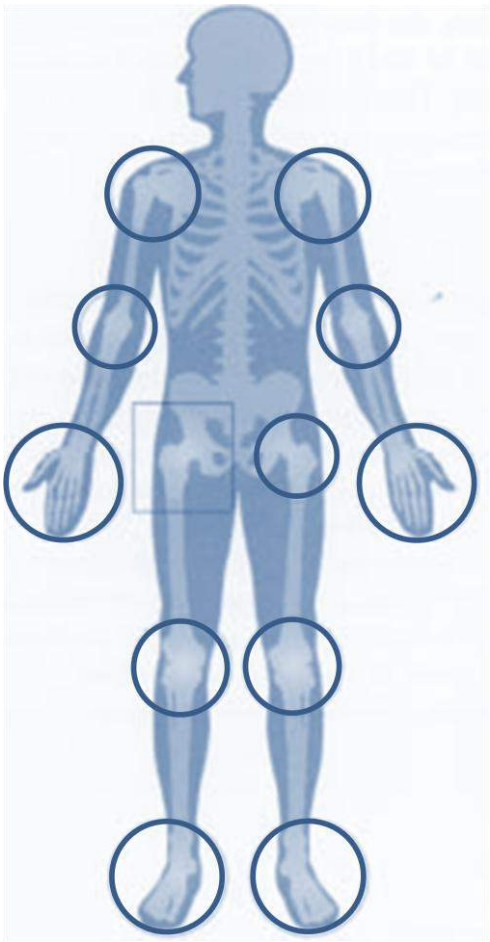
JAHRESBERICHT
ANNUAL-/SCIENTIFIC REPORT
2014/2015
Institute for Arthritis Research (iAR)

A collaborative project by four leading Research Institutions in Switzerland since 2009.
Turning Research into clinical application for Arthritis care

Ein vernetztes Projekt von vier führenden Schweizer Forschungsteams seit 2009.
Von der Forschung zur klinischen Behandlung rheumatischer Erkrankungen



EDITORIAL & VISION FOR THE FUTURE

Foreword by Prof. Cem Gabay
Scientific Coordinator of the Institute for Arthritis Research

Among the different forms of arthritis, inflammatory diseases such as rheumatoid arthritis are considered as the most severe conditions leading to disability, unemployment, and reduced life expectancy. Despite the development of novel therapies that have markedly changed their management and outcome, many patients do not respond to these treatments and none of these therapies is able to cure the diseases. Hence there are unmet needs that support the importance of basic and translational research in the field of arthritis.

The Institute of Arthritis Research (iAR) is a unique collaboration between several University centers in Switzerland, including Geneva, Lausanne, Zurich and Bellinzona with the common aim to understand basic cellular and molecular mechanisms associated with the development of arthritis and tissue damage.

The present report describes the research activities performed in the different iAR centers and their accomplishments regarding publications in peer-reviewed Journals. More specifically, the center in Bellinzona has a long-standing research activity in the field of adaptive immune responses with particular emphasis on the regulation of anti-pathogen and autoantibody production as well as in the mechanisms leading to T helper lymphocyte polarization. The center in Zürich has an expertise in the role of synovial fibroblasts in arthritis and in epigenetic modifications associated with inflammatory responses. The research in Lausanne is focused on innate immune responses, in particular toll-like receptor signaling during Leishmania infection as a model of tissue destruction that is reminiscent to the mechanisms associated with structural damage in arthritis. One of the research groups in Geneva is working on the role of interleukin-1-related cytokines in various models of arthritis and inflammatory diseases. The other group in Geneva is working on the role of autophagy in antigen presentation and inflammation in models of arthritis. In addition, the center in Geneva is also actively collaborating with industrial partners for the clinical development of anti-inflammatory drugs.

Recently, iAR has initiated an active collaboration with the Alpine Institute for Drug Discovery (AiDD) for the development of new compounds with anti-inflammatory activities. This choice is guided by the importance to link basic science discoveries with applied research with an ultimate aim to help patients with rheumatic diseases.

We would like to thank all the Foundations for their financial support to iAR.

Prof. Cem Gabay
Geneva, March 2016

STRATEGIC COLLABOARATION

The Alpine Institute for Drug Discovery (www.aidd.ch) was founded in 2013 by seasoned industry scientists. AiDD is an enterprise aiming at creating shared value for the community by maintaining industrial know-how in today's changing pharmaceutical landscape and is currently the unique Institute for Drug Discovery in the Alpine Region. AiDD initiates projects via public-private partnerships and makes its experts and technology suite available to assist academic partners with target identification, characterisation and validation efforts. AiDD also manages drug discovery projects through hit-to-lead and lead optima¬sation phases. Through collaborations bridging academia and industry, AiDD aims to be a partner of choice for academics pursuing translational therapeutic discovery and a stable, trusted provider of high-quality pre-medicines for pharmaceutical partners and venture investors.

In November 2015, AiDD and iAR signed an agreement according to a risk- and benefit-sharing principle and centered on an innovative approach to identify drug candidates with a differentiated mechanism of action based on allosteric modulation. The consortium is led by Professor Cem Gabay, Head of the Division of Rheumatology, Department of Internal Medicine Specialties, University of Geneva.

iAR and AiDD are working closely together on a G protein-coupled receptor abundantly ex¬pres-sed on immune cells. Engaging this receptor affects a range of cellular functions across different immune cell types thereby exerting a negative retro-control over inflammation and minimising the extent of collateral damages to host tissues during the course of inflammatory reactions.

Using an ultra sensitive cAMP biosensor, we have developed an HTS technology specifically designed to detect and optimise allosteric modulator drugs. This assay is used for an HTS campaign to identify novel chemical series with drug-like properties and with functional activity at the human target that will be further developed in the lead generation phase.

Ultimately, this program will lead to a novel broadly applicable anti-inflammatory, leveraging competencies, strengths and capabilities of each partner, thereby co-creating added value on early assets which can then be out-licensed to an industrial partner or serve as a basis for Start-up creation.

EDITORIAL & ZUKUNFTSVISION

Vorwort von Prof. Cem Gabay
Wissenschaftlicher Koordinator des Instituts für Arthritis Forschung

Unter den verschiedenen Varianten von Arthritis betrachtet man entzündliche Krankheiten wie rheumatische Arthritis als schlimmste Formen. Sie können zu Behinderung, Arbeitslosigkeit und verkürzter Lebenserwartung führen. Trotz der Entwicklung neuartiger Therapien, welche Handhabung und Wirkung markant verbessern, sprechen viele Patienten auf diese Behandlungen nicht an und keine dieser Therapien kann die Krankheit heilen. Durch diese unbefriedigende Situation wird die Dringlichkeit der Grundlagenforschung und der klinischen Forschung deutlich.

Das Institut für Arthritis Forschung (iAR) basiert auf einer einzigartigen Zusammenarbeit zwischen verschiedenen Universitätszentren der Schweiz, insbesondere in Genf, Lausanne, Zürich und Bellinzona. Es besteht das gemeinsame Ziel die grundlegenden zellulären- und molekularen Mechanismen welche zu Arthritis und Gewebeschädigungen führen zu verstehen. Der vorliegende Bericht skizziert die Forschungs-aktivitäten der verschiedenen iAR Zentren und die daraus resultierenden, von Experten begutachteten, Publikationen.

Das Forschungszentrum in Bellinzona bearbeitet seit Jahren diverse Forschungsprojekte im Bereich der adaptiven Immunreaktion mit spezieller Gewichtung der Regulation von Anti-Krankheitserreger und der Autoantikörper-Produktion sowie der Mechanismen welche zu T-Helper Lymphozyten-Polarisation führen. Das Zentrum in Zürich verfügt über extensive Kompetenz der Rolle synovialer Fibroblasten in Arthritis und in epigenetischen Modifikationen in Zusammenhang mit entzündlichen Reaktionen. Die Forschung in Lausanne ist fokussiert auf angeborene Immunreaktionen insbesondere des „toll-like“ receptor signaling bei Leishmania Infektionen als Modell einer Gewebezerstörung, welche an die Mechanismen im Zusammenhang mit strukturellen Schäden bei Arthritis erinnern. Eine der Forschungs-gruppen in Genf studiert die Rolle von Interleukin-1-verwandten Zytokinen in ver-schiedenen Modellen von Arthritis und Entzündungskrankheiten. Ein zweites Genfer Team erforscht die Funktion von autophagy in antigen presentation and inflammation models of arthritis. Zusätzlich engagiert sich das Zentrum in Genf, gemeinsam mit industriellen Partnern, für die klinische Entwicklung von Medikamenten gegen Entzündungskrankheiten.

Kürzlich hat das iAR für die Entwicklung neuer Präparate mit entzündungshemmender Wirkung eine Zusammenarbeit mit dem Alpine Institut for Drug Discovery (AiDD) gestartet. Dies unterstreicht den Stellenwert einer enger Zusammenarbeit von Grundlagenforschern mit Projekten der angewandter Forschung, was letztendlich den Patienten mit rheumatischen Erkrankungen helfen wird.

Wir bedanken uns bei allen Stiftungen für deren finanzielle Unterstützung des iAR.

Prof. Cem Gabay
Genf, März 2016

STRATEGISCHE ZUSAMMENARBEIT

Das Alpine Institute for Drug Discovery (www.aidd.ch) wurde 2013 durch ausgewiesene Wissenschafter mit langjähriger Industrie-erfahrung gegründet. AiDD, zur Zeit das einzige Institut für Drug Discovery in der Alpenregion, ist ein Unternehmen mit dem Ziel durch Erhalt und Ausbau des industriellen Know-hows in einer sich stetig verändernden pharma-zeutischen Welt gemeinsame Werte für die Allgemeinheit zu schaffen. AiDD initiiert Projekte via öffentlich-private Partnerschaften und stellt seine Experten und Technologie-Palette zur Verfügung, um akademische Partner bei Target Identifikation, Charakterisierung und Validierung zu unterstützen.

AiDD betreut auch Drug Discovery Projekte durch Hit-to-Lead- und Lead Optimisierungs-phasen. Durch gezielte Kooperationen von Hochschulen und Industrie positioniert sich AiDD als Partner erster Wahl für Akademiker welche translatorisch-therapeutische Forschung betreiben. AiDD verfolgt das Ziel, für pharma-zeutische Partner und Risikokapitalinvestoren ein stabiler, vertrauenswürdiger Lieferant von qualitativ hochstehenden Arzneimittel-Vor-läufern zu sein.

Im November 2015 unterschrieben AiDD und iAR einen Risiko- und Gewinnteilungsvertrag auf Grundlage eines innovativen Vorgehens zur Identifikation von Arzneimittel-Kandidaten durch einen differenzierten, auf allosterischer Modulation basierenden, Aktions-Mechanismus. Das Konsortium wird geleitet durch Professor Cem Gabay, Chef der Division of Rheumatology, Department of Internal Medicine Specialties, University of Geneva.

iAR und AiDD arbeiten eng zusammen an einem G protein-coupled receptor welcher in reichlichem Masse auf Immunzellen exprimiert wird. Das „Einschalten“ dieses Rezeptors beeinflusst eine Vielzahl von zellulären Funktionen verschiedener Immunzelltypen. Dies führt zu einer negativen Retro-Kontrolle von Entzündung und minimiert das Ausmass von Kollateralschäden am Wirtsgewebe während Entzündungsreaktionen. Durch die Anwendung eines ultra-sensitiven cAMP Biosensors hat AiDD eine HTS Technologie entwickelt, welche spezifisch dafür ausgelegt ist, allosterische Modulator Arzneimittel nachzuweisen und zu optimieren. Dieses Testverfahren wird angewendet in einer HTS Kampagne zur Identifikation neuer chemischer Verbindungen mit arzneimittel-ähnlichen Eigenschaften und mit funktioneller Aktivität beim humanen Target welche in der Lead-Generation Phase weiter entwickelt wird.

Letztendlich wird dieses Programm durch Nutzung der Kompetenzen, Stärken und Fähigkeiten jedes Partners zu einem neuartigen, breit anwendbaren Entzündungshemmer führen. So wird gemeinsam ein Mehrwert auf Anlagewerten in einem frühen Stadium kreiert. Diese können an interessierte industrielle Partner lizenziert werden oder als Basis für die Gründung eines Start-ups dienen.

FINANCIAL INFORMATION / FINANZ INFORMATION

(October 2014 – September 2015)

	2015	2014	
Cash at banks	1'357'016	2'097'323	Bankguthaben
Research Projects	1'000'000	1'930'000	Forschungsprojekte
Donations	375'000	2'350'000	Spenden
Acquisition screening apparatus	204'427	0	Anschaffung Screening Gerät
Administrative expenses / Fundraising	35'879	76'454	Verwaltungsaufwand / Fundraising

THANKS TO DONORS / DANK AN DIE SPENDER

The purchase of the Hamamatsu FDSS/µcell screening device (fluorescent and luminescent p recision highthroughput screening) was possible thanks to the support of Loterie Romande. The research projects were mainly supported by the Uniscientia-Stiftung, Schwyzer-Stiftung, Göhner-Stiftung and the Mäxi-Stiftung.

Thank you for your generous donations. We will continue to inform you about the further progress of the project.

Die Anschaffung eines Hamamatsu FDSS/µcell Screening Gerätes (precision highthroughput fluorescent and luminescent screening) wurde mit Unterstützung durch die Loterie Romande möglich. Die Forschungsprojekte wurden u.a. durch die Uniscientia-Stiftung, die Schwyzer-Stiftung, Göhner-Stiftung und die Mäxi-Stiftung unterstützt.

Herzlichen Dank für Ihre grosszügigen Spenden. Wir werden Sie weiterhin über den Verlauf des Projektes informieren.

Prof. Cem Gabay / Head, Division of Rheumatology University Hospitals of Geneva (Head of iAR)

Prof. Antonio Lanzavecchia / Institute for Research in Biomedicine in Bellinzona

Prof. Steffen Gay / University Hospital Zurich Head of Center of Experimental Rheumatology

Prof. Nicolas Fasel / University of Lausanne Director Department Biochemistry

CONTACTS

SCIENTIFIC COORDINATION
Prof. Cem Gabay
Hopitaux Universitaire de Genève
Head, Division of Rheumatology
Avenue Beau-Séjour 26
1211 Geneva
+41 22 372 35 00
cem.gabay@hcuge.ch

ADMINISTRATIVE COORDINATION
Martin Kuendig
c/o Lienhard AG
Bleicherweg 45
8027 Zurich
+41 79 211 64 82
martin.kuendig@iar-suisse.ch

SCIENTIFIC REPORT

University of Zurich

Also this year research in the Center of Experimental Rheumatology in Zurich focused on the elucidation of the activated phenotype of synovial fibroblast in RA patients. A major breakthrough was the characterization of the transcriptome including coding and small and long non-coding RNA of synovial fibroblasts isolated from different joints and diseases. This comprehensive analysis allowed us not only to describe differences in synovial fibroblasts from different joints, but also to see differences in mRNA, microRNA and long- non coding RNA expression between healthy, osteoarthritis and rheumatoid arthritis synovial fibroblasts. In the course of this experiment we could also for the first time show the expression of piwiRNA by synovial fibroblasts. These small non-coding RNAs have only recently been shown to be important in the silencing of retrotransposons in the human genome. Their function in synovial fibroblasts and their role in disease development are currently under investigation. Similarly, we analyze the function of the microRNA miR-204 in synovial fibroblasts, since we could show that this microRNA is downregulated in RA synovial fibroblasts at very early stages of disease and therefore might be a key molecule in promoting chronic activation of these cells.

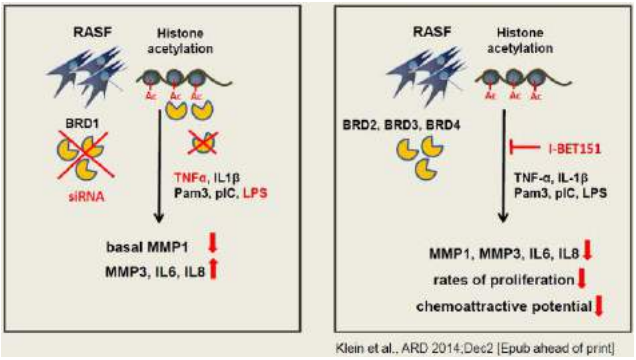
Newly this year we started to analyze the function of the TET proteins in synovial fibroblast and macrophage differentiation. These recently discovered enzymes promote demethylation of DNA and thereby might play an important role in the loss of DNA methylation marks that is found in immune and stromal cells from RA patients.

Our data show that changes in TET expression during macrophage differentiation regulate the production of the pro-inflammatory cytokine TNF, which makes them an attractive target for therapeutic interventions.

In macrophages and synovial fibroblasts, we also analyzed the effect of repeated LPS stimulation on histone marks. We found that specific positioning of certain histone marks either maintains or diminishes the production of cytokines and could thereby explain why synovial fibroblasts in contrast to macrophages are still producing most cytokines also after repeated LPS stimulations. We plan to follow up these experiments in collaboration with the groups in Lausanne and Geneva, which ad valuable expertise in TLR signaling and macrophage activation.

The Center of Experimental Rheumatology /Rheumatology Clinic University of Zürich was awarded for the 3rd time from the European League against Rheumatism (EULAR) be a Center of Excellence 2015-2020.

Steffen Gay was awarded a Master of the American College of Rheumatology in 2015.



BRD1 is a specific regulator of TNFα and LPS- induced pathways in RA synovial fibroblasts.

Auch dieses Jahr lag der Schwerpunkt der Forschung im Zentrum für Experimentelle Rheumatologie in Zürich in der Analyse des aktivierten Phänotyps von synovialen Fibroblasten in RA Patienten. Ein Durchbruch ist uns mit der Charakterisierung des Transkriptoms einschliesslich kodierender und kurzer und

langer nicht-kodierender RNA Moleküle in synovialen Fibroblasten aus unterschiedlichen Gelenken und Erkrankungen gelungen.

Diese umfassende Analyse erlaubte es uns nicht nur Unterschiede in synovialen Fibroblasten aus verschiedenen Gelenken zu beschreiben, sondern wir konnten auch Unterschiede in der Expression von mRNA, microRNA und langer nicht-kodierender RNA zwischen Gesunden und Patienten mit Osteoarthritis und rheumatoider Arthritis sehen.

Im Zuge dieses Experimentes konnten wir erstmalig die Expression von piwiRNA in synovialen Fibroblasten detektieren. Für diese kurzen nicht-kodierenden RNAs wurde erst kürzlich eine wichtige Rolle bei der Kontrolle von Retrotransposons im menschlichen Genom beschrieben. Ihre Funktion in synovialen Fibroblasten sowie ihre Rolle in der Pathogenese der RA untersuchen wir zur Zeit. Gleichermassen untersuchen wir die Funktion der microRNA miR-204 in synovialen Fibroblasten, da wir zeigen konnten, dass diese microRNA in RA synovialen Fibroblasten in einem sehr frühen Stadium der Erkrankung erniedrigt ist und daher ein Schlüsselmolekül in the chronischen Aktivierung dieser Zellen sein könnte.

Neu haben wir dieses Jahr mit der Analyse the Funktion der TET Proteine in synovialen Fibroblasten und Makrophagen Differenzierung begonnen. Diese kürzlich entdeckten Enzyme regulieren DNA Demethylierung und könnten daher eine wichtige Rolle beim Verlust von DNA Markierungen spielen, die in Immun- und Stromazellen von RA Patienten beschrieben wurde. Unsere Daten zeigen, dass Veränderungen der Expression von TET Proteinen während der Differenzierung von Makrophagen die Produktion des pro-inflammatorischen Zytokines TNF regulieren, was diese Proteine zu einem attraktiven Ziel für therapeutische Interventionen macht.

Ebenfalls in Makrophagen und synovialen Fibroblasten haben wir den Effekt wiederholter LPS Stimulationen untersucht. Wir konnten zeigen, dass spezifische Positionierung von bestimmten Histon Markierungen die Produktion von Zytokinen entweder erhält oder abschaltet, und konnten somit erklären warum synoviale Fibroblasten im Gegensatz zu Makrophagen auch noch nach wiederholter LPS Stimulierung die meisten ihrer Zytokine produzieren. Es ist geplant, diese Experimente in Kollaboration mit den Gruppen in Lausanne und Genf, die ihre Expertise in TLR Signalwegen und Makrophagen Aktivierung einbringen, weiter zu verfolgen.

Das Zentrum für Experimentelle Rheumatologie und die Rheumaklinik an der Universität Zürich wurde von der European League against Rheumatism (EULAR) zum 3. Mal zum Center of Excellence für 2015-2020 ausgezeichnet.

Steffen Gay wurde Master des American College of Rheumatology in 2015.

Publications

Kato M, Ospelt C, Gay RE, Gay S, Klein K. Dual role of autophagy in stress-induced cell death in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 66:40-48, 2014

Shah N, Hülsmeier AJ, Hochhold N, Neidhart M, Gay S, Hennet T. Exposure to mimivirus collagen promotes arthritis. J Virol 88:838-45, 2014

Ospelt C, Camici GG, Engler A, Kolling C, Vogetseder A, Gay RE, Michel BA, Gay S. Smoking induces transcription of the heat shock protein system in the joints. Ann Rheum Dis 73:1423-1426, 2014

Neidhart M, Karouzakis E, Jüngel A, Gay RE, Gay S. Inhibition of spermidine/spermine N1-acetyltransferase (SSAT1) activity - a new therapeutical concept in rheumatoid arthritis. Arthritis Rheumatol 66:1723-1733, 2014

Engler A, Niederer F, Klein K, Gay RE, Kyburz D, Camici GG, Gay S, Ospelt C. SIRT6 regulates the cigarette smoke-induced signalling in rheumatoid arthritis synovial fibroblasts. J Mol Med (Berl):92:757-767, 2014

Cerezo LA, Remáková M, Tomčik M, Gay S, Neidhart M, Lukanidin E, Pavelka K, Grigorian M, Vencovsky J, Senolt L. The metastasis-associated protein S100A4 promotes the inflammatory response of mononuclear cells via the TLR4 signalling pathway in rheumatoid arthritis. Rheumatology (Oxford) 53:1520-1526, 2014

Stähli BE, Yonekawa K, Altwegg LA, Wyss C, Hof D, Fischbacher P, Brauchlin A, Schulthess G, Krayenbühl PA, von Eckardstein A, Hersberger M, Neidhart M, Gay S, Novopashenny I, Wolters R, Frank M, Wischnewsky MB, Lüscher TF, Maier W. Clinical criteria replenish high-sensitive troponin and inflammatory markers in the stratification of patients with suspected acute coronary syndrome. PLoS One. 2014 Jun 3;9(6):e98626. doi: 10.1371/journal.pone.0098626. eCollection 2014.

Klawitter M, Hakozaki M, Kobayashi H, Krupkova O, Quero L, Ospelt C, Gay S, Hausmann O, Liebscher T, Meier U, Sekiguchi M, Konno SI, Boos N, Ferguson SJ, Wuertz K. Expression and regulation of toll-like receptors (TLRs) in human intervertebral disc cells. Eur Spine J.:23:1878-1891, 2014

Karouzakis E, Trenkmann M, Gay RE, Michel BA, Gay S, Neidhart. Epigenome analysis reveals TBX5 as a novel transcription factor involved in the activation of rheumatoid arthritis synovial fibroblasts. J Immunol 193:4945-4951, 2014

Kurinna S, Schäfer M, Ostano P, Karouzakis E, Chiorino G, Bloch W, Bachmann A, Gay S, Garrod D, Lefort K, Dotto GP, Beer HD, Werner S. A novel Nrf2-miR-29-desmocollin-2 axis regulates desmosome function in keratinocytes. Nat Commun 5:5099, 2014

Iwamoto N, Vettori S, Maurer B, Brock M, Pachera E, Jüngel A, Calcagni M, Gay RE, Whitfield ML, Distler JH, Gay S, Distler O. Downregulation of miR-193b in systemic sclerosis regulates the proliferative vasculopathy by urokinase-type plasminogen activator expression. Ann Rheum Dis. 2014 [Epub ahead of print]

Angiolilli C, Grabiec AM, Ferguson BS, Ospelt C, Malvar Fernandez B, van Es IE, van Baarsen LG, Gay S, McKinsey TA, Tak PP, Baeten DL, Reedquist KA. Inflammatory cytokines epigenetically regulate rheumatoid arthritis fibroblast-like synoviocyte activation by suppressing HDAC5 expression. Ann Rheum Dis. 2014 [Epub ahead of print]

Klein K, Kabala PA, Grabiec AM, Gay RE, Kolling C, Lin LL, Gay S, Tak PP, Prinjha RK, Ospelt C, Reedquist KA. The bromodomain protein inhibitor I-BET151 suppresses expression of inflammatory genes and matrix degrading enzymes in rheumatoid arthritis synovial fibroblasts. Ann Rheum Dis. 2014 [Epub ahead of print]

Whitaker JW, Boyle DL, Bartok B, Ball ST, Gay S, Wang W, Firestein GS. Integrative omics analysis of rheumatoid arthritis identifies non-obvious therapeutic targets. PLoS One. 2015 22;10(4):e0124254.

Aradi B, Kato M, Filkova M, Karouzakis E, Klein K, Scharl M, Kolling C, Michel BA, Gay RE, Buzas EI, Gay S, Jüngel A. Protein tyrosine phosphatase nonreceptor type 2

(PTPN2), an important regulator of IL-6 production in rheumatoid arthritis synovial fibroblasts. Arthritis Rheumatol. 2015 [Epub ahead ofprint]

Engler A, Tange C, Frank-Bertoncelj M, Gay RE, Gay S, Ospelt C. Regulation and function of SIRT1 in rheumatoid arthritis synovial fibroblasts. J Mol Med (Berl), 2015 [Epub ahead of print]

Ospelt C, Gay S. [Epigenetics in inflammatory systemic diseases].Internist (Berl). 55:124-7, 2014

Buzas EI, György B, Nagy G, Falus A, Gay S. Emerging role of extracellular vesicles in inflammatory diseases. Nat Rev Rheumatol. 2014 [Epub ahead of print]

Ramming A, Distler JH, Schett G, Gay S, Jüngel A. [Epigenetic modifications in autoimmune diseases.] Z Rheumatol 73:636-638, 2014

Frank-Bertoncelj M, Gay S. The epigenome of synovial fibroblasts: an underestimated therapeutic target in rheumatoid arthritis. Arthritis Res Ther 16:117, 2014

Klein K, Gay S. Epigenetics in rheumatoid arthritis. Curr Opin Rheumatol. 2015 Jan;27(1):76-82

Institute for Research in Biomedicine

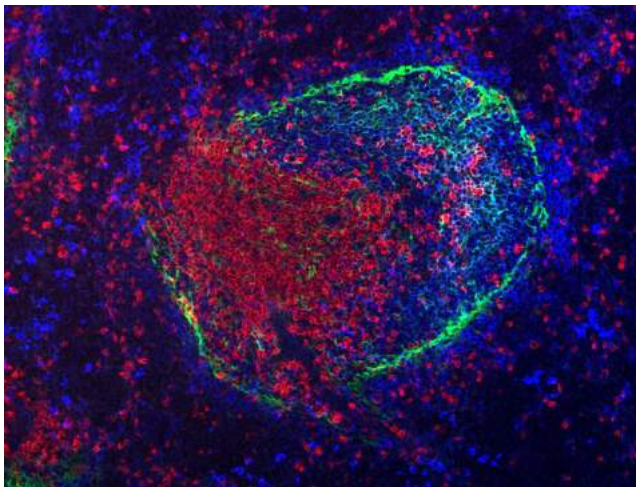
The role of T follicular helper and Th17 cells in physiology and pathology.

T follicular helper (Tfh) and Th17 cells are specialized subsets of CD4+ T cells that play an essential role in protective immunity, but are also implicated in immunopathology. We found that in lymphopenic conditions, antigenic stimulation induces the generation of high numbers of Tfh cells that are dysfunctional, since they fail to provide help to specific B cells and instead induce bystander B cell activation and production of autoantibodies (Baumjohann et al, 2013). Reconstitution with regulatory T cells restored Tfh-cell numbers and production of high affinity antibodies (Preite et al, 2015). These findings underline the importance of a quantitatively regulated Tfh-cell response for an efficient antibody response avoiding generation of autoantibodies. We also described two types of Th17 cells that differ in the requirements for differentiation and in effector function, in particular the ability to secrete IFN-γ or IL-10 (Zielinski et al, 2012).

Using the EAE model, we demonstrated that priming of pathogenic Th17 cells is dependent on the presence of pertussis toxin (PTX) at the time of immunization.

PTX induces early production of IL-1β by CD11b+ CCR2+ Gr1+ myeloid cells that are rapidly recruited to the antigen-draining lymph nodes. These data suggest that inflammatory monocytes and microbial infection can influence differentiation of pathogenic Th1/Th17 cells in autoimmune diseases through production of IL-1β.

Baumjohann D, et al. Immunity 2013, 38:596-605.
Preite S, et al. Eur J Immunol 2015, 45: 3010-3021.
Zielinski CE, et al. Nature 2012, 484:514-8.
Ronchi F, et al. Submitted.



Immunofluorescence staining of a mouse spleen section after immunization with ovalbumin. The white pulp comprises CD3ε+ T cells (red) and IgM+ follicular B cells (blue). This is surrounded by a ring of MAdCAM-1+ sinus lining cells (green) followed by an outer layer of IgM+ marginal zone B cells (blue).

Il ruolo dei linfociti T follicolari "helper" e Th17 in fisiologia e patologia

I linfociti follicolari T "helper" (Tfh) e Th17 sono due popolazioni di linfociti T CD4+ che svolgono un ruolo essenziale nell'immunità protettiva ma che sono anche implicati in immunopatologia. Abbiamo scoperto che in condizioni linfocitopeniche, la stimolazione antigenica induce l'espansione di un numero elevato di linfociti Tfh che sono disfunzionali, in quanto non danno "help" alle cellule B specifiche e, invece, attivano cellule B non-specifiche e la produzione di autoanticorpi (Baumjohann et al, 2013). In presenza di linfociti T regolatori il numero di linfociti Tfh è normalizzato così come la produzione di anticorpi specifici ad alta affinità (Preite et al, 2015). Questi risultati sottolineano l'importanza di una risposta dei linfociti Tfh quantitativamente regolata per l'induzione di una risposta anticorpale efficace, evitando la generazione di autoanticorpi. Abbiamo anche descritto due tipi di linfociti Th17 che differiscono nei requisiti per la differenziazione e la funzione effettrice, in particolare la capacità di secernere IFN-γ o IL-10

(Zielinski et al, 2012).

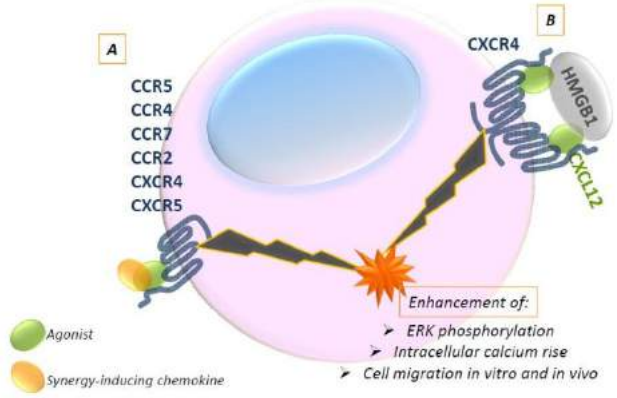
Utilizzando un modello sperimentale di sclerosi multipla, abbiamo dimostrato che il "priming" di linfociti Th17 patogenici è dipendente dalla presenza della tossina della pertosse (PTX) al momento dell'immunizzazione. La PTX induce la produzione di IL-1β da cellule mieloidi che sono rapidamente reclutate nei linfonodi drenanti l'antigene. Questi dati suggeriscono che nelle malattie autoimmuni cellule mieloidi infiammatorie e infezioni microbiche possono influenzare la differenziazione dei linfociti Th1 / Th17 patogenici attraverso l'induzione di IL-1β.

Chemokine Synergy-inducing molecules in Rheumatoid Arthritis

Chemokine structure/function studies led us to identify that chemokines can act in synergism with chemokine receptor agonists, forming heterocomplexes able to induce functional responses at lower agonist concentration. HMGB1, a nuclear protein released by necrotic and severely stressed cells, promotes cytokine release via its interaction with the TLR4 receptor and, as we recently described, cell migration via CXCR4, by forming a complex with CXCL12.

We are now studying, in collaboration with the group of Costantino Pitzalis at the William Harvey Institute (London, UK) and of Antonio Manzo at the University of Pavia (Italy) the molecules which cooperate in cell recruitment and activation at inflammatory sites which are crucial in Rheumatoid Arthritis and might be modulated by the anti-cytokine therapy. This study may shed new light on the mechanisms which significantly "push back" inflammation and that can be additional targets for novel anti-inflammatory strategies.

Schiraldi M. et al. J Exp Med 2012; 209:551-563.
Venereau E. et al. J Exp Med 2012; 209:1519-1528.
Venereau E. et al. Mol. Immunol. 2013, 55:76– 82.
Cecchinato V. et al. Journal of Leukocyte Biology. Epub ahead of print December 29, 2015.
Thelen M. and Uguccioni M. Encyclopaedia of Immunology 2015. In press.



Synergism induced by the formation of heterocomplexes.
A - Heterocomplex formation between two chemokines renders the agonist more potent on the selective receptor.
B – HMBG1 forms a heterocomplex with CXCL12, enhancing CXCL12 potency on the CXCR4.

Le molecole che sinergizzano con le chemochine nell'artrite reumatoide

Gli studi di struttura e funzione delle chemochine ci hanno portato a identificare quali di queste molecole possono agire in sinergia, formando complessi in grado di indurre delle risposte funzionali a una concentrazione di agonista che per se non avrebbe alcun effetto.

HMGB1, una proteina nucleare rilasciata dalle cellule necrotiche o stressate, promuove il rilascio di citochine tramite la sua interazione con il recettore TLR4 e, come abbiamo recentemente descritto, la migrazione di leucociti via CXCR4, formando un complesso con CXCL12. Stiamo ora studiando, in collaborazione con i gruppi di Costantino Pitzalis al William Harvey Institute (Londra, UK) e di Antonio Manzo presso l'Università di Pavia (Italia), le molecole che cooperano nel reclutamento e l'attivazione dei globuli bianchi nei siti di infiammazione che sono cruciali nell'artrite reumatoide e che potrebbero essere modulate da una terapia anti-citochine. Questo studio getta nuova luce sui meccanismi che riducono l'infiammazione in modo significativo e forniscono ulteriori target per lo sviluppo di nuove strategie anti-infiammatorie.

Publications

Pertussis toxin-driven IL-1β production by myeloid cells is required for priming of encephalitogenic Th1/Th17 cells
Ronchi F, Basso C, Preite S, Reboldi A, Baumjohann D, Perlini L, Lanzavecchia A, and F. Sallusto F.
Submitted. Thelen M. and Uguccioni M. Encyclopaedia of Immunology 2015. In press.

Somatic mutations and affinity maturation are impaired by excessive numbers of T follicular helper cells and restored by Treg cells or memory T cells.
Preite, S., D. Baumjohann, M. Foglierini, C. Basso, F. Ronchi, B. M. Rodriguez, D. Corti, A. Lanzavecchia and F. Sallusto
Eur J Immunol. 2015; 45:3010-3021.

Chemokine interaction with synergy-inducing molecules: fine tuning modulation of cell trafficking.
Cecchinato, V., G. D'Agostino, L. Raeli and M. Uguccioni
J Leukoc Biol. 2015;

HMGB1 and leukocyte migration during trauma and sterile inflammation.
Venereau, E., M. Schiraldi, M. Uguccioni and M. E. Bianchi
Mol Immunol. 2013; 55:76-82.

Persistent antigen and germinal center B cells sustain T follicular helper cell responses and phenotype.
Baumjohann, D., S. Preite, A. Reboldi, F. Ronchi, K. M. Ansel, A. Lanzavecchia and F. Sallusto
Immunity. 2013; 38:596-605.

Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta.
Zielinski, C. E., F. Mele, D. Aschenbrenner, D. Jarrossay, F. Ronchi, M. Gattorno, S. Monticelli, A. Lanzavecchia and F. Sallusto
Nature. 2012; 484:514-518.

Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release.
Venereau, E., M. Casalgrandi, M. Schiraldi, D. J. Antoine, A. Cattaneo, F. De Marchis, J. Liu, A. Antonelli, A. Preti, L. Raeli, S. S. Shams, H. Yang, L. Varani, U. Andersson, K. J. Tracey, A. Bachi, M. Uguccioni and M. E. Bianchi
J Exp Med. 2012; 209:1519-1528.

HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4.
Schiraldi, M., A. Raucci, L. M. Munoz, E. Livoti, B. Celona, E. Venereau, T. Apuzzo, F. De Marchis, M. Pedotti, A. Bachi, M. Thelen, L. Varani, M. Mellado, A. Proudfoot, M. E. Bianchi and M. Uguccioni
J EXP MED. 2012; 209:551-563

University of Lausanne

A growing amount of evidence supports the involvement of TLRs in the pathology of rheumatoid arthritis (RA). For example, TLR 2, 3 and 4 were found to be expressed by RA synovial fibroblasts. Moreover, stimulating TLR2 or TLR3 of RA macrophages with different ligands induced their own up-regulation, thus increasing macrophage sensitivity to stimuli. Due to the TLR role in the recognition of CpG DNA, dsRNA or ssRNA, viral infections could play a role in RA, furnishing TLR ligands and initiating a potent, type 1 interferon driven, pro-inflammatory response. Indeed, several viruses, including alphaviruses, HCV, HIV and parvovirus B19 have been found in synovial tissue and have been implicated in the development of RA. Taken together there is evidence to suggest that TLRs could play a fundamental role in the initiation and self-perpetuation of RA, inducing the production of pro-inflammatory cytokines, which lead to recruitment of inflammatory cells and consequent tissue damage, resulting in cell death and release of more TLR ligands, creating a vicious cycle.

In Lausanne, we focused on the importance of TLR3 which can be activated by dsRNA viruses. Using a model system based on Leishmania parasites which harbor a viral cytoplasmic dsRNA, we looked for signaling pathways implicated in the survival of macrophages and determined a TLR3 dependent axis which induced phosphorylation of AKT1. In addition, we demonstrated the importance of IL-17 in the inflammatory response and spreading of inflammation at secondary sites. We also showed that inflammation can be reactivated by subsequent infection and determined the importance of TLR3 and its activation in the poor responsiveness to specific drugs.

In clinically related investigations, we could demonstrate that Leishmania parasites harboring the dsRNA could be predictive of clinical complications such as first-line treatment failure, increased and reactivated inflammation, and symptomatic relapse, symptoms which are relevant in RA. Our data could guide treatment strategies, to better predict, avoid, and manage the complications of such hyper-inflammatory process and could have an impact on potential synergistic therapeutic effects on inflammatory diseases.

Publications

Bourreau E., Ginouves M., Prévot G., Hartley M.A., Gangneux J.P., Robert-Gangneux F., Dufour J., Sainte-Marie D., Bertolotti A., Pratlong F. et al., 2016. Presence of Leishmania RNA Virus 1 in Leishmania guyanensis Increases the Risk of First-Line Treatment Failure and Symptomatic Relapse. Journal of Infectious Diseases 213(1) pp. 105-111. [DOI] [Pubmed]

Casanova M., Gonzalez I.J., Sprissler C., Zalila H., Dacher M., Basmaciyan L., Späth G.F., Azas N., Fasel N., 2015. Implication of different domains of the Leishmania major metacaspase in cell death and autophagy. Cell Death and Disease 6 pp. e1933. [DOI] [Pubmed]

Ashok D., Schuster S., Ronet C., Rosa M., Mack V., Lavanchy C., Marraco S.F., Fasel N., Murphy K.M., Tacchini-Cottier F. et al., 2014. Cross-presenting dendritic cells are required for control of Leishmania major infection. European Journal of Immunology 44(5) pp. 1422-1432. [DOI] [Web of Science] [Pubmed]

Hartley M.A., Drexler S., Ronet C., Beverley S.M., Fasel N., 2014. The immunological, environmental, and phylogenetic perpetrators of metastatic leishmaniasis. Trends in Parasitology 30(8) pp. 412-422. [DOI] [Web of Science] [Pubmed]

Ives A., Masina S., Castiglioni P., Prével F., Revaz-Breton M., Hartley M.A., Launois P., Fasel N., Ronet C., 2014. MyD88 and TLR9 dependent immune responses mediate resistance to Leishmania guyanensis infections, irrespective of Leishmania RNA virus burden. PLoS One 9(5) pp. e96766. [DOI] [Web of Science] [Pubmed]

Martin R., Gonzalez I., Fasel N., 2014. Leishmania metacaspase: an arginine-specific peptidase. Methods in Molecular Biology 1133 pp. 189-202. [DOI] [Web of Science] [Pubmed]

Zangger H., Hailu A., Desponds C., Lye L.F., Akopyants N.S., Dobson D.E., Ronet C., Ghalib H., Beverley S.M., Fasel N., 2014. Leishmania aethiopica field isolates bearing an endosymbiontic dsRNA virus induce pro-inflammatory cytokine response. PLoS Neglected Tropical Diseases 8(4) pp. e2836. [DOI] [Web of Science] [Pubmed]

University of Geneva

Cytokines are small peptides that a key role in cell communications. The interleukin (IL)-1 family of cytokines comprises 11 members, including IL-1 (a, b), IL-1 receptor antagonist (IL-1Ra), IL-18, IL-33, IL-36 (a, b, g), IL-36Ra, IL-37 and IL-38. IL-1 exerts pro-inflammatory activities that are tightly controlled by different inhibitors, including IL-1Ra and the decoy receptor IL-1R type 2 (IL-1R2). IL-1R2 is predominantly expressed by neutrophils and its extracellular domain is released upon neutrophil stimulation. We found that IL-1R2 knockout mice exhibit a more severe form of arthritis.

We have previously shown that IL-36, but not IL-1, stimulates mouse dendritic cells (DC) and naïve CD4+ T cells and is able to induce Th1 responses. We compared the stimulatory effects of IL-36 and IL-1 in human DC and macrophages and found that IL-1 was more potent than IL-36. In contrast, IL-36 and IL-1 were equipotent in stimulating Langerhans cells and keratinocytes. Future studies will be conducted with knockout mice in which the expression of IL-36R is targeted in keratinocytes and dendritic cells.

IL-18 exerts pro-inflammatory activities that are controlled by IL-18 binding protein (IL-18BP). Serum IL-18BP is present in large excess, thus preventing exaggerated IL-18 responses. Our laboratory was involved in the development of an immunoassay to measure unbound free IL-18. By using this ELISA we found that elevated free IL-18 levels in adult onset Still's disease (AOSD), a systemic inflammatory condition, as well as in a severe hereditary inflammatory condition associated with a mutation of the inflammasome component NLRC4. These findings led to 1) a clinical trial with recombinant IL-18BP in AOSD and 2) with the treatment of a child with a severe form of NLRC4 mutation. Our group is involved in the laboratory and clinical extension of this project.

We have shown that salt-inducible kinases (SIK) play a major role in the regulation of inflammatory cytokine production by human macrophages and dendritic cells. In addition, we recently observed that SIK are also involved in the regulation of osteoclastogenesis. These findings indicate that SIK may represent a future target for the treatment of arthritis.

Research Focus 1

The aim of our current work is focused on better understanding the role of the interleukin-1 family of cytokines using experimental models of arthritis and other experimental models of inflammatory diseases. In particular we have generated several lines of transgenic mice to explore the role of interleukin-1 cytokines in vivo. More recently we have been working on the role of IL-36 in immune response related to skin and articular inflammation.

We are also working on IL-18 in inflammatory rheumatic diseases using both human samples and experimental models in the mouse. The results led to an ongoing clinical trial using an IL-18 antagonist in adult onset-Still's disease, an inflammatory rheumatic condition.

In addition, we are currently examining the role of signaling pathways involved in the modulation of inflammatory responses in macrophages and other myeloid cells. Our research includes also translational aspects with the use of biological samples from patients with rheumatoid arthritis to identify biomarkers of disease severity and response to therapy, as well as to understand the pathogenic role of specific autoantibodies.

Group Leader: Cem Gabay, MD Cem.Gabay@hcuge.ch
Members: Gaby Palmer – PhD, Praxedis Martin, PhD – Jennifer Palomo, PhD – Maria Stella Lombardi, PhD – Sylvette Bas, PhD – Damien Dietrich, MD-PhD student – Charlotte Girard, MD-PhD student – Dominique Talabot-Ayer, technician – Emiliana Rodriguez, technician – Corine Gilliéron, technician

Original articles

Lombardi M.S., et al: SIK inhibition in human myeloid cells modulates TLR and IL-1R signaling and induces an anti-inflammatory phenotype. J Leukoc Biol 2015 (in press)

Martin P., et al: Atherosclerosis severity is not affected by a deficiency in IL-33/ST2 signaling. Immun Inflamm Dis. 2015; 3: 239-246

Segueni N., et al: Limited contribution of IL-36 versus IL-1 and TNF pathways in host response to mycobacterial infection. PlosOne 2015; 10: e0126058

Talabot-Ayer D., et al: Severe neutrophil-dominated inflammation and enhanced myelopoiesis in IL-33-overexpressing CMV/IL-33 Mice. J Immunol 2015; 194: 750-60

Martin P., et al: Mouse neutrophils express the decoy type 2 interleukin-1 receptor (IL-1R2) constitutively and in acute inflammatory conditions. J Leukoc Biol 2013; 94: 791-802

Lamacchia C., et al: Endogenous IL-1a is chromatin-associated protein in mouse macrophages. Cytokine 2013; 63: 135-144

Lamacchia C., et al: The severity of experimental arthritis is independent of IL-36 receptor signaling. Arthritis Res Ther 2013; 15: R38

Martin P., et al: Disease severity in K/BxN serum transfer-induced arthritis is not affected by IL-33 deficiency. Arthritis Res Ther 2013; 15: R13

Vigne S., et al: IL-36 Signaling Amplifies Th1 Responses by Enhancing Proliferation and Th1 Polarization of Naïve CD4+ T cells. Blood 2012; 120: 3478-87

Lamacchia C., et al: Mice deficient in hepatocyte-specific interleukin-1 receptor agonist show delayed resolution of concanavalin A-induced hepatitis. Eur J Immunol. 2012; 42: 1294-303

Lamacchia C., et al: Articular inflammation is controlled by myeloid cell-derived interleukin-1 receptor antagonist during the acute phase of arthritis in mice. Ann Rheum Dis 2012; 71: 281-7

Talabot-Ayer D, et al: Distinct serum and synovial fluid interleukin (IL)-33 levels in rheumatoid arthritis, psoriatic arthritis and osteoarthritis. Joint Bone Spine. 2012; 79: 32-7

Research Focus 2

The aim of our current work is to address the contribution of macroautophagy to the pathogenesis of autoimmune and inflammatory arthritis through different aspects. One part of our research is focused on using models of arthritis in mice deficient for autophagy in their antigen presenting cells (dendritic cells and macrophages).

The secund part of our work is to analyze in human samples the role of macroautophagy in the initiation of the immune and inflammatory response during both Rheumatoid arthritis (RA) and B27 spondylarthropathies. More precisely, we are currently working on the degradation, processing and citrullination of selected auto-antigens through macroautophagy and its relevance in RA. Finally we have identified a new role for macroautophagy in the internalization and degradation of MHC class 1 molecules and we are currently analyzing the contribution of the pathway to the misfolding of B27.

Group Leader: Monique Gannagé, MD, PhD Monique.ghannage@unige.ch
Members : Jennifer Niven, Postdoctoral associate- Alexandre Ghounaris, PhD student- Gracia Gangath master student- Assunta Caruso, technician

Publications

The tumor antigen NY-ESO-1 mediates direct recognition of melanoma cells by CD4+ T cells after intercellular antigen transfer Fonteneau J, Brilot F, Munz C, Gannagé M J Immunol. 2016 Jan 1;196(1):64-71.

Macroautophagy in endogenous processing of self- and pathogen-derived antigens for MHC class II presentation .Duares F, Niven J, Hugues S*and Gannagé M* Front Immunol. 2015 Sep 22;6:459. doi: 10.3389/fimmu.2015.00459. eCollection 2015. Review.

Guidelines for the Use and Interpretation of Assays for Monitoring Autophagy Klionsky D, Abdelmohsen K, Abe A,… Gannagé M ,… Zong, Antonio Zorzano, and Zughaier S . Autophagy. 2016 Jan 2;12(1):1-222.

S100B Up-Regulates Macrophage Production of IL1β and CCL22 and Influences Severity of Retinal Inflammation. Niven J, Hoare J, McGowan D, Devarajan G, Itohara S, Gannagé M, Teismann P, Crane I. 2015 Jul 23;10(7):e0132688. doi: 10.1371/journal.pone.0132688. eCollection 2015.

Checking the garbage bin for problems in the house, or how autophagy assists in antigen presentation to the immune system. Romao S, Gannagé M, Münz C. Semin Cancer Biol. 2013 Oct;23(5):391-6.

Antigen processing for MHC presentation via macroautophagy.bGannage M, da Silva RB, Münz C. Methods Mol Biol. 2013;960:473-88

Press Release February 2015

iAR strives for drug discovery: thanks to LOTERIE ROMANDE

Investigations undertaken by the association IAR (aIAR) have always endeavoured to find new ways to relieve and reverse the destructive inflammation of arthritis. After years of dedicated research, our members have identified several druggable targets that may realise these goals and thus, the aIAR has recognised the need to make the critical expansion to drug discovery. The goal of this endeavour is to screen existing compound libraries for small molecules able to inhibit or activate key pathways previously identified in the pathological process of arthritis. This screening has been made possible by **la Loterie Romande** who has provided aIAR with a precision high-throughput fluorescent and luminescent screening apparatus: the Hamamatsu FDSS/µcell. This kinetic imaging plate reader for cell-based assays allows simultaneous measurements of multiple parameters with no time lag between conditions.

Building on their previous studies, investigators at the aIAR will initiate the FDSS/µcell drug screen on targets that have showed promising preliminary results. Specifically, the initial focus will be on:

1) Inhibitory compounds of a kinase important in acute inflammation and,
2) Activators of G-protein-coupled receptors, which are important regulators of tissue-damaging inflammatory processes.

So far, all inhibitory compounds on this kinase of interest have had significant off-target effects, necessitating high-throughput screening on large compound libraries such as defined in this project. Testing will be started on experimental cell-lines expressing reporter molecules and then followed by assays in synovial fibroblasts of arthritic patients.

The drug screening effort supported by **la Loterie Romande** will be instrumental in shedding light on the therapeutic feasibility of these promising pathways to relieve and reverse the destructive inflammatory processes of arthritis.

For more information: Prof. Nicolas Fasel
Département de Biochimie
Université de Lausanne
Chemin des Boveresses 155
1066 Epalinges
nicolas.fasel@unil.ch