DBM FACTS

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"The impact factor is what matters!" Research experience in Africa | The new Department of Biomedicine Histology Core Facility | Summertime – Time for Open Water Swim Races

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Departement Biomedizin Basel

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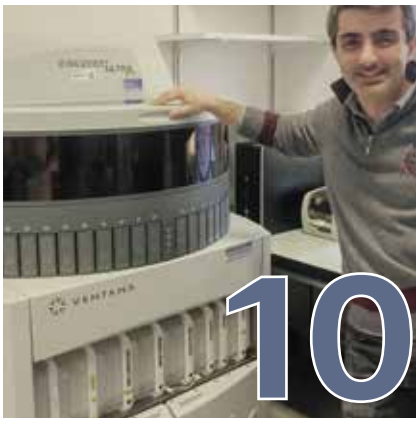

The new Department of Biomedicine Histology Core Facility
from Diego Calabrese

Sommerzeit ist Seezeit
from Vera Lorenz

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IMPRESSUM

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EDITORIAL

Radek Skoda
Leiter DBM

Liebe Leserinnen und Leser

Ein schöner Sommer und unser DBM-Symposium und Barbecue liegen bereits hinter uns und neue Herausforderungen warten auf uns.
Für den Neubau DBM hat die nächste wichtige Planungsphase – das Bauprojekt – begonnen. Die Grundlagen aus dem Vorprojekt werden nun von den diversen Fachplanern detailliert ausgearbeitet.
Nach sieben Jahren in der Leitung des DBM wechselt Frank Neumann per Oktober 2019 in das Vizerektorat Forschung, wo er Prof. Torsten Schwede unterstützen wird. Wir bedauern seinen Weggang, danken ihm sehr für die grossen Beiträge zur Weiterentwicklung des DBM und wünschen ihm viel Erfolg am neuen Arbeitsort.
Ende Juli ist Armin Bieri nach 38 Jahren am Departement Forschung/Biomedizin in die wohlverdiente Pension gegangen. Auch ihm danken wir herzlich für seine über die ganzen Jahre hinweg geleistete Arbeit und seinen grossen Einsatz. Wir wünschen ihm viel Freude und Zufriedenheit im neuen Lebensabschnitt. Sein Nachfolger Massimo Usan wird am 1. Oktober 2019 am DBM Hebelstrasse beginnen.
Thomas Klimkait berichtet in dieser Ausgabe über seine langjährigen Forschungserfahrungen in Tansania (ab Seite 2). Diego Calabrese stellt uns die neue «Histology Facility» vor, die er seit 1. Januar 2019 leitet (ab Seite 10). Exzellente Publikationen aus dem DBM warten auf Sie ab Seite 13. Vera Lorenz lässt den Sommer noch einmal aufleben und schwimmt mit uns durch den Bodensee (ab Seite 27). Mit auf die Reise in seine Heimat Kroatien nimmt uns Šime Brkić (ab Seite 30).

Eine spannende Lektüre!

Dear Readers,

A wonderful summer and our DBM Symposium and barbecue are already over and new challenges await us.
Construction, the next important phase of the new DBM building has begun. The basics of the preliminary project are now being developed in detail by the various specialist planners.
In October 2019 Frank Neumann will, after seven years in the management of the DBM, move to the Vice Rectorate for Research where he will support Prof. Torsten Schwede. We regret his departure, thank him very much for the great contributions to the development of the DBM and wish him every success in the new place of work.
At the end of July Armin Bieri took his well-deserved retirement after 38 years at the Department of Research / Biomedicine. We also thank him for dedication and work over the years. We wish him every joy and satisfaction in the new phase of his life. His successor, Massimo Usan, will start at the DBM Hebelstrasse on 1st October 2019.
In this issue Thomas Klimkait tells us about his years of research experience in Tanzania (page 2). Diego Calabrese introduces us to the new Histology Facility which he has been managing since 1st January 2019 (page 10). The many excellent publications from the DBM are waiting for you on page 13. Vera Lorenz brings the summer to life again and brings us for a swim in the Bodensee (page 27). Šime Brkić brings us with him on a journey to his homeland of Croatia (page 30)

Enjoy reading!

"The impact factor is what matters!" Research experience in Africa

Isn't this what matters to every scientist: Striving for a paper in Cell, Nature or NEJM…
Impact defines our quality of scientific life, right!?

There is certainly truth to this and, looking scientifically back onto my own past, I have little reason to complain: Having had the chance to discover and describe the viral release factor Vpu, at that time an HIV protein with unknown function; having been part of the pharmaceutical team that developed atazanavir, a novel HIV-specific protease inhibitor, which helped to transform HIV treatment into modern combination therapy; and the work of my DBM-research group contributed to the field valuable tools for HIV resistance testing and viral tropism research. Throughout my career, it has been in the center of my scientific interest to work on the 'applicability of science' and to help propagate clinical translation. And precisely this was the driver for the other side of my scientific curiosity: How can our Northern high impact clinical research be utilized to create a higher impact in disadvantaged settings, in remote healthcare centers, and technologically unreached hospitals and laboratories?

Simply take a look at the advancements in the field of virology: Until the 1990s, it appeared entirely impossible to dream about modern medication or laboratory technology for most African countries with their largely rural healthcare-settings. There are more than 35 million HIV-infected people globally with 2/3 of the disease burden located in Africa; countries with about 24% prevalence in the age group of the working population, orphaned children and collapsing economies. But then,

"photo, photo, make a photo!" Lovely faces posing in the main street in Ifakara

Yes, this is the main road to Ifakara during rainy season! Imagine, it is the only route for transporting clinical samples to the diagnostic center…, and it can easily take 6 hrs to Morogoro

Landing strip at Ifakara during rainy season:
The pilot carefully checked the conditions before touch-down… "there's just a little water on the ground, not very deep"

as a huge blessing, these were the years, where global funds made large sums available to fight AIDS, to provide free medication and to globally improve the health situation in these hardest hit countries! And, at the very same time, many new inventions on molecular diagnostics and in HIV research were developed, offering tremendous new opportunities.

Impact via improved medical care

In the Northern hemisphere, HIV was already largely under control by 2008, with a low remaining risk of infection for the "general population", and the "Swiss statement" announced the impressive news that successfully treated persons will NOT transmit the virus to their part-

M. Weisser leading the weekly clinical report of the entire clinical and lab team on Monday morning (CDCI, Ifakara)

Entrance to the Molecular Lab at the Ifakara Health Institute in Ifakara, Tanzania

ners. But this was NOT at all applicable to many African countries even by 2014. Standard HIV diagnostics and virus tests remained completely unavailable in rural settings in central Tanzania, and no modern medical support had been established in the North of Lesotho, where the adult HIV-prevalence was exceeding 23%. In previous years global programs have provided life-saving medication, and HIV treatment has also been sustainably established in the large rural district of Morogoro (Tanzania), where the Swiss Tropical Public Health Institute and the Infectious Disease Unit of the University Hospital Basel (team of Manuel Battegay) have established the 'Chronic Disease Clinic Ifakara' with solid state-of-the-art therapy and patient management. The unit is hosted by the local Ifakara Health Institute and St. Francis Hospital.

Meanwhile, modern laboratory diagnostics remained essentially absent in 2010: No determination of the HIV viral load in blood (an essential key indicator for a successful therapy) was possible, simple kit-based tests for immune

Meticulous morning sweep to keep streets clean (it just won't help against the potholes…), and with a happy smile on the face.

Creative 'bottom-mount' of an automatic door closer: "We did not have enough space above the door..."

"By car you will never make it to the airport in 3 hours: Securing my luggage on a 'Boda-boda-Taxi' in Dar-es-Salaam on a rainy day – and indeed, I caught my flight!

"Wide load" – or rather "on the way to a huge omelet..."?? One of the very creative transport ideas common in Tanzania

function were often available only with large supply gaps, and lab certification or accreditation were completely out of reach for the laboratories.

Mandated by the Basel institutions, my laboratory managed to establish the first very inexpensive home-brew methods that were applicable to the African HIV-1 strains and determined HIV in patients' blood and cellular HIV-DNA as evidence for mother-to-child transmission. With this, the local laboratories gained the capability to provide a treatment monitoring tool and to support HIV infant diagnosis. In this process, a true adventure, we had to solve issues of excessively long times between blood-draw and analysis, which often led to hemolysis and virus degradation; -20°C freezers that failed in the local hot environmental conditions with shocking frequencies, and a chronic shortage of cooled storage space challenged our capabilities in "international logistics", the omnipresent lack of adequate technological education stimulated us to sharpen our teaching skills, leading me to often truly enjoy the sophisticated Swiss army knife in my pocket...

Finally, during 2018, certified diagnostic systems by the main diagnostic suppliers became available, so that the laboratories can now rely on the international capacity of diagnostic companies and provide reliable, high throughput platforms for HIV testing and monitoring. As a consequence, viral load monitoring today is pretty well organized for the entire district and in preparation for laboratory accreditation with only minor hick-ups in the kit supply.

In Tanzania, bicycles are omnipresent and used as the typical way to transport loads up to 150 kg!

I should mention here that the often quite challenging development time was enchanted by the little signs of creativity of the local specialists who often showed how solutions can be found even where technical equipment is limited…

This is where the electrical wiring (and sometimes also the technical nightmare) starts… Snapshot in front of a diagnostic laboratory.

Impact through new powerful diagnostic tools

The advent of combination therapy for HIV (cART), introduced at several clinics in Tanzania and Lesotho chiefly by the clinical teams from Basel, brought HIV disease management to an entirely new level. But along with this grew a new urgent need for the ability to assess the development of viral resistance to the treatment drugs. The molecular, genotypic determination of changes in the viral genes targeted by the applied drugs would enable the timely detection of drug-resistant viruses and permit therapy-adjustment. This could save on ineffective medication, and prevent a further evolution of viral resistance in the affected patient and an onward spread of resistant HIV variants. Together with Ingrid Felger, STPHI, and support by Marcel Tanner, we worked on translating the dream to establish the DNA sequencing capability in the affiliated laboratories of the respective clinics in Ifakara

4 truck batteries serve as power backup for the capillary sequencer (No need for a sophisticated and expensive APC…). And it works… as long as nobody forgot to check the water levels!

Laboratory head Me Klaas and T. Klimkait in discussion about accreditation at Butha-Buthe hospital in Lesotho

Happy faces in the discussion of a quick-test result. N. Labhardt during a home-visit campaign in Northern Lesotho (photo: C.Heuss, SolidarMed)

Not only the lab team but also the principal of Seboche Hospital, Sr. Lebina,celebrate the first successful sequence result for HIV resistance testing on the ABI machine here in Northern Lesotho.

(Tanzania) and Butha-Buthe/Seboche (Lesotho). With superb help from technology specialists, who serviced such instruments in Switzerland, we refurbished ABI 4-capillary sequencers and sent them across the long dirt roads to our African settings. Our ambitious aim was the provision of on-site services and to build local scientific capacity.
However, it does not take particularly much fantasy to anticipate the various road-blocks that would follow, and sometimes we felt like we had been too naïve with our plans…
After laborious customs clearance in the respective countries, and once the instruments had survived transport on the bumpy roads to the rural destinations, new obstacles surfaced immediately: how could we overcome the frequent power failures that are VERY typical for these sites? How to bridge if power is off for several hours? The instruments would not only lose all analyzed information, but could also be damaged by the interruption itself. You can hardly imagine the tons of local creativity and the many hours the computer specialists, electricians, maintenance managers and SOP-writers needed to invest to make the dream come true and reach the point where our African collaborators had their OWN first HIV-sequence and resistance information in hand.

Also financially, the local laboratories and clinics made a huge effort to make this new technological development possible and, at Butha-Buthe hospital, the small new facility, built to host the Ampliprep instrument for HIV viral load testing was affectionately dubbed "Little Switzerland". We share the pride of the local experts that their new development on-site can finally make an important difference for good clinical care and support the establishment of sustainable health care systems in Tanzania and Lesotho.
But despite all that, serious setbacks can occur almost anytime that require our attention and energy to maintain good international connection and interaction. Recently an unfortunate power failure with its sudden (unannounced) return accompanied by very high voltage

Posing in front of the new Molecular Lab for HIV viral load, lovingly called "little Switzerland" by the technicians, since it hosts a diagnostic system from CH (Butha-Buthe)

spikes 'killed' the laser of our sequencer and the steering hardware. Despite emergency treatment reanimation was not possible and, naturally, no funds were available for replacement. Here the invaluable work and support of the responsible clinical researcher, Niklaus Labhardt, who has obviously lost his heart to Lesotho, opened ways to re-activate the plan. Employing highly innovative approaches, Niklaus drives the establishment of state-of-the-art HIV therapy and diagnostics. A recent study, which employs repeated home-visits for an HIV testing campaign, impressively demonstrated how new strategies of reaching patients can improve their linkage to medical care and thereby effectively contribute to controlling HIV spread in the population.

Impact by regional capacity building

The provision of expertise and medical care over several years utilizing the expertise of specialists from Switzerland reflects a key step in building capacity and for making an impact in medicine. This must, however, lead to the establishment of a national and local expertise; and this is a massive challenge for rural African environments such as in Ifakara (Tanzania) or Butha-Buthe (Lesotho). We, as the partners from Basel, are therefore committed to establishing the basis for such self-sustainable operations. Even within our African partner countries the big competition from capital cities with more attractive healthcare institutions leads to a very significant loss of well-trained, highly capable doctors and scientists. Our main focus is on strengthening capacity and knowledge of the young, who are locally anchored through their families. By supporting their further education and by offering international exchange and training we are convinced that we can **generate an impact that can go beyond the mathematical impact factor of publications in renowned journals**. And yet, the collaborations have begun to yield fruits emerging in joint publications of our joint work!

Thomas Klimkait

Snapshot in the rain – taken from the car during the road-passage through Mikumi National Park

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The new Department of Biomedicine Histology Core Facility

What is histology

Histology is a term derived from two Greek words: ἱστός (histós), tissue, and λόγος (lógos), study. Thus, histology is the study of a tissue, of the cells that compose it and the interactions that exist between them.

The discipline was formally born only after the formulation of the cell theory, in 1838 by M.J. Schleiden and T. Schwann, when the cell was identified as the structural and functional unit of all living tissues. However, many intellectuals were already fascinated by biological tissues (primarily plants at that time) before this, and began studying them, to understand their composition and functions.

Interestingly enough, the first histological studies were more of an expensive hobby for the European aristocracy, who were able to cope with the cost of the necessary equipment, than a true scientific discipline.

During the 19th and 20th centuries there were significant developments in equipment and techniques. The Hematoxylin and Eosin staining, the most important diagnostic tool for pathologists nowadays, was introduced in 1876. The Gram's method, fundamental for the identification of microscopical pathogens, responsible of mortal diseases at that time, was invented in 1884. The first report of an immunohistochemistry experiment was in 1890 from Von Behring, who discovered serum antibodies, and used them to cure diphtheria and tetanus. But the first actual tissue immunostaining only occurred in 1923.

Histology is therefore an ancient discipline which, however, has not lost its scientific value over the centuries and continues to be a key element of many scientific publications.

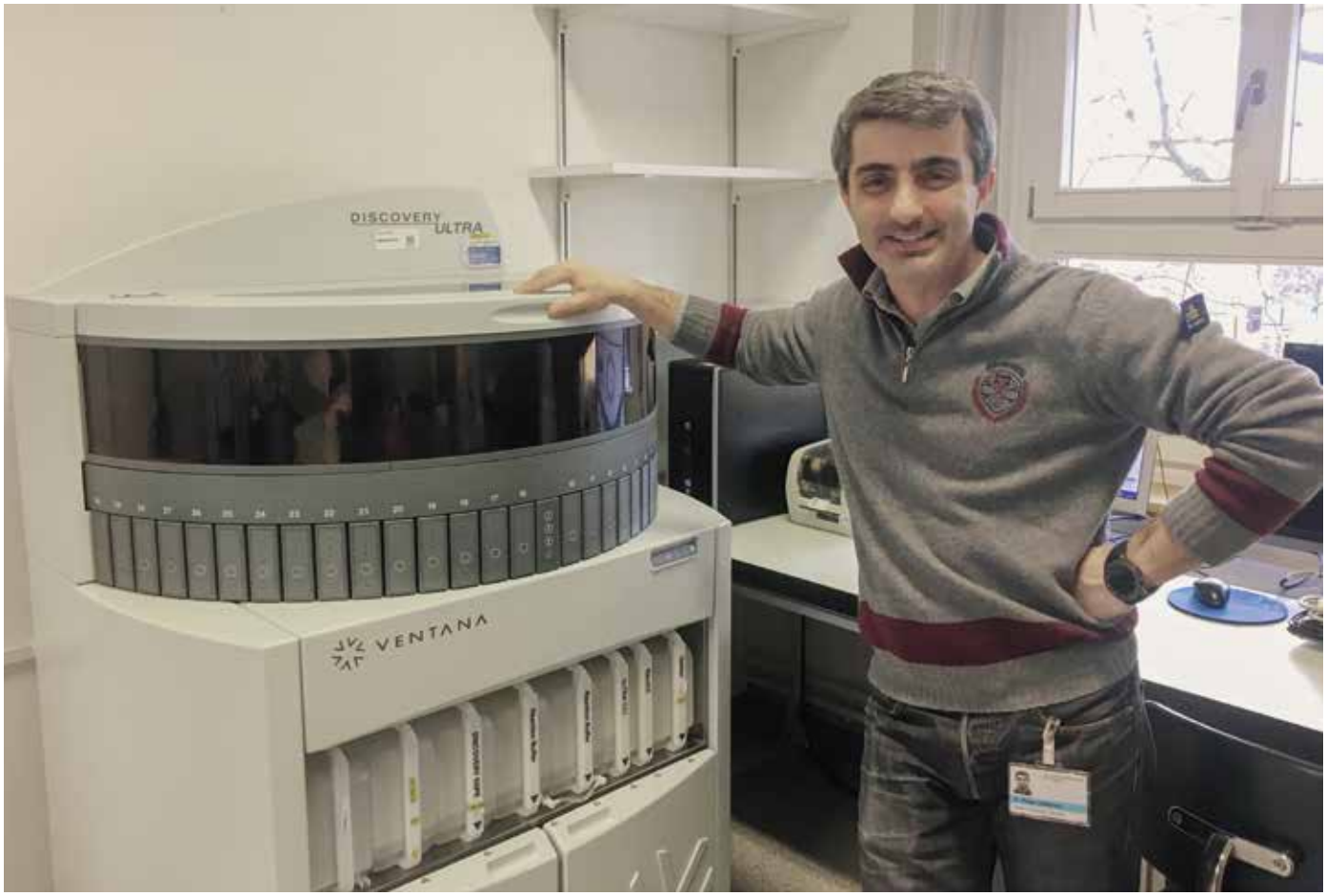
Histology at DBM

The Department of Biomedicine (DBM) is strongly committed to medicine and translational research. As such, histology plays an important role in most of the research projects developed at DBM.

The DBM Histology Core Facility was born in 2015, based on a voluntarily initiative of myself and members of the Tissue Engineering group. The basic idea was to reorganize the histology laboratory, already present at DBM in Hebelstrasse, to improve the working conditions and, in the long term, to expand the experimental possibilities. Over the following three years, in parallel with my research activities as a post doc of the Hepatology group, I trained hundreds of users (i.e. for a total of 380 hours of training) on basic histological techniques. In parallel, I worked in synergy with the Department, for a slow re-equipment process of the Facility.

At beginning of 2019 the Facility was relocated to the Anatomy Institute, in Pestalozzistrasse. This was a delicate and strongly debated decision: undoubtedly this relocation was inconvenient for the Hebelstrasse researchers, now forced to move to another building for those experiments done before in house. On the other hand, the facility has expanded in terms of space, equipment and experimental capabilities, thus becoming not only a room crowded with equipment, but a real facility.

Since the beginning of this experience, I have spent most of my time training the facility users. The facility is a departmental laboratory based on the "do it yourself" principle. Dozens of users work every day, side by side, in a common space and using common equipment. Anyone who has worked in a research laboratory knows that this can be the perfect recipe for a potential disaster. To avoid this risk, it is necessary that every user has the basic knowledge that allows him/her to work safely, without negatively affecting the work of other users, and that is instrumental to achieving the desired result. Training is



therefore focused on safety, basic histology and practical use of the equipment. This recipe has proved successful over the years, although occasionally it is still possible to observe some rare examples of "creative and alternative science", so to speak. Since January 2019 I have been hired as head of the Facility, so users can now also count on me full time, for suggestions and support in designing and conducting their own experiments.

Thanks to the new organization, then, in addition to the DIY platform, the hard core of the facility, we are also able to offer a service. The facility is now developing protocols for manual and automated immunohistochemistry, immunofluorescence and in-situ hybridization for those groups that want to outsource these activities.

Finally, starting from 2020, the facility will also be the reference center for a multi-center phase 3 clinical trial on ovarian cancer. The facility will be in charge of the histological analysis and biobanking of all of the samples collected in Switzerland and in several European Countries.

In summary, the facility wants to be first and foremost a training center for young researchers, interested in deepening their knowledge in histology. Secondly, we want to provide continuous support to those groups involved in the development of new histological methods. Ultimately, we want to be a reference center for those activities, such as clinical trials, that require infrastructures, knowledge and quality standards that are above the average.

What about the future? The motto of Joseph von Gerlach, a medical doctor and histologist lived in XIX century, was: "arte ungendi innititur histologia", which means the histology is based on staining skill. The future will not be that different in principle. In a scientific world dominated by the -omics techniques, in the future we will focus a lot on multiplexing our methods. New technologies are already on the market, but we are only at the beginning of a new revolution in this field.

The future of the Histology Core Facility will be all about support, development and divulgation of the ancient histology art.

Diego Calabrese

Dissertationen

Am 6. Juli 2018 konnte **David Grünig** von der Forschungsgruppe «Clinical Pharmacology» (Departement Biomedizin Hebelstrasse) seine Dissertation mit Erfolg beenden. Er befasste sich in seiner Dissertation mit dem Thema: «Molecular mechansims of drug induced hepatic steatosis».

Am 13. August 2018 stellte sich **Dino Lüthi** von der Forschungsgruppe «Psychopharmacology Research» (Departement Biomedizin Hebelstrasse) den Fragen des Dissertationskomitees. Der Titel seiner Dissertation hiess: «Pharmacological and toxilogical investigations of new psychoactive substances».

Am 29. September 2018 stellte sich **Zahra Ehsaei** von der Forschungsgruppe «Embryology and Stem Cell Biology» (Departement Biomedizin Mattenstrasse) den Fragen des Dissertationskomitees. Der Titel ihrer Dissertation lautete: «Differentiation potential, lineage commitment and gene expression profile of human cortical neural progenitor cells derived from pluripotent stem cells».

Mit der Doktorprüfung am 14. Dezember 2018 schloss **Tanzila Mukhtar** von der Forschungsgruppe «Embryology and Stem Cell Biology» (Departement Biomedizin Mattenstrasse) erfolgreich ihre Dissertationszeit ab. Das Thema ihrer Doktorarbeit lautete: «Hippo Signalling in mammalian cortical development».

Am 30. April 2019 konnte **Barbara Szczerba** von der Forschungsgruppe «Cancer Metastasis» (Departement Biomedizin Mattenstrasse) ihre Dissertation mit Erfolg beenden. Sie befasste sich in ihrer Dissertation mit dem Thema: «Single-Cell Resolution Characterization of Circulating Tumor Cell Clusters».

Am 21. Juni 2019 stellte sich **Jasmin Grählert** von der Forschungsgruppe «Immunobiology» (Departement Biomedizin Hebelstrasse) den Fragen des Dissertationskomitees. Der Titel ihrer Dissertation lautete: «Iron metabolism dictates NK cell function».

Auszeichnungen

Ana Correia ausgezeichnet
Ana Correia von der Forschungsgruppe «Tumor Heterogeneity, Metastasis and Resistance» (Departement Biomedizin Hebelstrasse) hat am Personalized Oncology Meeting, das vom 23. bis 25. Juni 2019 in Basel stattgefunden hat, den «Peter Meier-Abt Personalized oncology conference award 2019» erhalten.

Nina Khanna erhält Wissenschaftspreis der Stadt Basel
Der diesjährige Wissenschaftspreis der Stadt Basel geht an Nina Khanna Gremmelmaier von der Forschungsgruppe „Infection Biology" (Departement Biomedizin Hebelstrasse). Der Preis ist mit 20'000 CHF dotiert und wird an Nina Khanna für ihre herausragenden Leistungen in der Erforschung von Therapien zur Bekämpfung von Antibiotikaresistenzen vergeben.

Herzliche Gratulation!

Science 364, 254 (2019) IF 41.058

Amygdala ensembles encode behavioral states

Jan Gründemann[1,2*†], Yael Bitterman[1*], Tingjia Lu[1], Sabine Krabbe[1], Benjamin F. Grewe[3,4], Mark J. Schnitzer[5], Andreas Lüthi[1,6†]

Abstract

Internal states, including affective or homeostatic states, are important behavioral motivators. The amygdala regulates motivated behaviors, yet how distinct states are represented in amygdala circuits is unknown. By longitudinally imaging neural calcium dynamics in freely moving mice across different environments, we identified opponent changes in activity levels of two major, nonoverlapping populations of basal amygdala principal neurons. This population signature does not report global anxiety but predicts switches between exploratory and nonexploratory, defensive states. Moreover, the amygdala separately processes external stimuli and internal states and broadcasts state information via several output pathways to larger brain networks. Our findings extend the concept of thalamocortical "brain-state" coding to include affective and exploratory states and provide an entry point into the state dependency of brain function and behavior in defined circuits.

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Annals of the Rheumatic Diseases Letter, Volume 78, Issue 7 IF 12.350

Serial IL-6 measurements in patients with tocilizumab-treated large-vessel vasculitis detect infections and may predict early relapses

Christoph T Berger[1,2] Birke Rebholz-Chaves,[3] Mike Recher,[1,4] Tobias Manigold,[3] Thomas Daikeler[3]

Tocilizumab (TCZ) has been approved for giant cell arteritis (GCA). Interleukin-6 (IL-6) receptor blockade suppresses clinical disease and is steroid sparing.[1 2] Since IL-6 induces the acute-phase response, the clinically used inflammation markers (C reactive protein (CRP), erythrocyte sedimentation rate (ESR)) are suppressed during TCZ treatment. Whether serum IL-6 is useful in monitoring disease activity and detecting infections in TCZ-treated GCA is unknown.

We longitudinally measured IL-6 in 23 patients with intravenous TCZ–treated GCA, two patients with polymyalgia rheumatica and one patient with Takayasu arteritis of our GCA cohort (EKBB-239/09), and in 13/26 patients additionally before TCZ treatment. Patient characteristics are shown in *table 1*. At each visit, clinical and laboratory parameters (white blood cell (WBC), CRP, ESR) were assessed. Relapse was defined as the need for treatment intensification following new or increasing symptoms, or rising CRP/ESR not otherwise explained.[2]

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Clinical Cancer Research 25(10) May 15, 2019 IF 10.199

A Variant of a Killer Cell Immunoglobulin-like Receptor Is Associated with Resistance to PD-1 Blockade in Lung Cancer

Marcel P. Trefny[1], Sacha I. Rothschild[1,2], Franziska Uhlenbrock[1], Dietmar Rieder[3], Benjamin Kasenda[2], Michal A. Stanczak[1], Fiamma Berner[4], Abhishek S. Kashyap[1], Monika Kaiser[1], Petra Herzig[1], Severin Poechtrager[5], Daniela S.Thommen[6], Florian Geier[7], Spasenija Savic[8], Philip Jermann[8], Ilaria Alborelli[8], Stefan Schaub[9], Frank Stenner[1,2], Martin Früh[10], Zlatko Trajanoski[3], Lukas Flatz[4], Kirsten D. Mertz[5], Alfred Zippelius[1,2], and Heinz Läubli[1,2]

Purpose: PD-(L)1–blocking antibodies have clinical activity in metastatic non–small cell lung cancer (NSCLC) and mediate durable tumor remissions. However, the majority of patients are resistant to PD-(L)1 blockade. Understanding mechanisms of primary resistance may allow prediction of clinical response and identification of new targetable pathways.

Experimental Design: Peripheral blood mononuclear cells were collected from 35 patients with NSCLC receiving nivolumab monotherapy. Cellular changes, cytokine levels, gene expression, and polymorphisms were compared between responders and nonresponders to treatment. Findings were confirmed in additional cohorts of patients with NSCLC receiving immune checkpoint blockade.

Results: We identified a genetic variant of a killer cell immunoglobulin-like receptor (KIR) *KIR3DS1* that is associated with primary resistance to PD-1 blockade in patients with NSCLC. This association could be confirmed in independent cohorts of patients with NSCLC. In a multivariate analysis of the pooled cohort of 135 patients, the progression-free survival was significantly associated with presence of the *KIR3DS1* allele (HR, 1.72; 95% confidence interval, 1.10–2.68; $P = 0.017$). No relationship was seen in cohorts of patients with NSCLC who did not receive immunotherapy. Cellular assays from patients before and during PD-1 blockade showed that resistance may be due to NK-cell dysfunction.

Conclusions: We identified an association of the *KIR3DS1* allelic variant with response to PD-1–targeted immunotherapy in patients with NSCLC. This finding links NK cells with response to PD-1 therapy. Although the findings are interesting, a larger analysis in a randomized trial will be needed to confirm KIRs as predictive markers for response to PD-1–targeted immunotherapy.

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Regulation of glioma cell invasion by 3q26 gene products PIK3CA, SOX2 and OPA1

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Abstract Diffuse gliomas progress by invading neighboring brain tissue to promote postoperative relapse. Transcription factor *SOX2* is highly expressed in invasive gliomas and maps to chromosome region 3q26 together with the genes for PI3K/AKT signaling activator PIK3CA and effector molecules of mitochondria fusion and cell invasion, MFN1 and OPA1. Gene copy number analysis at 3q26 from 129 glioma patient biopsies revealed mutually exclusive *SOX2* amplifications (26%) and *OPA1* losses (19%). Both forced *SOX2* expression and *OPA1* inactivation increased LN319 glioma cell invasion *in vitro* and promoted cell dispersion *in vivo* in xenotransplanted *D. rerio* embryos. While PI3 kinase activity sustained SOX2 expression, pharmacological PI3K/AKT pathway inhibition decreased invasion and resulted in SOX2 nucleus-to-cytoplasm translocation in an mTORC1-independent manner. Chromatin immunoprecipitation and luciferase reporter gene assays together demonstrated that SOX2 trans-activates *PIK3CA* and *OPA1*. Thus, SOX2 activates PI3K/AKT signaling in a positive feedback loop, while *OPA1* deletion is interpreted to counteract *OPA1 trans*-activation. Remarkably, neuroimaging of human gliomas with high *SOX2* or low *OPA1* genomic imbalances revealed significantly larger necrotic tumor zone volumes, corresponding to higher invasive capacities of tumors, while autologous necrotic cells are capable of inducing higher invasion in *SOX2* overexpressing or *OPA1* knocked-down relative to parental LN319. We thus propose necrosis volume as a surrogate marker for the assessment of glioma invasive potential. Whereas glioma invasion is activated by a PI3K/AKT-SOX2 loop, it is reduced by a cryptic invasion suppressor SOX2-OPA1 pathway. Thus, PI3K/AKT-SOX2 and mitochondria fission represent connected signaling networks regulating glioma invasion.

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PGC-1β modulates statin-associated myotoxicity in mice

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Abstract

Statins inhibit cholesterol biosynthesis and lower serum LDL-cholesterol levels. Statins are generally well tolerated, but can be associated with potentially life-threatening myopathy of unknown mechanism. We have shown previously that statins impair PGC-1β expression in human and rat skeletal muscle, suggesting that PGC-1β may play a role in statin-induced myopathy. PGC-1β is a transcriptional co-regulator controlling the expression of important genes in mitochondrial biogenesis, antioxidative capacity and energy metabolism. The principle aim of the current study was to investigate the interaction between atorvastatin and PGC-1β in more detail. We therefore treated wild-type mice and mice with selective skeletal muscle knockout of PGC-1β (PGC-1β$^{(i)skm-/-}$ mice) with oral atorvastatin (5 mg/kg/day) for 2 weeks. At the end of treatment, we determined body parameters, muscle function, structure, and composition as well as the function of muscle mitochondria, mitochondrial biogenesis and activation of apoptotic pathways. In wild-type mice, atorvastatin selectively impaired mitochondrial function in glycolytic muscle and caused a conversion of oxidative type IIA to glycolytic type IIB myofibers. Conversely, in oxidative muscle of wild-type mice, atorvastatin enhanced mitochondrial function via activation of mitochondrial biogenesis pathways and decreased apoptosis. In PGC-1β$^{(i)skm-/-}$ mice, atorvastatin induced a switch towards glycolytic fibers, caused mitochondrial dysfunction, increased mitochondrial ROS production, impaired mitochondrial proliferation and induced apoptosis in both glycolytic and oxidative skeletal muscle. Our work reveals that atorvastatin mainly affects glycolytic muscle in wild-type mice and demonstrates the importance of PGC-1β for oxidative muscle integrity during long-term exposure to a myotoxic agent.

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Antigen Extraction and B Cell Activation Enable Identification of Rare Membrane Antigen Specific Human B Cells

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Determining antigen specificity is vital for understanding B cell biology and for producing human monoclonal antibodies. We describe here a powerful method for identifying B cells that recognize membrane antigens expressed on cells. The technique depends on two characteristics of the interaction between a B cell and an antigen-expressing cell: antigen-receptor-mediated extraction of antigen from the membrane of the target cell, and B cell activation. We developed the method using influenza hemagglutinin as a model viral membrane antigen, and tested it using acetylcholine receptor (AChR) as a model membrane autoantigen. The technique involves co-culturing B cells with adherent, bioorthogonally labeled cells expressing GFP-tagged antigen, and sorting GFP-capturing, newly activated B cells. Hemagglutinin-specific B cells isolated this way from vaccinated human donors expressed elevated CD20, CD27, CD71, and CD11c, and reduced CD21, and their secreted antibodies blocked hemagglutination and neutralized viral infection. Antibodies cloned from AChR-capturing B cells derived from patients with myasthenia gravis bound specifically to the receptor on cell membrane. The approach is sensitive enough to detect antigen-specific B cells at steady state, and can be adapted for any membrane antigen.

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Quantitative reduction of RyR1 protein caused by a single-allele frameshift mutation in *RYR1* ex36 impairs the strength of adult skeletal muscle fibres

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Abstract

Here we characterized a mouse model knocked-in for a frameshift mutation in *RYR1* exon 36 (p.Gln1970fsX16) that is isogenic to that identified in one parent of a severely affected patient with recessively inherited multiminicore disease. This individual carrying the *RYR1* frameshifting mutation complained of mild muscle weakness and fatigability. Analysis of the RyR1 protein content in a muscle biopsy from this individual showed a content of only 20% of that present in a control individual. The biochemical and physiological characteristics of skeletal muscles from RyR1Q1970fsX16 heterozygous mice recapitulates that of the heterozygous parent. RyR1 protein content in the muscles of mutant mice reached 38% and 58% of that present in total muscle homogenates of fast and slow muscles from wild-type (WT) littermates. The decrease of RyR1 protein content in total homogenates is not accompanied by a decrease of $Ca_v1.1$ content, whereby the $Ca_v1.1$/RyR1 stoichiometry ratio in skeletal muscles from RyR1Q1970fsX16 heterozygous mice is lower compared to that from WT mice. Electron microscopy (EM) revealed a 36% reduction in the number/area of calcium release units accompanied by a 2.5-fold increase of dyads (triads that have lost one junctional sarcoplasmic reticulum element); both results suggest a reduction of the RyR1 arrays. Compared to WT, muscle strength and depolarization-induced calcium transients in RyR1Q1970fsX16 heterozygous mice muscles were decreased by 20% and 15%, respectively. The RyR1Q1970fsX16 mouse model provides mechanistic insight concerning the phenotype of the parent carrying the RYR1 ex36 mutation and suggests that in skeletal muscle fibres there is a functional reserve of RyR1.

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TGF-β Upregulated Mitochondria Mass through the SMAD2/3→C/EBPβ→PRMT1 Signal Pathway in Primary Human Lung Fibroblasts

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Abstract

Tissue remodeling of subepithelial mesenchymal cells is a major pathologic condition of chronic obstructive pulmonary disease and asthma. Fibroblasts contribute to fibrotic events and inflammation in both airway diseases. Recent mechanistic studies established a link between mitochondrial dysfunction or aberrant biogenesis leading to tissue remodeling of the airway wall in asthma. Protein arginine methyltransferase-1 (PRMT1) participated in airway wall remodeling in pulmonary inflammation. This study investigated the mechanism by which PRMT1 regulates mitochondrial mass in primary human airway wall fibroblasts. Fibroblasts from control or asthma patients were stimulated with TGF-β for up to 48 h, and the signaling pathways controlling PRMT1 expression and mitochondrial mass were analyzed. PRMT1 activity was suppressed by the pan-PRMT inhibitor AMI-1. The SMAD2/3 pathway was blocked by SB203580 and C/EBPβ by small interference RNA treatment. The data obtained from unstimulated cells showed a significantly higher basal expression of PRMT1 and mitochondrial markers in asthmatic compared with control fibroblasts. In all cells, TGF-β significantly increased the expression of PRMT1 through SMAD2/3 and C/EBPβ. Subsequently, PRMT1 upregulated the expression of the mitochondria regulators PGC-1α and heat shock protein 60. Both the inhibition of the SAMD2/3 pathway or PRMT1 attenuated TGF-β–induced mitochondrial mass and C/EBPβ and α-SMA expression. These findings suggest that the signaling sequence controlling mitochondria in primary human lung fibroblasts is as follows: TGF-β→SMAD2/3→C/EBPβ→PRMT1→PGC-1α. Therefore, PRMT1 and C/EBPβ present a novel therapeutic and diagnostic target for airway wall remodeling in chronic lung diseases.

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Cytochrome P450 enzymes contribute to the metabolism of LSD to nor-LSD and 2-oxo-3-hydroxy-LSD: Implications for clinical LSD use

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Abstract In recent years, experimental research on lysergic acid diethylamide (LSD) in humans has gained new momentum. In humans, LSD is metabolized rapidly into several metabolites but knowledge of the involved metabolizing enzymes is limited. The aim of the current study was to identify the cytochrome P450 (CYP) isoforms involved in the metabolism of LSD to 6-norlysergic acid diethylamide (nor-LSD) and 2-oxo-3-hydroxy-LSD (O-H-LSD) *in vitro*, in order to evaluate potential effects of enzyme polymorphisms or prescription drugs on LSD pharmacokinetics. Additionally, interactions of LSD and both metabolites with 5-hydroxytryptamine (5-HT) receptors were assessed.

LSD was incubated with human liver microsomes over 4 h and the production of nor-LSD and O-H-LSD was quantified by liquid chromatography tandem mass spectrometry. Metabolism was inhibited by the addition of specific CYP inhibitors. Additionally, recombinant CYPs were used to verify the inhibition results obtained with microsomes and induction of metabolism was investigated in human hepatocyte-derived cells. Radioligand binding and calcium mobilization assays were used to determine 5-HT receptor affinities and activities, respectively.

Human liver microsomes displayed minor metabolite formation (<1% metabolized) over 4 h. CYP2D6, 2E1, and 3A4 significantly contributed to the formation of nor-LSD, and CYP1A2, 2C9, 2E1, and 3A4 were significantly involved in the formation of O-H-LSD. These findings could be verified using recombinant CYPs. Enzyme induction with rifampicin distinctly increased the formation of both metabolites, whereas treatment with omeprazole only slightly increased formation of nor-LSD. LSD and nor-LSD were pharmacologically active at the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. Nor-LSD mainly differed from the parent compound by having a lower affinity to the 5-HT_{2C} receptor. O-H-LSD displayed substantially weaker affinity and activity at serotonergic receptors in comparison to LSD. To conclude, human liver microsomes converted only small amounts of LSD to nor-LSD and O-H-LSD but several CYPs significantly contributed. Genetic polymorphisms and drug interactions could therefore influence pharmacokinetics and pharmacodynamics of LSD. Nor-LSD likely has hallucinogenic activity similar to LSD, whereas O-H-LSD is inactive. Drug-drug interaction studies in humans are required to further assess the clinical relevance of these findings.

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Mechanisms of insulin resistance by simvastatin in C2C12 myotubes and in mouse skeletal muscle

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Abstract

Statins inhibit cholesterol biosynthesis and lower serum LDL-cholesterol levels. They are generally well tolerated, but can cause insulin resistance in patients. Therefore, we investigated the mechanisms underlying the statin-induced insulin resistance.

We used mice and C2C12 myotubes (murine cell line): mice (n = 10) were treated with oral simvastatin (5 mg/kg/day) or water (control) for 21 days and C2C12 cells were exposed to 10 μM simvastatin for 24 h.

After intraperitoneal glucose application (2 g/kg), simvastatin-treated mice had higher glucose but equal insulin plasma concentrations than controls and lower glucose transport into skeletal muscle. Similarly, glucose uptake by C2C12 myotubes exposed to 10 μM simvastatin for 24 h was impaired compared to control cells. In simvastatin-treated C2C12 myotubes, mRNA and protein expression of the insulin receptor (IR) β-chain was increased, but the phosphorylation (Tyr1361) was impaired. Simvastatin decreased numerically Akt/PKB Thr308 phosphorylation (via insulin signaling pathway) and significantly Akt/PKB Ser473 phosphorylation (via mTORC2), which was explained by impaired phosphorylation of mTOR Ser2448. Reduced phosphorylation of Akt/PKB impaired downstream phosphorylation of GSK3 , leading to impaired translocation of GLUT4 into plasma membranes of C2C12 myotubes. In contrast, reduced phosphorylation of AS160 could be excluded as a reason for impaired GLUT4 translocation.

In conclusion, simvastatin caused insulin resistance in mice and impaired glucose uptake in C2C12 myotubes. The findings in myotubes can be explained by diminished activation of Akt/PKB by mTORC2 and downstream effects on GSK3β, impairing the translocation of GLUT4 and the uptake of glucose.

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Non-immunological toxicological mechanisms of metamizole-associated neutropenia in HL60 cells

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Abstract

Metamizole is an analgesic and antipyretic, but can cause neutropenia and agranulocytosis. We investigated the toxicity of the metabolites N-methyl-4-aminoantipyrine (MAA), 4-aminoantipyrine (AA), N-formyl-4-aminoantipyrine (FAA) and N-acetyl-4-aminoantipyrine (AAA) on neutrophil granulocytes and on HL60 cells (granulocyte precursor cell line). MAA, FAA, AA, and AAA (up to 100 μM) alone were not toxic for HL60 cells or granulocytes. In the presence of the myeloperoxidase substrate H_2O_2, MAA reduced cytotoxicity for HL60 cells at low concentrations (<50 μM), but increased cytotoxicity at 100 μM H_2O_2. Neutrophil granulocytes were resistant to H_2O_2 and MAA. $Fe2^+$ and $Fe3^+$ were not toxic to HL60 cells, irrespective of the presence of H_2O_2 and MAA. Similarly, MAA did not increase the toxicity of lactoferrin, hemoglobin or methemoglobin for HL60 cells. Hemin (hemoglobin degradation product containing a porphyrin ring and $Fe3^+$) was toxic on HL60 cells and cytotoxicity was increased by MAA. EDTA, N-acetylcystein and glutathione prevented the toxicity of hemin and hemin/MAA. The absorption spectrum of hemin changed concentration-dependently after addition of MAA, suggesting an interaction between $Fe3^+$ and MAA. NMR revealed the formation of a stable MAA reaction product with a reaction pathway involving the formation of an electrophilic intermediate. In conclusion, MAA, the principle metabolite of metamizole, increased cytotoxicity of hemin by a reaction involving the formation of an electrophilic metabolite. Accordingly, cytotoxicity of MAA/hemin could be prevented by the iron chelator EDTA and by the electron donors NAC and glutathione. Situations with increased production of hemin may represent a risk factor for metamizole-associated granulocytopenia.

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Insulin prevents and reverts simvastatin-induced toxicity in C2C12 skeletal muscle cells

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Abstract

Simvastatin is an inhibitor of the 3-hydroxy-3-methylglutaryl-CoA reductase used for decreasing low density lipoprotein (LDL)-cholesterol in patients. It is well-tolerated but can cause myopathy. Our aims were to enlarge our knowledge regarding mechanisms and effects of insulin on simvastatin-associated myotoxicity in C2C12 myotubes. Simvastatin (10 μM) reduced membrane integrity and ATP content in myotubes treated for 24 hours, which could be prevented and partially reversed concentration- and time-dependently by insulin. Furthermore, simvastatin impaired the phosphorylation of Akt (Protein Kinase B) mainly at Ser473 and less at Thr308, indicating impaired activity of the mammalian Target of Rapamycin Complex 2 (mTORC2). Impaired activation of Akt increased mRNA expression of the muscle atrophy F-Box (MAFbx), decreased activation of the mammalian Target of Rapamycin Complex 1 (mTORC1) and stimulated apoptosis by impairing the Ser9 phosphorylation of glycogen synthase kinase 3β. Decreased phosphorylation of Akt at both phosphorylation sites and of downstream substrates as well as apoptosis were prevented concentration-dependently by insulin. In addition, simvastatin caused accumulation of the insulin receptor β-chain in the endoplasmic reticulum (ER) and increased cleavage of procaspase-12, indicating ER stress. Insulin reduced the expression of the insulin receptor β-chain but increased procaspase-12 activation in the presence of simvastatin. In conclusion, simvastatin impaired activation of Akt Ser473 most likely as a consequence of reduced activity of mTORC2. Insulin could prevent the effects of simvastatin on the insulin signaling pathway and on apoptosis, but not on the endoplasmic reticulum (ER) stress induction.

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Para-Halogenation Affects Monoamine Transporter Inhibition Properties and Hepatocellular Toxicity of Amphetamines and Methcathinones

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Halogenated derivatives of amphetamine-type stimulants are appearing on the drug market, often with altered pharmacological profile and sometimes different legal status compared to the non-halogenated substances. The aim of the present study was to investigate the pharmacological profile and hepatocellular toxicity of *para*-halogenated amphetamines and cathinones. The potential of amphetamine, 4-fluoroamphetamine, 4-chloroamphetamine, methcathinone, 4-fluoromethcathinone, and 4-chloromethcathinone to inhibit the monoamine transporters for norepinephrine, dopamine, and serotonin was determined in transporter-transfected human embryonic kidney 293 cells. Cell membrane integrity, ATP content, oxygen consumption rate, and superoxide levels were measured in human hepatoma HepG2 cells after exposure to the substances for 24 h. All compounds inhibited the norepinephrine transporter at submicromolar concentrations and the dopamine transporter at low micromolar concentrations. The selectivity of the compounds to inhibit the dopamine *versus* serotonin transporter decreased with increasing size of the *para*-substituent, resulting in potent serotonin uptake inhibition for the halogenated derivatives. All substances depleted the cellular ATP content at lower concentrations (0.25–2 mM) than cell membrane integrity loss occurred (≥0.5 mM), suggesting mitochondrial toxicity. The amphetamines and 4-chloromethcathinone additionally impaired the mitochondrial respiratory chain, confirming mitochondrial toxicity. The following toxicity rank order for the *para*-substituents was observed: chloride > fluoride > hydrogen. In conclusion, *para*-halogenation of stimulants increases the risk for serotonergic neurotoxicity. Furthermore, *para*-halogenation may increase hepatic toxicity mediated by mitochondrial impairment in susceptible users.

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Antisense oligonucleotide targeting CD39 improves anti-tumor T cell immunity

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Background: Cancer cells are known to develop mechanisms to circumvent effective anti-tumor immunity. The two ectonucleotidases CD39 and CD73 are promising drug targets, as they act in concert to convert extracellular immune-stimulating ATP to adenosine. CD39 is expressed by different immune cell populations as well as cancer cells of different tumor types and supports the tumor in escaping immune recognition and destruction. Thus, increasing extracellular ATP and simultaneously reducing adenosine concentrations in the tumor can lead to effective anti-tumor immunity.
Methods: We designed locked nucleic acid (LNA)-modified antisense oligonucleotides (ASOs) with specificity for human or mouse CD39 that do not need a transfection reagent or delivery system for efficient target knockdown. Knockdown efficacy of ASOs on mRNA and protein level was investigated in cancer cell lines and in primary human T cells. The effect of CD39 knockdown on ATP-degrading activity was evaluated by measuring levels of ATP in tumor cell supernatants and analysis of T cell proliferation in the presence of extracellular ATP. The in vivo effects of CD39-specific ASOs on target expression, anti-tumor immune responses and on tumor growth were analyzed in syngeneic mouse tumor models using multi-color flow cytometry.
Results: CD39-specific ASOs suppressed expression of CD39 mRNA and protein in different murine and human cancer cell lines and in primary human T cells. Degradation of extracellular ATP was strongly reduced by CD39-specific ASOs. Strikingly, CD39 knockdown by ASOs was associated with improved $CD8^+$ T cell proliferation. Treatment of tumor-bearing mice with CD39-specific ASOs led to dose-dependent reduction of CD39-protein expression in regulatory T cells (Tregs) and tumor-associated macrophages. Moreover, frequency of intratumoral Tregs was substantially reduced in CD39 ASO-treated mice. As a consequence, the ratio of $CD8^+$ T cells to Tregs in tumors was improved, while PD-1 expression was induced in CD39 ASO-treated intratumoral $CD8^+$ T cells. Consequently, CD39 ASO treatment demonstrated potent reduction in tumor growth in combination with anti-PD-1 treatment.
Conclusion: Targeting of CD39 by ASOs represents a promising state-of-the art therapeutic approach to improve immune responses against tumors.

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Journal for ImmunoTherapy of Cancer (2019) 7:16 IF 3.826

A novel anti-HER2 anthracycline-based antibody-drug conjugate induces adaptive anti-tumor immunity and potentiates PD-1 blockade in breast cancer

Lucia D'Amico[1†], Ulrike Menzel[2†], Michael Prummer[3], Philipp Müller[1,7], Mélanie Buchi[1], Abhishek Kashyap[1], Ulrike Haessler[2], Alexander Yermanos[2], Rémy Gébleux[4], Manfred Briendl[4], Tamara Hell[4], Fabian I. Wolter[4,8], Roger R. Beerli[4], Iva Truxova[5], Špíšek Radek[5], Tatjana Vlajnic[6], Ulf Grawunder[4], Sai Reddy[2] and Alfred Zippelius[1*]

Abstract

Increasing evidence suggests that antibody-drug conjugates (ADCs) can enhance anti-tumor immunity and improve clinical outcome. Here, we elucidate the therapeutic efficacy and immune-mediated mechanisms of a novel HER2-targeting ADC bearing a potent anthracycline derivate as payload (T-PNU) in a human HER2-expressing syngeneic breast cancer model resistant to trastuzumab and ado-trastuzumab emtansine. Mechanistically, the anthracycline component of the novel ADC induced immunogenic cell death leading to exposure and secretion of danger-associated molecular signals. RNA sequencing derived immunogenomic signatures and TCRβ clonotype analysis of tumor-infiltrating lymphocytes revealed a prominent role of the adaptive immune system in the regulation of T-PNU mediated anti-cancer activity. Depletion of CD8 T cells severely reduced T-PNU efficacy, thus confirming the role of cytotoxic T cells as drivers of the T-PNU mediated anti-tumor immune response. Furthermore, T-PNU therapy promoted immunological memory formation in tumor-bearing animals protecting those from tumor rechallenge. Finally, the combination of T-PNU and checkpoint inhibition, such as α-PD1, significantly enhanced tumor eradication following the treatment. In summary, a novel PNU-armed, HER2-targeting ADC elicited long-lasting immune protection in a murine orthotopic breast cancer model resistant to other HER2-directed therapies. Our findings delineate the therapeutic potential of this novel ADC payload and support its clinical development for breast cancer patients and potentially other HER2 expressing malignancies.

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International Journal of Molecular Sciences 2019, 20, 875 IF 3.687

IgE Downregulates PTEN through MicroRNA-21-5p and Stimulates Airway Smooth Muscle Cell Remodeling

Lei Fang [1], Xinggang Wang [2], Qingzhu Sun [1,3], Eleni Papakonstantinou [1,4], Chongteck S'ng [5], Michael Tamm [1], Daiana Stolz [1] and Michael Roth [1,*]

Abstract

The patho-mechanism leading to airway wall remodeling in allergic asthma is not well understood and remodeling is resistant to therapies. This study assessed the effect of immunoglobulin E (IgE) in the absence of allergens on human primary airway smooth muscle cell (ASMC) remodeling in vitro. ASMCs were obtained from five allergic asthma patients and five controls. Proliferation was determined by direct cell counts, mitochondrial activity by expression of cytochrome c, protein expression by immunoblotting and immuno-fluorescence, cell migration by microscopy imaging, and collagen deposition by cell based ELISA and RNA expression by real time PCR. Non-immune IgE activated two signaling pathways: (i) signal transducer and activator of transcription 3 (STAT3)→miR-21-5p→downregulating phosphatase and tensin homolog (PTEN) expression, and (ii) phosphatidylinositol 3-kinases (PI3K)→protein kinase B (Akt)→mammalian target of rapamycin (mTOR)→ribosomal protein S6 kinase beta-1 (p70s6k)→peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC1-α)→peroxisome proliferator-activated receptor-γ (PPAR-γ)→cyclooxygenase-2 (COX-2)→mitochondrial activity, proliferation, migration, and extracellular matrix deposition. Reduced PTEN expression correlated with enhanced PI3K signaling, which upregulated ASMC remodeling. The inhibition of microRNA-21-5p increased PTEN and reduced mTOR signaling and remodeling. Mimics of microRNA-21-5p had opposing effects. IgE induced ASMC remodeling was significantly reduced by inhibition of mTOR or STAT3. In conclusion, non-immune IgE alone is sufficient for stimulated ASMC remodeling by upregulating microRNA-21-5p. Our findings suggest that the suppression of micoRNA-21-5p may present a therapeutic target to reduce airway wall remodeling.

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Molecular Toxicological Mechanisms of Synthetic Cathinones on C2C12 Myoblasts

Xun Zhou [1], Dino Luethi [1], Gerda M. Sanvee [1], Jamal Bouitbir [1,2], Matthias E. Liechti [1,2] and Stephan Krähenbühl [1,2,*]

Synthetic cathinones are popular psychoactive substances that may cause skeletal muscle damage. In addition to indirect sympathomimetic myotoxicity, these substances could be directly myotoxic. Since studies in myocytes are currently lacking, the aim of the present study was to investigate potential toxicological effects by synthetic cathinones on C2C12 myoblasts (mouse skeletal muscle cell line). We exposed C2C12 myoblasts to 3-methylmethcathinone, 4-methylmethcathinone (mephedrone), 3,4-methylenedioxymethcathinone (methylone), 3,4-methylenedioxypyrovalerone (MDPV), alpha-pyrrolidinovalerophenone (α-PVP), and naphthylpyrovalerone (naphyrone) for 1 or 24 h before cell membrane integrity, ATP content, mitochondrial oxygen consumption, and mitochondrial superoxide production was measured. 3,4-Methylenedioxymethamphetamine (MDMA) was included as a reference compound. All investigated synthetic cathinones, as well as MDMA, impaired cell membrane integrity, depleted ATP levels, and increased mitochondrial superoxide concentrations in a concentration-dependent manner in the range of 50–2000 µM. The two pyrovalerone derivatives α-PVP and naphyrone, and MDMA, additionally impaired basal and maximal cellular respiration, suggesting mitochondrial dysfunction. Alpha-PVP inhibited complex I, naphyrone complex II, and MDMA complex I and III, whereas complex IV was not affected. We conclude that, in addition to sympathetic nervous system effects and strenuous muscle exercise, direct effects of some cathinones on skeletal muscle mitochondria may contribute to myotoxicity in susceptible synthetic cathinone drugs users.

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Nose to back: Compatibility of nasal chondrocytes with environmental conditions mimicking a degenerated intervertebral disc

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Abstract

Nasal chondrocytes (NCs) have gained increased recognition for cartilage tissue regeneration. To assess NCs as a source for cell therapy treatment of intervertebral disc (IVD) degeneration, tissue-forming properties of NCs under physiological conditions mimicking the degenerated IVD were compared to those of mesenchymal stromal cells (MSCs) and articular chondrocytes (ACs), two cell sources presently used in clinical trials. Cells were cultured in a combination of low glucose, hypoxia, acidity and inflammation for 28 d. Depending on the conditions, cells were either cultured in the absence of instructive growth factors or underwent chondrogenic instructional priming by addition of transforming growth factor β1 (TGFβ1) for the first 7 d. Histology, immunohistochemistry, biochemistry, enzyme-linked immunosorbent assay (ELISA) and quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) analyses demonstrated limited cell maintenance and accumulation of cartilaginous extracellular matrix for MSCs in IVD conditions. ACs maintained a steady accumulation of glycosaminoglycans (GAGs) throughout all non-acidic conditions, with and without priming, but could not synthesise type II collagen (Col2). NCs accumulated both GAGs and Col2 in all non-acidic conditions, independent of priming, whereas MSCs strongly diminished their GAG and Col2 accumulation in an inflamed environment. Supplementation with inflammatory cytokines or an acidic environment affected NCs to a lower extent than ACs or MSCs. The data, overall indicating that in an inflamed IVD environment NCs were superior to ACs and MSCs, encourage further assessment of NCs for treatment of degenerative disc disease.

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Pharmacological characterization of the aminorex analogs 4-MAR, 4,4´-DMAR, and 3,4-DMAR

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Abstract

4,4´-Dimethylaminorex (4,4´-DMAR) is a novel psychoactive substance (NPS) that appeared on the illicit drug market in addition to the psychostimulant 4-methylaminorex (4-MAR). Both substances are methylated derivatives of aminorex, an amphetamine-like anorectic used in the 1960ies and withdrawn from the marked due to severe cardiovascular toxicity. The aim of the present study was to characterize the *in vitro* pharmacological profiles of 4-MAR, 4,4´-DMAR, and 3,4-dimethylaminorex (3,4-DMAR, direx). We assessed norepinephrine (NE), dopamine (DA), and serotonin (5-HT) transporter inhibition potencies and monoamine release in transporter-transfected human embryonic kidney (HEK) 293 cells. We also assessed monoamine receptor and transporter binding affinities. 4,4´-DMAR potently inhibited all monoamine transporters (IC_{50}<1 µM) with greater potency than 3,4-methlyenedioxymethamphetaime (MDMA) and displayed a higher serotonergic over dopaminergic preference, relatively similar to MDMA (DA transporter / 5-HT transporter inhibition ratio of 0.4 and 0.08 for 4,4´-DMAR and MDMA, respectively). In contrast, 4-MAR preferentially inhibited the NE and DA transporter, exhibiting a pharmacological profile more similar to amphetamine. Both 4-MAR and 4,4´-DMAR were also substrate releasers at the DAT. 3,4-DMAR only weakly inhibited the NE transporter and showed no relevant activity at the DA and 5-HT transporter. Binding affinities of all three aminorex derivatives at various monoamine receptors were negligible (K_i values >2 µM). The *in vitro* pharmacological profiles indicate that 4,4´-DMAR has comparable psychoactive properties and serotonergic toxicity to MDMA and may be more potent. 4-MAR is a psychostimulant similar to amphetamine or methamphetamine. 3,4-DMAR likely has only weak psychostimulant properties.

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European Journal of Pharmacology Volume 855, (2019) 103-111 IF 3.040

Monoamine receptor interaction profiles of 4-aryl-substituted 2,5-dimethoxyphenethylamines (2C-BI derivatives)

Dino Luethi[a], Robert Widmer[a], Daniel Trachsel[b], Marius C. Hoener[c], Matthias E. Liechti[a,*]

Abstract

Many ring-substituted phenethylamines exert psychedelic effects that are thought to be primarily mediated by interactions with serotonergic 5-hydroxytryptamine 2 (5-HT_{2A}) receptors. The 2,5-dimethoxyphenethylamine (2C derivative) core structure with small lipophilic substituents at the 4-position seems to be particularly favorable for psychedelic effects. In contrast, 2C derivatives with bulky lipophilic substituents at the 4-position of the phenyl ring tend to display antagonist behavior at serotonin 5-HT2 receptor sites. To gain a better understanding of agonist and antagonist behavior of substituted phenethylamines, binding affinities and functional activation and inhibition of a series of 4´-aryl substituted 2,5-dimethoxyphenethylamine (2C-BI derivatives) at various monoamine receptors were determined. In addition, the interactions of the compounds with monoamine transporters were assessed. Various 2C-BI derivatives potently bound to human serotonergic and adrenergic receptors and to rat and mouse trace amine-associated receptor 1. Additionally, 2C-BI-8 and 2C-BI-12 activated serotonin 5-HT_{2A} and 5-HT_{2B} receptors at submicromolar concentrations. 2C-BI-1 and 2C-BI-7 were the only 2C-BI derivatives to activate human trace amine-associated receptor 1. 2C-BI-3 and 2C-BI-4 interacted with monoamine transporters but with low overall potency. In conclusion, the tested 2C-BI derivatives displayed diverse pharmacological profiles. The relatively high affinities of various 2C-BI derivatives at the serotonin 5-HT_{2A} receptor indicate a high steric tolerance of the binding pocket. Potent partial activation of the serotonin 5-HT_{2A} receptor by 2C-BI-8 and 2C-BI-12 suggests that these substances may potentially exert psychedelic effects similar to other compounds of the 2C family.

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Above you can find the abstracts of recent articles published by members of the DBM. The abstracts are grouped according to the impact factor of the journal where the work appeared. To be included, the papers must meet the following criteria:

1. The first author, last author or corresponding author (at least one of them) is a member of the DBM.
2. Department of Biomedicine and University of Basel affiliation must be mentioned in authors list as published by the journal.
3. The final version of the article must be available (online pre-publications will be included when the correct volume, page numbers etc. becomes available).

We are focussing on original publications. Due to page constraints, abstracts of publications that appeared in lower ranked journals may not be able to be included. Review articles are generally not considered, unless they appeared in the very top journals (e.g. Cell, Science, Nature, NEJM, etc.). The final decision concerning inclusion of an abstract will be made by the chair of the Department of Biomedicine.

If you wish that your article will appear in the next issue of DBM Facts please submit a pdf file to the Departmental Assistant, Manuela Bernasconi: manuela.bernasconi@unibas.ch

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Congratulations

Solène Rea Breitling Ritz
Geboren am 19.Dezember 2018

Emma Piscuoglio
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Yannick Handschin
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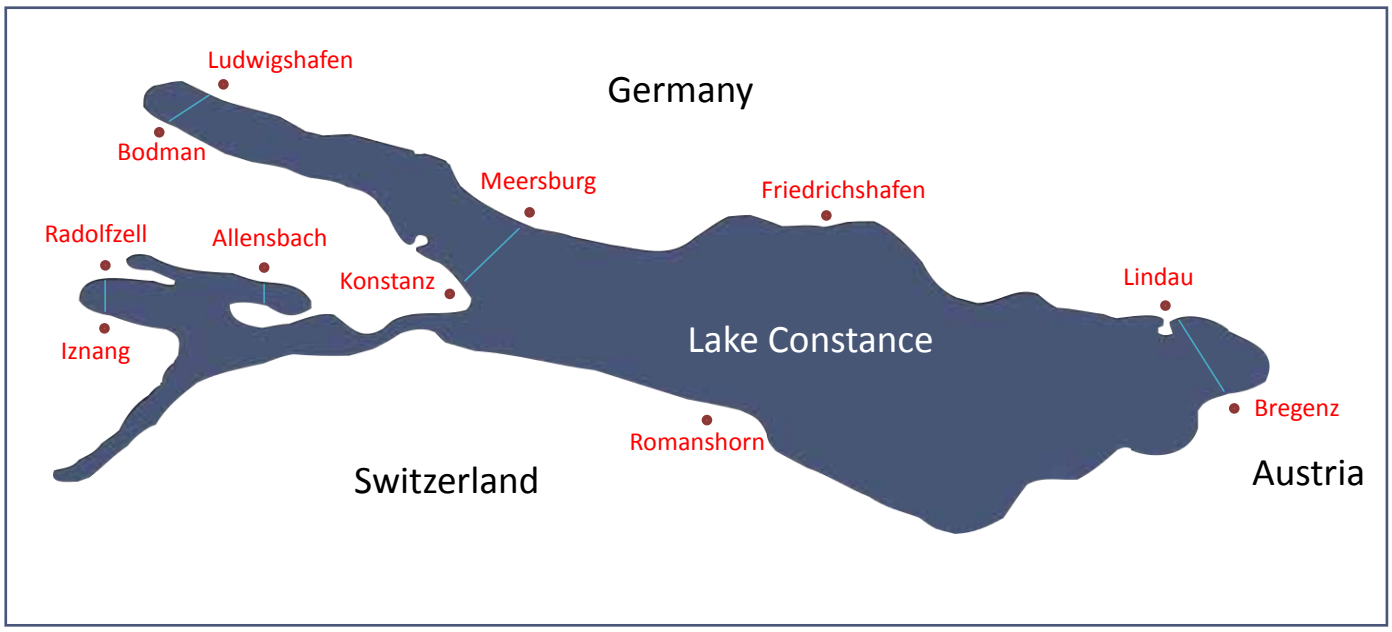
Livia Nuciforo
Geboren am 16.Juni 2019

Lucy Mileah Siegrist Codilupi
Geboren am 14. April 2019

Herzlich willkommen, allerseits!

Sommerzeit ist Seezeit
Summertime – Time for Open Water Swim Races

And, there are a lot of possibilities on Lake Constance. One such possibility is between the beautiful little town Radolfzell, where I live, and the village Iznang, located on the peninsula Höri. Last year 111 swimmers plunged into the lake – the youngest participant being only 13 years old, and the oldest participant 80 years old. The weather was great and water temperature was just perfect around 22°C, not too cold and not too warm. The swimmers were transferred by the organisers to the starting point in Iznang by buses. A few years ago, a pontoon ferry was still in use, so the participants could sail across the lake, and in 2015 it even had a brass band on board. Unfortunately the ferry was scrapped. The total swimming distance is approximately 2.5 km and the fastest swimmer



arrived at the finish point just after 28 min. Instead of rushing, myself and my friends just enjoyed the opportunity to swim safely across the lake. For security reasons each participant wears a red cap and numerous boats and volunteers are observing the race. If someone is not feeling well she/he will get fished out by an accompanying boat. After 63 min we reached the lido of Radolfzell and were welcomed with an applause and a cup of hot tea. After the last swimmer had arrived the award ceremony started and every participant who finished the race received a completion certificate.

This year, unfortunately, a thunderstorm led to a cancellation of this event. But the other open water races between Ludwigshafen – Bodman (2.3 km), Konstanz – Meersburg (5 km), Allensbach – Reichenau (1.5 km) and many more locations, could be conducted. If you like swimming, I really recommend you to attend one of the open water swimming events on Lake Constance. They are a lot of fun.

Vera Lorenz

Today: Šime Brkić, Myeloid Malignancies

Hi everyone! My name is Šime and I am a Postdoc in the Myeloid Malignancies group. I have been at DBM since 2013 when I started my PhD in the Cell and Gene Therapy group. Here, I will present my home country Croatia to you. Let's start with the facts about Croatia (or *Hrvatska* in Croatian – I know, it is a tough one to pronounce). The country has a particular shape and it is said it resembles a dragon. Croatia is located in southeast Europe and its population stands at around 4.2 million (little more then half of the population of Switzerland). The country's capital is Zagreb and approximately one quarter of the population lives in Zagreb and its metropolitan area. Croatia is known as a tourist destination due to its beautiful coast and nature which attract more then 10 million tourists per year. With more then 1100 islands (50 of which are inhabited) it is a perfect sailing destination. In a relatively small country with the surface of 56 594 km² you can find wide fertile planes in the northeast, mountains in the central part and the coast on the Adriatic sea. It is rich in water resources so Croatia has the third highest freshwater reserves per capita in Europe.

Croatia had a very rich and turbulent history. Over the centuries, different parts of its territory were under Austrian, Hungarian, French or Venetian governance. Today, Croatia is an EU member state. All the

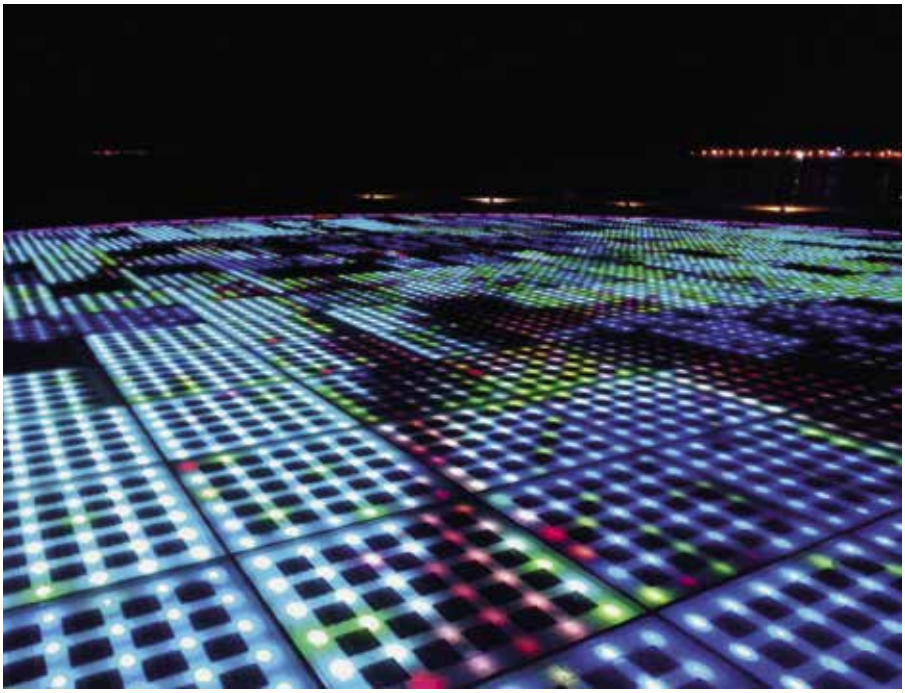
Top: Zadar

Bottom: Sea Organ

Left: Dubrovnik

cultures left something behind, and in addition to architecture, the dialects of the Croatian language are also reminiscent of the past. The dialects from the northern part of Croatia will thus contain a lot of Germanisms, while the ones on the coast have a lot words with Italian origin.

My hometown Zadar is located on the Adriatic coast, in the Dalmatia region. That makes me a Dalmatian, but without the black spots. Dalmatian islands are quite unique as they run parallel to the coast. As this is rare, in geography, this type of coastline is called the Dalmatian type. Zadar is almost 3000 years old and it is the longest continuously inhabited city in Croatia. It is home to the Sea Organ (*Morske orgulje*), a one of a kind set of pipes that have been installed within the sea pier and that produce a melody when hit by a wave. The city of Zadar is known to have scenic sunsets, so during one visit to the city, Winston Churchill said that Zadar had the most beautiful sunset in the world. Thus, a special installation, called The Greeting to the Sun (*Pozdrav Suncu*), has been built next to the



Sea Organ. It is composed of solar panels that absorb light during the day and emit it in different colours during the night. Next time when in Zadar, come at sunset and enjoy.

Even though Croatia is a small country, it has a good pool of athletes. Croatian athletes are to in the world in different disciplines such as football, handball, water polo and skiing. Croatian skier Janica Kostelić is the only woman to have won four gold medals in alpine skiing at the Winter Olympics. The Croatian national football team achieved great success at the last World Cup by winning a silver medal. People in Croatia cheered so passionately during the semi-final match that the local seismologists in Zagreb could detect the shaking of the Earth's surface after the goal that brought Croatia to final was scored.

Every person that lives close to the seaside will tell you that the sea has a unique energy and one creates a special connection with it. As a child, I spent my time on the beach during summer fishing and thereby learning about different sea organisms. The sea holds endless secrets and it was the perfect environment to feed the desire for exploration that I have had since I was a child. The sea is also a rich source of food

Top: Greeting to the Sun

Middle: Octopus Meal

Bottom left: Šime Brkić young days

Bottom middle: Peka

Bottom right: Kornati

and many sea organisms are very tasty. I especially enjoy octopus or squid when prepared traditionally, under the iron bell, called *peka*, that is covered with fire. Paired with some potatoes they make a very delicious meal.

Coffee is a very important part of the Croatian culture. People can sit for hours and chat over a coffee. They will discuss anything and everything. An invitation to a coffee is appropriate for almost any form of social meeting. It can be a casual meeting of friends, family get-together, or it can be an important business meeting or even a job interview. Even though people in Croatia invite each other to grab a coffee, maybe this coffee will turn out into unexpected night-out. Next time when in Croatia, and you see bars full of people, remember, coffee in Croatia is much more than just a coffee.

Did you know?

- Around 10% of the Croatian territory is protected as a national or natural park.
- Croatia is the birthplace of the tie (kravata).
- King's Landing in the popular TV series "Game of Thrones" is actually the Croatian city of Dubrovnik.
- The White House in the USA was, in part, built with the stones from the Croatian island of Brač.
- Nikola Tesla was born in Croatia.

Basic expressions in Croatian

Bok	Hi
Hvala	Thank you
Molim	Please
Da/Ne	Yes/No
Kako se zoveš?	What's your name?
Kako si?	How are you?
Hoćemo na kavu?	Shall we go for a coffee?

« Hast Du einen Feind,
suche ihn im Schatten Deiner Hütte. »

Aus Afrika