scandens* leaves hexanic extract is the only one to exert a repellency on the two mosquitoes species (*An. minimus* at the concentration of 2.5% and *Ae. aegypti* at 5%). The obtained results confirm the traditional use of *S. scandens*, and show that Cambodian plants extracts could be a promising environmental friendly alternative to synthetic insecticides for which resistances have recently been observed.

References:[1] Roberts D. *et al.* Am Mosq Control Assoc (1997) 13:13-17. [2] Suwansirisilp K. *et al.* J Pestic Sci (2013) 86(2):309-320.

DISCOVERY OF NEW THIENOPYRIMIDINONE DERIVATIVES DISPLAYING ANTIMALARIAL PROPERTIES TOWARD BOTH ERYTHROCYTIC AND HEPATIC STAGES OF *PLASMODIUM*

Anita Cohen, a Peggy Suzanne, b Jean-Charles Lancelot, b Pierre Verhaeghe, c Aurélien Lesnard, b Sébastien Hutter, a Michèle Laget, a Aurélien Dumètre, a Lucie Paloque, d Eric Deharo,d Maxime D. Crozet,c Pascal Rathelot,c Patrick Dallemagne,b Audrey Lorthiois,e Carol Hopkins Sibley,f Patrice Vanelle,c Alexis Valentin,d Dominique Mazier,e* Sylvain Rault,b* Nadine Azasa*

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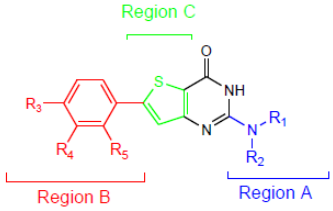
Malaria is a devastating pathology which in 2012 affected 207 million people and caused 627 000 deaths. Important and durable progress has been recorded for a few years, with malaria-specific mortality rates reduced by 42% between 2000 and 2012 [1]. Nonetheless, the growing drug resistance of parasites remains a real and ever-present danger, attributable mainly to *P. falciparum*. Artemisinin-based combination therapies have now been recommended as the first line of treatment in endemic areas. However, a *P. falciparum* treatment by artemisinin derivatives failure was identified and confirmed since 2009. In this context, all innovative practices and encouraging results are being sponsored and shared [2], so as to accelerate the development and licensing of new antimalarial drugs. N NH S N R1 O R3Region B Region A R4 R5 R2 Region C HepG2 CC50 = 15.0 - 49.9 µM P. falciparum K1 IC50 = 45 - 800 nM (erythrocytic activity) SI (HepG2/P. falciparum K1) = 9 - 533 1a: Specific activity between t = 0 - 32 h of the erythrocytic cycle (ring and trophozoïtestages) P. falciparum 3D7 IC50 = 35 - 344 nM (erythrocytic activity) P. yoelii IC50 = 35 - 120 nM (hepatic activity) 1a: 45% reduction in parasitemia of P. berghei infected mice at 5 mg/Kg IP No mutagenicity, no genotoxicity Best antiplasmodial results (40 products) 1-120

A preliminary antiplasmodial screening of some compounds belonging to various chemical families from our chemical library first revealed that the thieno[3,2-*d*]pyrimidin-4(3*H*)-one scaffold displayed a promising anti-infectious profile toward the human malaria parasite. Then, 120 new derivative compounds were synthesized and evaluated *in vitro*. Forty of them showed good *in vitro* antiplasmodial activity toward both chloroquino-sensitive and resistant *P. falciparum* strains, in comparison with 3 antimalarial drug references. These compounds were neither cytotoxic, toward the human HepG2 and murine CHO cell lines, nor mutagenic (Negative Ames tests), the corresponding selectivity indexes ranging from 9 to 533. Structure-activity relationships were then studied and precisely defined [3,4].



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Best antiplasmodial results (40 products)
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1a: 45% reduction in parasitemia of *P. berghei* infected mice at 5 mg/Kg IP
No mutagenicity, no genotoxicity

[1] Andrews, K.; Aregawi, M.; Cibulskis, R.; Fergus, C.; Lynch, M.; Newman, R.; Szilagyi, Z.; Williams, R. World malaria report, World Health Organization, **2013**.
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Importance of the deglutamylase CCP5B in *Leishmania major* apoptosis

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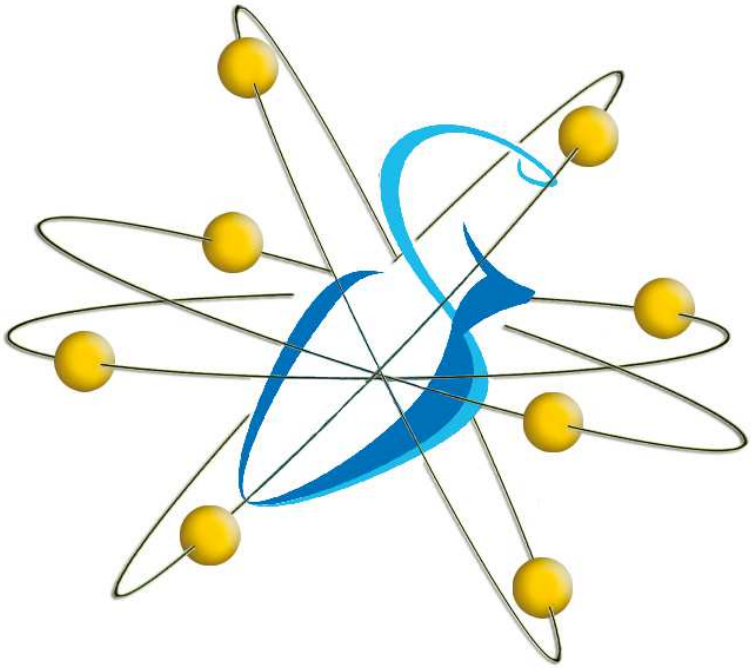
Leishmania has a cytoskeleton primarily made of stable microtubules, the latter constituting the most abundant part of the cytoskeleton. They constitute the mitotic spindle, the flagellar axoneme, the basal body and most importantly the subpellicular corset, made of a dense network of microtubules cross-linked to each other and to the plasma membrane, forming a helical pattern along the long axis of the cell. Microtubules in these parasites are subject to a series of post-translational modifications including polyglutamylation and deglutamylation that respectively adds or removes glutamate amino acids on glutamate residues within the primary sequence of the target protein. Post translational modifications along microtubules are likely to encode novel information for the cell, both linked to the nature, length and spacing patterns of these modifications, defining what has been called 'the tubulin code'. We show here that the deglutamylase CCP5B is implicated in *L. major* apoptosis, its overexpression inducing cell survival after miltefosine addition. Furthermore, we show that *L. major* presents microtubule desorganisation during apoptosis, inducing the formation of a tubulin ring when observed with fluorescence microscope. One hypothesis developed in mammal cells is that this microtubule ring would prevent cells from entering necrosis.



URMITE UM 63 CNRS 7278 IRD 198 INSERM U1905

Aix-Marseille Université

Implementation of computer tools for the real-time epidemiological surveillance of abnormal events based on clinical microbiology laboratory data.

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Infectious diseases represent major unpredictable causes of mortality worldwide. Efficient surveillance systems are warranted. We aim to implement surveillance systems for the surveillance of pathogens isolated from patients admitted in Marseille University hospitals using data routinely produced by the microbiology laboratory of the Timone hospital. The objectives were to monitor the incidence of all the bacterial species isolated at least once in our laboratory, and the incidence of antibiotic-resistance profiles of 15 bacterial species of interest.

We first implemented two historical Microsoft Excel databases. The first database contained all the available data referring to patients from which bacterial species were isolated from January 2002 through December 2013 at the Timone laboratory. The second database included all the available antibiotic-susceptibility testing results performed at our laboratory between October 2012 and March 2013. The databases were then cleaned.

The first historical database (500,174 lines, finally 206,623 lines (128,590 patients)) allowed us to implement BALYSES (BActerial real-time LaboratorY-based SurveillancE System) for the monitoring of the incidence of the 671 bacterial species isolated at least once at the Timone laboratory since 2002. The system automatically compare the weekly number of patients infected each bacterial species to the weekly historical mean number of infected patient plus two standard deviations. The second historical database (initially 12,062 lines) allowed to implement MARSS (Marseille Antibiotic Resistance Surveillance System) for the monitoring of the weekly incidence of the main β-lactam susceptibility phenotypes of 15 bacterial species of interest. From May 2013 to May 2015, our surveillance systems emitted 111 validated alarms. 55 were true epidemiological events, 33 were declared to the Agence Régionale de la Santé, and 7 led to publications.

We are currently implementing a web-based platform to merge all our surveillance systems for more accurate monitoring and investigation of the emitted alarms.

Genomic analyses and molecular testing of giant marseilleviruses

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Marseillevirus, a giant virus associated with amoebae, is the founding member of the newly described family *Marseilleviridae*. This family encompasses three phylogenetic lineages which comprise viruses isolated from the environment and humans samples collected on three different continents. The presence of marseilleviruses in humans was first suggested by the isolation of Senegalvirus in human stools. Then, a marseillevirus was discovered by a metagenomic study in the blood from blood donors, and marseillevirus DNA was detected in the blood from asymptomatic persons, and the lymph node from a patient with adenitis. These data raise the issue of the potential pathogenicity of marseilleviruses in humans. We aimed at updating the core- and pan-genomes of the family *Marseilleviridae* to improve current knowledge on their genetic and proteic diversity and to implement real time PCR systems to detect marseilleviruses in environmental and human samples.

The annotation of the genomes from three new marseillevirus isolated from soil and sewage samples collected in Marseille and Paris allowed describing three new proteins, never yet described in representatives from this family. The size of the marseillevirus pangenome increased to 626 proteins. Beyond, the present work highlighted the mosaicism of marseillevirus genomes by detecting lateral gene transfers between these giant viruses and other viruses, bacteria, and eukaryotes, including the amoebal host. Our analyses further revealed a great genetic diversity of these viruses, which hampered the implementation of PCR systems targeting all the viral lineages. We could develop and optimize systems targeting the lineage A, which comprises marseilleviruses previously associated with humans.

Whole genome sequence to decipher the Resistome and virulence genes of clinical multidrug-resistant bacterium

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We characterized and deciphered the resistome and the virulence factors of *Shewanella algae* MARS14, a multidrug-resistant clinical strain using the whole genome sequencing (WGS) strategy. The bacteria was isolated from the bronchoalveolar lavage of a hospitalized patient in the Timone Hospital in Marseille, France who developed pneumonia after plunging into the Mediterranean Sea. The genome size of *S. algae* MARS 14 was 5,005,710 bp with 52.8% GC content. The resistome includes members of class C and D beta-lactamases, a macrolide resistance gene along with numerous multidrug-efflux pumps. We also found the presence of several hemolysins genes, a complete flagellum system gene cluster and genes responsible for biofilm formation. Moreover, we reported for the first time in a clinical strain of *Shewanella spp*. the presence of a bacteriocin (marinocin). The WGS analysis of this pathogen provides insight into its virulence factors and resistance to antibiotics.

Comprendre le support de la résistance intrinsèque à la colistine chez *Proteus vulgaris.*

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Depuis plusieurs années, face à l'augmentation de la résistance chez les bactéries à Gram négatif, la colistine a été de nouveau utilisée comme antibiotique de dernière ligne dans les infections sévères à germes multi-résistants. Malheureusement, le regain d'intérêt pour ce peptide antimicrobien a entrainé l'émergence de résistances acquises notamment chez les entérobactéries, mais aussi l'augmentation des infections à germes intrinsèquement résistants à la colistine. Les mécanismes de résistance à la colistine et aux polymyxines en général sont complexes et mal connus, surtout chez les bactéries naturellement résistantes. Alors que les toutes les bactéries du genre *Proteus* sont naturellement résistantes à la colistine, nous avons été surpris d'isoler récemment une souche de *Proteus vulgaris* anormalement sensible à la colistine à partir d'un prélèvement humain, et nous proposons dans ce travail d'étudier et de de comprendre le support moléculaire de la résistance à la colistine chez cette espèce par une approche génomique comparative et des tests in vitro. Après séquençage de la souche sensible CSUR P1868_S et d'une souche résistante CSUR P1867_R nous rechercherons les gènes absents, tronqués ou mutés dans la souche sensible L'utilisation de la microscopie électronique et de l'analyse du lipide A par spectrométrie de masse nous aidera à sélectionner les gènes possiblement impliqués dans la résistance. Le génome de la souche P1868_S avait une taille de 4,28 Mb et un GC% de 37,8% contre 3,9 Mb et 38,1% pour la souche P1867_R. Il contenait également de nombreux gènes de résistance notamment aux aminosides, aux β-lactamines, aux fluoroquinolones et au cotrimoxazole, porté par plusieurs plasmides. La comparaison des deux génomes a permis d'identifier 20 gènes absents dans la souche sensible dont un opéron codant pour le métabolisme de l'acide sialique et 2 gènes impliqués dans la synthèse de l'antigène-O. Nous avons mis en évidence l'absence de sucres au niveau de la membrane externe et notamment l'absence de fixation d'amino-4-arabinose au niveau du lipopolysaccharide. Deux gènes de l'opéron *arn* responsable de la fixation de ce sucre étaient mutés dans la souche sensible. L'étude de l'expression de ces gènes par QPCR permettra de montrer que ces gènes ne sont plus fonctionnels, expliquant ainsi la sensibilité accrue de cette souche à la colistine. En conclusion, la fixation de sucres sur le lipide A est une voie clé dans la résistance aux polymyxines mais il n'est pas le seul et est sous la dépendance de nombreux gènes de régulation. La bactérie a développé au cours du temps des systèmes de défense contre les peptides antimicrobiens et les mécanismes induisant cette résistance restent complexes.