Preisverleihung 2021

STIFTUNG PROFESSOR DR. MAX CLOËTTA

Heft Nr. 49

Prof. Dr. Bart Deplancke

«A technology-centric view of how our genome encodes cellular and phenotypic diversity»

Prof. Dr. Anne Müller

«The two faces of *Helicobacter pylori*»

STIFTUNG
PROFESSOR DR. MAX CLOËTTA

achtundvierzigste Preisverleihung

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INHALT

VORWORT

Prof. Dr. Fritjof Helmchen

Es ist uns eine ausserordentliche Freude, die diesjährige Feier zur Verleihung des Cloëtta-Preises in Zürich wie gewohnt mit vielen Gästen persönlich durchführen zu können, nachdem die letztjährige Veranstaltung aufgrund der Corona-Pandemie notgedrungen «hybrid» stattfand, also im kleinsten Kreis mit Preisträgerin und Preisträger und mit einer Online-Übertragung der Feier und Festvorträge. Ich denke, die meisten von uns spüren nach mehr als einem Jahr Einschränkungen wieder ein sehr grosses Bedürfnis nach direkter Teilnahme an Veranstaltungen und dem unmittelbaren persönlichen Austausch, sei es im Familienkreis, im Berufsumfeld oder bei kulturellen Ereignissen. Die Lockerungen der Massnahmen zur Eindämmung der Corona-Pandemie, auch mithilfe des Covid-Zertifikats, sind daher sehr zu begrüssen und werden, hoffentlich bald, in eine – wenn auch vielleicht neue – Normalität führen. Nichtsdestotrotz wird uns das Corona-Virus weiterhin begleiten, und es hat unserer Gesellschaft deutlich vor Augen geführt, wie grundlegend wichtig der medizinische Fortschritt ist, um bei akuten wie auch bei schwelenden gesundheitlichen Gefährdungen wirkungsvolle Antworten parat zu haben. Die Stiftung Prof. Dr. Max Cloëtta hat sich ganz der Unterstützung und Förderung der medizinischen Forschung in der Schweiz verpflichtet und leistet seit bald fünf Jahrzehnten Aussergewöhnliches in diesem Bereich, insbesondere auch durch die Vergabe von Fortbildungsstipendien «Klinische Medizin Plus» an junge Mediziner und Medizinerinnen. Es ist ja die heutige Nachwuchsgeneration, welche zukünftig mit neuen Herausforderungen konfrontiert sein wird. Je besser ausgebildet diese nächste Mediziner-Generation ist und je mehr ihre Forschungstätigkeiten neben der Beanspruchung im klinischen Alltag gefördert werden und möglich bleiben, desto besser aufgestellt wird unsere Gesellschaft als Ganzes sein.

Wir freuen uns sehr, auch dieses Jahr zwei junge Forscherpersönlichkeiten mit dem Cloëtta-Preis 2021 auszuzeichnen in Würdigung ihrer beeindruckenden wissenschaftlichen Leistungen und ihrer herausragenden Beiträge zum Erkenntnisgewinn in der Medizin in ganz unterschiedlichen Forschungsbereichen.

Prof. Dr. Anne Müller von der Universität Zürich ist eine ausgewiesene Helicobacter-Expertin. Die Besiedlung des menschlichen Magens durch das Bakterium *Helicobacter pylori* ist eine der häufigsten chronischen bakteriellen Infektionen. Neben Geschwüren kann eine Helicobacter-Infektion auch Krebs und andere Krankheitsbilder auslösen. Doch warum geschieht dies nur manchmal und hat die Helicobacter-Besiedlung vielleicht auch Vorteile? Frau Prof. Müller wird uns über ihre spannenden Forschungsergebnisse auf der Molekular- und Zellebene in diesem Gebiet berichten.

Prof. Dr. Bart Deplancke von der EPFL, Lausanne, untersucht in seinen Forschungsarbeiten, wie aus den genetischen Bauplänen die enorme Vielfalt an Zellen und komplexen Organismen erwachsen kann. Neue Technologien und bioinformatische Analysetechniken, an deren Entwicklung Prof. Deplancke beteiligt war, erlauben es nun, die molekularen Regelmechanismen, welche das Auslesen der genetischen Information umsetzen, im Detail zu verstehen. Prof. Deplancke wird uns dies am Beispiel der Entstehung von Fettzellen und Fettgewebe erläutern.

Ende dieses Jahres wird Prof. Adriano Fontana, langjähriger Klinikdirektor der Klinik für Immunologie am Universitätsspital Zürich, aus dem Stiftungsrat ausscheiden. Er hat mit seinem breiten wissenschaftlichen Wissen und vor allem auch seiner klinischen Kompetenz über viele Jahre die Arbeit der Cloëtta Stiftung entscheidend mitgeprägt, insbesondere auch als Präsident des Stiftungsrats von 2011 bis 2017. Lieber Adriano, wir danken Dir von Herzen für Deinen grossartigen Einsatz und wir werden Dich wahrlich vermissen!

Erfreulicherweise konnten wir mit Prof. Sabine Werner von der ETH Zürich bereits eine hervorragende und breit vernetzte Forscherin als Nachfolgerin für den Stiftungsrat gewinnen. Sie ergänzt mit ihrer Expertise den wissenschaftlichen Ausschuss des Stiftungsrats perfekt, und wir freuen uns auf die gemeinsame Zusammenarbeit.

Zum Schluss ein grosses Dankeschön an Anja Witte, die im April 2021 zur neuen Geschäftsführerin der Stiftung Prof. Dr. Max Cloëtta gewählt wurde. Dazu nochmals herzlichen Glückwunsch! Bei ihr laufen alle Fäden zusammen, und sie vermag es grossartig, daraus ein tragfähiges und sinnvolles Gewebe zu flechten. Für ihr grosses Engagement, ihre hervorragende Administration aller Stiftungsgeschäfte, und insbesondere ihre kreativen, vorausschauenden Ideen und Anregungen sind wir ihr sehr dankbar.

Anja Witte

Geschäftsführerin

Stiftungsrat

Die Zusammensetzung des aktuellen Stiftungsrates aus sechs hochkarätigen Medizinprofessorinnen und -professoren und drei anerkannten Experten auf dem Gebiet der Finanzen und des Rechts bewährt sich weiterhin. Ende Jahr kommt es zu einem Wechsel im Stiftungsrat: Prof. emer. Dr. med. Adriano Fontana scheidet nach 13 Jahren, in denen er von 2011 bis 2017 die Präsidentschaft innehatte, mit Erreichen seines 75. Altersjahres statutenkonform aus, und Prof. Dr. Sabine Werner tritt neu in den Stiftungsrat ein, wo sie ebenfalls Mitglied des Wissenschaftlichen Ausschusses sein wird.

Adriano Fontana, Cloëtta-Preisträger von 1993, bereichert den Stiftungsrat seit 2008 mit seinem fundierten Fachwissen, seinen pragmatischen und weitsichtigen Lösungsansätzen sowie seinem unermüdlichen Einsatz für klinisch tätige Forscherinnen und Forscher. Er und seine Expertise werden im Stiftungsrat schmerzlich vermisst werden, und es ist zu hoffen, dass die Stiftung ihn bei Bedarf auch weiterhin kontaktieren darf. An dieser Stelle ein herzliches und dickes Dankeschön für all die im Sinne des Stiftungszweckes geleistete Arbeit!

Mit Sabine Werner kommt eine weitere Cloëtta-Preisträgerin (2008) und exzellente Wissenschafterin in den Stiftungsrat. Neben ihrer Tätigkeit als ordentliche Professorin für Zellbiologie an der ETH Zürich und Vize-Präsidentin des Departements Biologie ist Sabine Werner gewähltes Mitglied der European Academy of Sciences (EURASC), der European Molecular Biology Organization (EMBO) und der Leopoldina (Deutsche Akademie der Wissenschaften). Weiterhin ist sie Mitglied des Stiftungsrats des Schweizerischen Nationalfonds, des Senats der Helmholtz-Gemeinschaft und des wissenschaftlichen Beirats der Krebsliga Schweiz und der Wilhelm Sander Stiftung. Wir freuen uns ausserordentlich, dass Sabine Werner, trotz ihres bereits vielfältigen Engagements, zugesagt hat, den Stiftungsrat bei der Unterstützung und Förderung der medizinischen Forschung sowie der damit verbundenen naturwissenschaftlichen Hilfsdisziplinen in der Schweiz und im Ausland zu verstärken.

Ende Mai erreichte uns die traurige Nachricht vom Hinschied unseres Cloëtta-Preisträgers von 1991 und ehemaligen Stiftungsratsmitglieds Prof. Dr. med. Peter J. Meier-Abt. Von 2010 bis 2014 hat er die Geschicke der Stiftung Prof. Dr. Max Cloëtta massgeblich mitgeformt und sich unentwegt dafür eingesetzt, dass in der Klinik tätige Ärzte sich auch im Gebiet der medizinischen Forschung entwickeln konnten. Sein Einsatz für den wissenschaftlichen Nachwuchs, sein immenses Fachwissen, sein unerschöpfliches Engagement, seine Weitsicht und sein Interesse für den einzelnen Menschen behalten wir in bester Erinnerung und sind ihm dafür von Herzen dankbar.

Einmal mehr bedanken wir uns ausdrücklich bei den Mitgliedern des Stiftungsrates, die engagiert ihr Fachwissen und ihre Erfahrung einbringen, sowie bei den Expertinnen und Experten, deren Gutachten die Entscheidungsfindung auch bei der Auswahl der Cloëtta-Preisträger unterstützen. Erst diese breit abgestützte Kompetenz ermöglicht es der Stiftung, ihren Zweck wirkungsvoll umzusetzen.

Cloëtta-Preis

Der Stiftungsrat und die Geschäftsstelle freuen sich, 2021 zwei hochkarätige Preisträger aus der medizinischen Grundlagenforschung mit dem Cloëtta-Preis auszuzeichnen: Der erste Preis geht an Prof. Dr. Bart Deplancke, ordentlicher Professor am Institut für Bioengineering und Vizedekan für Innovation an der School of Life Sciences der ETH Lausanne. Mit Prof. Dr. Anne Müller wird eine ordentliche Professorin und Direktorin am Institut für Molekulare Krebsforschung der Universität Zürich geehrt. Unser herzlicher Dank gilt den Verantwortlichen der Universität Zürich, wo wir dieses Jahr zu Gast sein dürfen, und ihrem Vertreter in unserem Stiftungsrat, Prof. Dr. Fritjof Helmchen, für die tatkräftige Unterstützung bei der Organisation der diesjährigen Preisverleihung.

Forschungsstellen

Die Forschungsstellen der Stiftung Prof. Dr. Max Cloëtta sind für den akademischen Mittelbau in der Schweiz von grosser Bedeutung. Finanziert werden Stellen an schweizerischen Hochschulen, Kliniken oder Instituten für bereits ausgebildete und selbstständig arbeitende Forscherinnen und Forscher bis max. 40 Jahre. Mit diesem Programm will die Stiftung einem Mangel an Forschernachwuchs in der Schweiz entgegenwirken und den Stelleninhabenden helfen, die manchmal nicht einfache Phase bis zur Berufung auf eine ordentliche Professur zu überbrücken. Die Stipendien werden alle zwei Jahre vergeben, im Jahr 2021 wurden zwei neue Stellen aus der Ausschreibung 2020 besetzt.

2021 finanzierte die Stiftung Prof. Dr. Max Cloëtta folgende Forschende an Schweizer Universitäten mit dreieinhalb- bis fünfjähriger Unterstützungsperiode:

Dr. Sophie Croizier, 1984, Universität Lausanne,
Center for Integrative Genomics.
Projekt: «Stress Regulation of Energy Metabolism»
Unterstützungsdauer: 1.9.2021–31.08.2026

Dr. András Jakab, 1985, Universitäts-Kinderspital Zürich,
Center for MR-Research.
Projekt: «From axons to therapy: Characterizing the connectivity of the human thalamus with 3D multi-scale imaging»
Unterstützungsdauer: 1.10.2020–31.12.2024

Dr. Mathias Hauri-Hohl, 1975, Universitäts-Kinderspital Zürich,
Abt. Stammzellentransplantation.
Projekt: «Improving T-cell reconstitution and enhancing central tolerance mechanism in hematopoietic stem cell transplantation»
Unterstützungsdauer: 1.1.2016–31.5.2021 (Sistierung 1.4.2018–31.8.2018)

Dr. Paula Nunes-Hasler, 1980, Universität Genf,
Institut für Pathologie und Immunologie.
Projekt: «Exploring the ER-phagosome connection during antigen cross-presentation»
Unterstützungsdauer: 1.10.2019–30.9.2024

Dr. Salvatore Piscuoglio, 1982, Universität Basel,
Departement Biomedizin.
Projekt: «Biomarker identification to guide surgical intervention after neoadjuvant chemoradiotherapy in rectal cancer»
Unterstützungsdauer: 1.7.2021–30.6.2026

Dr. Alexandre Theocharides, 1975, UniversitätsSpital Zürich,
Klinik für Hämatologie.
Projekt: «The role of cell-extrinsic factors in hematopoietic stem cell malignancies»
Unterstützungsdauer: 1.6.2015–30.9.2021 (Sistierung 1.9.2019–31.12.2020)

Dr. Grégory Verdeil, 1976, Universität Lausanne,
Abteilung für fundamentale Onkologie und Ludwig Cancer Centre.
Projekt: «Finding and characterizing new targets to overcome T cell exhaustion for immunotherapy of cancer»
Unterstützungsdauer: 1.8.2017–30.4.2021

Klinische Medizin Plus

Seit 2010 vergibt die Stiftung Prof. Dr. Max Cloëtta Stipendien «Klinische Medizin Plus». Medizinerinnen und Medizinern werden während oder unmittelbar nach Abschluss ihrer Facharztausbildung Stipendien von drei bis maximal zwölf Monaten für die Absolvierung einer Spezialausbildung an einer renommierten, vornehmlich ausländischen Institution ausgerichtet.

2021 kommen folgende Medizinerinnen und Mediziner in den Genuss eines Stipendiums:

Dr. med. Zacharenia Kallinikou, 1984, Junior Staff, Kardiologie,
Universitätsspital und Universität Fribourg.
Projekt: Trainingkurs in Cardiovascular Magnetic Resonance (CMR)
Guest Institution: CMR Unit, Royal Brompton Hospital, London, UK,
1.5.2021–31.7.2021

Dr. med. Guillaume Maitre, 1985, Fellow in Critical Care Medicine, Hôpital du Valais, Sion.
Projekt: Clinical advanced pediatric critical care fellowship
Guest Institution: McGill University, Montreal, Canada, 1.1.2021–31.12.2021

Dr. med. Thomas Nestelberger, 1986, Resident, Klinische Kardiologie am Universitätsspital Basel.
Projekt: Clinical Research Fellowship: Incidence, Predictors, Biochemical Signatures and Prognostic Value of Spontaneous Coronary Artery Dissection
Guest Institution: Vancouver General Hospital and University of British Columbia in Vancouver, Kanada, 1.7.2020–30.06.2021

Dr. med. Stergios Tsartsalis, 1988, Resident Physician, Department of Psychiatry, Geneva University Hospitals.
Projekt: Research fellowship: Transcriptomic investigation of microglial and astrocyte populations in Alzheimer's disease: a human brain post-mortem single-nucleus RNA sequencing study
Guest Institution: Department of Brain Sciences, Imperial College London, Department of Brain Sciences, London, UK, 1.7.2021–31.10.2021

Dr. med. Julia Velz, 1983, Resident, Klinik für Neurochirurgie am UniversitätsSpital Zürich.
Projekt: 1. Specialized training to gain knowledge and expertise in the field of Pediatric Neurosurgery
2. Investigation of the underlying genetical and immunological mechanisms in medulloblastoma, the most common malignant pediatric brain tumor
Guest Institution: Department of Pediatric Neurosurgery at Hôpital Necker-Enfants Malades, Paris, Frankreich, 1.7.2020–30.6.2021

Wechsel in der Geschäftsführung

Seit Anfang 2019 darf ich die Stiftung Prof. Dr. Max Cloëtta in ihrer Fördertätigkeit vonseiten Geschäftsstelle unterstützen. Im September 2020 hat Brigitt Küttel nach fast 25 Jahren ihren Rücktritt als Geschäftsführerin bekannt gegeben und dem Stiftungsrat empfohlen, mir die Geschäftsführung ad interim zu übertragen. Im April 2021 habe ich dankend und mit Stolz die Wahl zur Geschäftsführerin der Stiftung Prof. Dr. Max Cloëtta angenommen. Ich freue mich ausserordentlich, gemeinsam mit dem engagierten Stiftungsrat und der Geschäftsstelle die erfolgreiche Arbeit der Stiftung fortzuführen und sie für die Zukunft aufzustellen.

Grosse Anerkennung und mein aufrichtiger Dank gehen an meine Vorgängerin, Brigitt Küttel. Sie hat die Geschäfte der Stiftung seit 1996 mit Umsicht und Sorgfalt geführt und wird immer Teil der Cloëtta-Family bleiben.

Diese Cloëtta-Family von aktuellen und ehemaligen Preisträgerinnen und Preisträgern, Stipendiatinnen und Stipendiaten und allen aktuellen und ehemaligen Mitgliedern des Stiftungsrates darf 2023 das 50-jährige Bestehen der Stiftung feiern. Die Vorbereitungen dafür sind bereits angelaufen, und wir freuen uns schon jetzt darauf, alle am 28. September 2023 zum grossen Symposium im Swiss Re Centre for Global Dialogue in Rüschlikon zu begrüssen!

THE CLOËTTA PRIZE 2021
IS AWARDED TO

PROFESSOR

BART DEPLANCKE

BORN IN 1975 IN KORTRIJK, BELGIUM

INSTITUTE OF BIOENGINEERING AND VICE-DEAN OF INNOVATION
AT THE SCHOOL OF LIFE SCIENCES
AT ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

FOR HIS GROUND-BREAKING CONTRIBUTIONS
TO THE FIELD OF BIOENGINEERING AND SYSTEMS BIOLOGY AND
TO THE ELUCIDATION OF KEY MECHANISMS OF OBESITY

ZURICH, 26TH NOVEMBER 2021

IN THE NAME OF THE FOUNDATION BOARD:

THE PRESIDENT

THE VICE PRESIDENT

A MEMBER

BART DEPLANCKE

PERSONAL INFORMATION

Bart DEPLANCKE, PhD; Date of birth: 21/08/1975; Nationality: Belgian; Web site: http://deplanckelab.epfl.ch/ Researcher unique identifier(s) (ORCID): 0000-0001-9935-843X

EDUCATION

2002	Ph.D. in Immunobiology (Division of Nutritional Sciences), University of Illinois, USA
1998	M.S. in Biochemical Engineering, Ghent University, Belgium
1995	B.S. in Bio-engineering, Ghent University, Belgium

EMPLOYMENT HISTORY

2020–present	Full Professor in Systems Biology and Genetics, Institute of Bioengineering, SV, EPFL
2018-present	Vice-Dean of Innovation at the School of Life Sciences (SV), Ecole Polytechnique Féderale de Lausanne (EPFL), Switzerland
2013–present	Swiss Institute of Bioinformatics Group Leader
2014–2019	Associate Professor in Systems Biology and Genetics, Institute of Bioengineering, SV, EPFL
2007–2014	Assistant Professor in Systems Biology and Genetics, Institute of Bioengineering, School of Life Sciences, EPFL (CH)
2003–2007	Postdoctoral Fellow, Program in Gene Function and Expression, Program in Molecular Medicine, University of Massachusetts Medical School, Worcester (USA)

2002–2003	Postdoctoral Fellow, Department of Cancer Biology in Dana-Farber Cancer Institute & Department of Genetics, Harvard Medical School, Boston (USA)
1998–2002	Research Fellow, Division of Nutritional Sciences, University of Illinois (USA)
1997–1998	Research Assistant, Department of Biochemical & Microbial Technology, Ghent University (BE)

INSTITUTIONAL RESPONSIBILITIES (a selection)

Vice-Dean of Innovation at the School of Life Sciences (since 2018)

EPFL Innovation Council (INC) (since 2018)

Faculty Direction Member (since 2018)

Member of the **Doctoral School in Quantitative and Computaional Biology Reviewing Committee** (since 2018)

Main Coordinator of the **Catalyse4Life EPFL SV Research Innovation Program** (since 2017)

EPFL Life Sciences SV-IT Steering Committee Member (2017–present)

Main Coordinator of the 2016 EPFL School of **Life Sciences Audit**

SUPERVISION OF > 20 GRADUATE STUDENTS AND 15 POSTDOCTORAL FELLOWS (since 2008)

TEACHING ACTIVITIES (summary)

Genetics & Genomics (4 credits; 7x3 h; 3rd year Bachelor); Single Cell Biology (4 credits; 7x3 h; 1st year Master)

GOVERNING ACTIVITIES

Search Committee Member for candidates of Assistant Professorships at the EPFL (Bioinformatics, Bio- engineering, BioMEMS, BioPhotonics, Biostatistics, Cancer, Informatics & Communication, Metabolism, Microbiology, Statistics); INSERM ATP-Avenir (France; Genetics & Genomics); KU Leuven (Belgium); Institute of Technology (Italy; Systems and synthetic biology); ETH Zurich (Systems Biology & Bioinformatics); University of Lausanne (Computational Biology; Switzerland) and University of Fribourg (Environmental Biology / Omics)

Ad-hoc paper reviewer (since 2006, a selection): Cell, Development, eLife, Genome Biology, Genome Research, Molecular Cell, Molecular Systems Biology, Nature, Nature Biotechnology, Nature Cell Biology, Nature Communications, Nature Genetics, Nature Methods, Nature Medicine, Nature Reviews Genetics, Science

Editorial Activities: Editorial Board Member of BMC Genomics and Nucleic Acids Research; 2017 Issue of Current Opinion in genetics and Development (Genome architecture and Expression topic)

Member: 1) Center for Organismal Studies Scientific Advisory Board, University of Heidelberg (from 2019); 2) Health 2030 Genome Center Strategic board, Geneva, CH (from 2018); 3) "Integrative Biology of the Cell" Research Center Scientific Advisory Board, Paris-Saclay University (from 2018); 4) Swiss National Science Foundation National Research Council (from 2017); 5) Lausanne Integrative Metabolism Network Association Board of Directors (from 2017); 6) The ETH Domain Strategic Focus Area "Personalized Health and Related Technologies" Executive Committee (from 2017)

Reviewer for Grant Proposals and Programs: 1) Member of the Swiss National Science Foundation National Research Council, 2017-present; 2) Member of the Advanced Postdoc Mobility fellowship Evaluation commission of the Swiss National Science Foundation (SNSF, since 2014); 3) Reviewer of Starting, Advanced and Synergy ERC grants (since 2016); 4) National Infrastructures in Health and Biology Reviewer for the Investments for the Future (ANR) Program (France, 2016); 5) Functional Genomics Center Zürich (2014), 6) Swedish Foundation for Strategic Research (www.stratresearch.se); 7) FWO grants and postdoctoral fellowships (Belgium); 8) SNSF-based proposals: Div III (Biology and Medicine), Interdisciplinary Research, Sinergia, Ambizione

ORGANISATION OF SCIENTIFIC MEETINGS

1) Organizing Committee Member of the 2019 European Drosophila Research Conference, Lausanne (CH), September 2019; 2) Organizing Committee Member of the First (Leuven (Belgium), December 2017) and Second (Janelia Research Campus (USA), March 2019) Fly Cell Atlas meetings; 3) Organizing Committee Chairman for the 2017 International SystemsX.ch Conference (Zürich, CH); 4) Organizing Committee member of the 2010, 2015 & 2016 EPFL International Life Science Symposia; 5) Co-organizer (with Prof. Jeff Jensen, EPFL) of the Conference "Systems Genetics and Evolution of Non-human (Model) Organisms" (>100 participants), Ascona, CH (2014)

INVITED PRESENTATIONS (> 100 at International Symposia or Institutes, > 60 in the last 5 years)

FELLOWSHIPS AND AWARDS

2021 Cloëtta Prize for outstanding contributions to biomedical research.

Highest Level SNSF Project Grant Rating with invitation to **Bonus of Excellence** Program (2018)

Elected to the **National Research Council (2017) of the Swiss National Science Foundation**

EPFL Teaching Ambition Award (2012) for dedication to undergraduate teaching

Peter Reeds Young Investigator Award for 2005 by the American Society for Nutritional Sciences;

Henri Benedictus-BAEF Fellow of the King Baudouin Foundation and the Belgian American Educational Foundation in Biomedical Engineering, 2002–2003

College of Agricultural, Consumer and Environmental Sciences Doctoral Student Research Award for best PhD Thesis, University of Illinois, 2001.

CURRENT FUNDING ID

2020–2024 – Principal PI – SNSF Project Grant – Uncovering novel molecular principles underlying regulatory variation using variable chromatin modules;

2020–2021 – Principal PI – EPFL Open Science Fund – ASAP: an open, robust and interactive web-based portal for (single cell) omics analyses;

2019–2021 - Collaboration (2 groups, PI: Christian Wolfrum) – Precision Health and related Technologies (PHRT) Pioneer Grant - Adipose tissue heterogeneity and function in the development of metabolic diseases;

2020–2024 – H2020 – Innovative Training Network – ENHPATHY: Molecular basis of human enhanceropathies

2019–2023 – Consortium (5 groups, PI: Bart Deplancke) – Swiss National Science Foundation (SNSF) Sinergia – Elucidating the human mesenchymal bone marrow stromal hierarchy in health and disease;

2019–021 – Consortium (4 groups, PI: Christian Wolfrum) – PHRT Project Grant – Targeting the brown fat: Personalized strategies for treatment of metabolism;

2019–2022 – Principal PI – SNSF Project Grant – Dissecting the molecular and physiological function of Aregs in white adipose biology.

PUBLICATION RECORD

As of August 2021: h-index: 51; >10.7k citations (Source: Google Scholar); 114 total publications 88 research papers (36 as last author), 9 reviews, 3 perspectives, 6 protocols and 8 consortium-based.

Complete List: https://scholar.google.ch/citations?user=EMV2SUMAAAAJ&hl=en

TRANSLATIONAL ACTIVITIES

Co-founder Alithea Genomics (2020): a company specializing in high-throughput transcriptomics solutions for drug screening and biobank functionalization. Winner of the 2021 Swiss-wide VentureKick competition.

Co-founder and Former Chairman and Board Member of Genohm SA (2011): a company delivering software (big data management) products and services for pharma and R&D labs (www.genohm.com). Acquired by Agilent Technologies in May 2018.

Patents:

Patent application entitled **"High Throughput One-Hybrid System"**. Inventors: University of Massachusetts; Dana Farber Cancer Institute. Inventors: A.J.M. Walhout, M. Vidal, **B. Deplancke**. International publication number: **WO2005/005960**.

Patent application entitled **"Microfluidic device and method for isolation of nucleic acids"**. Inventors: A. Isakova, **B. Deplancke**. Applicant: Ecole Polytechnique Fédérale de Lausanne (EPFL). International publication number: **WO2016/059619**.

Patent application entitled **"Soft microbotic device for high throughput single cell studies"**. Inventors: Johannes Bues, Riccardo Dainese, Marjan Biocanin, **B. Deplancke**. Applicant: Ecole Polytechnique Fédérale de Lausanne (EPFL). International publication number: **WO2018/051242**.

Patent application (provisonal) entitled: **"A microfluidic device for rapid, multiplexed, bead-less, chromatin immunoprecipitation with on-chip DNA processing"**. Inventors: Riccardo Dainese, **B. Deplancke**. Applicant: Ecole Polytechnique Fédérale de Lausanne (EPFL). Application number: **PCT/IB2017/057889 (2017)**.

SELECTED PUBLICATIONS

(*, first author; #, corresponding)

M. Litovchenko, A.C.A Meireles-Filho, M.V. Frochaux, R.P.J Bevers, A. Prunetto, A.M. Anduaga, B. Hollis, V. Gardeux, V.S. Braman, J.M.C. Russeil, S. Kadener, M. Dal Peraro, **B. Deplancke**. Extensive tissue-specific expression variation and novel regulators underlying circadian behavior, ***Science Advances, 7***:eabc3781, 2021.

R.P.J. Bevers*, M. Litovchenko*, A. Kapopoulou, V.S. Braman, V.M. Lemos Da Silva, M.R. Robinson, J. Auwerx, B. Hollis, **B. Deplancke**. Mitochondrial haplotypes affect metabolic phenotypes in the *Drosophila* Genetic Reference Panel, *bioRxiv,* 2018; ***Nature Metabolism, 1***:226–1242, 2019.

W. Chen*, P.C. Schwalie*, E.V. Pankevich, C. Gubelmann, S.K. Raghav, R. Dainese, M. Cassano, M. Imbeault, S. Min Jang, J. Russeil, T. Delessa, D. Trono, C. Wolfrum, **B. Deplancke**. ZFP30 promotes adipogenesis through the KAP1-mediated activation of a retrotransposon-derived *Pparg2* enhancer, ***Nature Communications, 10***:1809, 2019.

P.C. Schwalie*, H. Dong*, M. Zachara*, J. Russeil, D. Alpern, N. Akchiche, C. Caprara, W. Sun, K.U. Schlaudraff, G. Soldati, C. Wolfrum#, **B. Deplancke#**. A stromal cell population that inhibits adipogenesis in mammalian fat depots, ***Nature***, 559:103–108, 2018.

Isakova, R. Groux, M. Imbeault, P. Rainer, D. Alpern, R. Dainese, A. Giovanna, D. Trono, P. Bucher, **B. Deplancke**. SMiLE-seq identifies binding motifs of single and dimeric transcription factors. ***Nature Methods, 14***:316–322, 2017

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A TECHNOLOGY-CENTRIC VIEW OF HOW OUR GENOME ENCODE CELLULAR AND PHENOTYPIC DIVERSITY

B. Deplancke[1,2]

Summary

Our genome is a sublime, but also mysterious biological entity that, despite being shared among all cells of our body, gives rise to enormous cellular heterogeneity. Moreover, the genome of one human is on average 99.9% identical to that of an unrelated individual, yet there is great phenotypic variation in the human population, for example in height, fitness, aging and susceptibility to disease. The overarching, underlying question is how does our genome manage to produce such stunning cellular and phenotypic diversity, even though the set of genetic instructions that is encoded by our genome is virtually identical between cell types and mostly so between human individuals? This is clearly a fundamental question in biology and one that has already occupied numerous labs in the world, including my own, for many years. While initially hampered by a general lack of adequate experimental and analytical tools, efforts to better understand the function of our genome were greatly boosted by the availability of the human genome DNA sequence at the turn of this century. This in turn spurred a technological and analytical revolution, resulting in the development of tools and resources that allowed us to holistically probe our genome in the context of development, homeostasis and disease, first at the bulk tissue level, but increasingly in recent years at the single cell level. In this review, I summarize how in the now 15 years of its existence, my lab contributed to this revolution, developing both novel methods and analytical tools that allowed us to gain new insights into the inner workings of this beautiful set of DNA molecules: our genome.

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Introduction

The genome, our book of life

Our genome is a most beautiful book, one that: "wrote itself, continually adding, deleting and amending over four billion years" (Matt Ridley, Author of "The Genome")

The genome is the fascinating genetic material that one can find in almost all cells of the body. We know that it contains all the instructions that are required to generate and maintain a specific organism, which is why it is often referred to as the book, or even better yet, the manual of life. Yet, how these instructions are encoded in the genome, also known as "the regulatory code", and how they are interpreted and executed by the non-DNA machinery in a cell remains poorly understood. To crack this regulatory code, a first requirement is of course to be able to assess the content of the manual. This is why the release of the nearly complete genome sequences of humans and model organisms such as the mouse and *Drosophila* in the beginning of the 21st century was such a fundamental turning point as it for the first time provided a glimpse at all the parts that may be required to build a complex organism such as a human, including the protein-coding genes, but also the so-called non-coding sequences that may control which genes are expressed where, when and at which level. Considerable progress has since been made to annotate the ~25,000 genes encoded by both the human and mouse genomes. However, much less is still known about the sequences, i.e. regulatory elements, and corresponding networks that control the expression of these genes. This is because we now realize that the regulatory code is cryptic and degenerate, in contrast to the genetic code, which renders it very difficult to infer function from sequence information alone.

In my lab, we have had a continuous interest in dissecting how readers of this regulatory code – proteins that are called transcription factors (TFs) and their co-regulators – work together and interact with the genome to interpret its embedded set of instructions that allow a cell to change its identity and for example become a new cell type (Chen et al., 2019; Deplancke, 2009; Gubelmann et al., 2014; Pradhan et al., 2017a; Raghav et

al., 2012). This is conceptually comparable to seeing a beautiful figure made out of distinct Lego blocks (the "genes") and trying to decipher what the instructions could be that enabled a person to create this figure, i.e. putting the appropriate type of Lego blocks on top of one another in the right configuration and right color (or "expressing the genes at the right time, level, and place") (**Fig. 1**). To assist the Lego enthusiast, a manual is typically provided in the Lego box, guiding the user to build his/her creation in step-wise fashion. Of course, our genome does not come with a manual and it is up to us, scientists, to reverse engineer its instructions. In other words, given a specific cell type, can we infer the underlying manual, i.e. its core parts, starting with regulatory elements and TFs, and how these parts together give rise to that cell type. The interactions between regulatory elements and TFs constitute gene regulatory networks (GRNs). The ultimate function of these networks is to coordinate the progression of distinct regulatory states in space and time, which lies at the heart of nearly all biological processes such as differentiation, cycling, responses to environmental stimuli etc. Thus, to understand how GRNs orchestrate differential gene expression programs underlying a biological process of interest and essentially define the manual of life, it is crucial to devise technologies that allow us to identify all implicated regulatory elements and corresponding TFs as well as how, when, and where they interact, a great challenge to which our lab has consistently contributed contributed (Alpern et al., 2019; Gubelmann et al., 2013; Hens et al., 2011; Isakova et al., 2017; Simicevic et al., 2013)(Dainese et al., 2020).

Fig. 1. The Lego Analogy. *While the fundamental building blocks (Lego pieces) are the same between these distinct creations, by tinkering with the number, shape and color of these pieces and with when they are used during the building process, one can create vastly different Lego animals. Similarly, while the genes are the same in each cell, by modulating which genes are expressed, where and when, very different cell types can be generated. This analogy even holds for within and between species' comparisons. Indeed, even between human and mouse, > 80% of the genes are shared, which is why already back in 1975, King and Wilson, when comparing human and chimpanzee genes, argued in their seminal Science paper that "their macromolecules are so alike that regulatory mutations may account for their biological differences" (King and Wilson, 1975). In other words, it is the regulatory code, the manual of life, that drives cellular heterogeneity and variations in this code result in phenotypic diversity and can even account for species' differences. Copyright@ Legotruman.*

notypic variation. Imagine that someone makes slight modifications in the Lego manual: how would the resulting Lego figure look like? The same? Better or worse? It turns out that in nature, all three scenarios are possible, but how a slight modification in the manual can induce an altered phenotype, e.g. differential disease susceptibility, is again poorly understood. This is not surprising given that, as indicated above, we still have a hard time deciphering the manual of life for one cell type, let alone the development of an entire organism or a variation thereof! Addressing this fundamental question is further complicated by the fact that environmental factors may also play a role in affecting a specific phenotype (e.g. smoking increases your risk of lung cancer). This is why in my lab, we

also study the effect of genomic variation not only in humans (Kilpinen et al., 2013; Waszak et al., 2015), but also in model organism populations such as that of the fly (Bevers et al., 2019; Bou Sleiman et al., 2020, 2015; Frochaux et al., 2020; Litovchenko et al., 2021) given that we can raise these animals in highly standardized settings, effectively eliminating environmental confounders.

Adipose tissue: our blanket of life

"The devil has put a penalty on all things we enjoy in life, either we suffer in health, or we suffer in soul, or we get fat" (Albert Einstein)

Humans, as other mammals, benefit from a subcutaneous fat layer that insulates and cushions the outer world, hence the long-lasting perception that this layer serves merely as a comfort blanket. However, the rapid rise in obesity, reaching pandemic levels, and especially its many co-morbidities such as diabetes, cardiovascular disease and even cancer, have forced the scientific community to revisit the physiological importance of adipose tissue. This has led to a true scientific (re)valorization of this prevalent tissue, recognizing not only its biomechanical and insulating roles but also its key involvement in systems metabolism & physiology, tissue growth, regeneration and repair, as well as innate immunity.

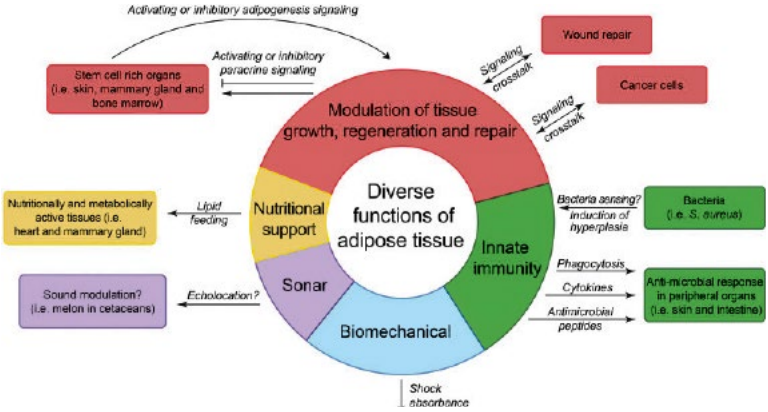

Fig. 2. Key functions of adipose tissue. *From (Zwick et al., 2018).*

While all of us can easily locate our respective fat layers and clearly recognize the biomedical importance of studying adipose biology, it may come as a surprise that we still have a relatively poor understanding of how adipocytes are formed, both in terms of their respective stem / precursor origin and the structure and function of adipogenic regulatory networks. This is partially due to the highly heterogeneous and unstructured nature of adipose tissue depots, which are present in multiple anatomical locations and consist of a mixture of different cell types, whose origin and identity differs between distinct fat depots. Nevertheless, adipogenesis is still one of the best studied differentiation paradigms, greatly aided by suitable *in vitro* models such as the mouse 3T3-L1 cells that closely mimic the molecular processes underlying especially the terminal phase of adipocyte differentiation. Consequently, much progress has been made in at least defining a core GRN that controls 3T3-L1 terminal adipogenesis. The first step in this process is triggered by the TFs C/EBPβ and δ, which are induced immediately after the addition of pro-adipogenic stimuli, but are initially inactive. Once a subsequent C/EBPβ-dependent clonal cell expansion round is completed, then these TFs activate the transcription of the pro-adipogenic master regulators PPARγ and C/EBPα. Indeed, the latter TFs are thought to control the expression of adipogenic genes and genes mediating cell cycle withdrawal. In addition, they also cross-regulate each other effectively maintaining a terminal differentiation state. Finally, no other factor has thus far been identified that can promote adipogenesis in the absence of PPARγ. However, while PPARγ is necessary, it is clearly not sufficient, implying the involvement of several other TFs that need to be integrated in the overall adipogenic GRN. This constituted the state of the art when back in 2007, we took it upon us to devise new technologies that would improve our ability to map mammalian GRNs, aiming to then specifically apply these technologies to study the GRNs' underlying adipogenesis. Little did we know then that this quest would take us well beyond the mapping of GRNs alone. Indeed, driven by new revolutionary single cell omic methods, we ventured into resolving adipose stem cell and precursor heterogeneity, uncovering a new cell type that may well fundamentally change our view of how adipose tissue development and homeostasis is controlled, as will be detailed below.

Thus, the principal questions that my lab aims to address are:

1. How the genome steers the development of specific cell types and adipocytes in particular.
2. How variation in this genome makes each of us different.

With this manuscript, I intend to review the technological contributions that my lab has made to addressing these questions as well as the scientific findings that emerged from our studies with respect to understanding genome function. I thereby restricted the biological scope of this review to our studies involving mouse and human models with a specific focus on our adipose biology work, which was honored with the Cloëtta Prize. I intentionally restricted the listed references primarily to our own papers, referring the reader for a more comprehensive and impartial representation of the pertinent literature to already published reviews of my group (Deplancke, 2009; Deplancke et al., 2016; Ferrero et al., 2020; Pradhan et al., 2017b) as well as references in our publications that are listed here.

Development of technologies to map (adipogenic) gene regulatory networks

The state of a cell is defined by its gene and ultimately protein expression profile, which itself depends on how the cell's entire regulatory circuit or network is wired at that point in time. Transitions to a different cell state (e.g. during differentiation or re-programming) are then mediated by transcriptional changes through circuit re-wiring. Thus, to understand cell behavior and function, we need to achieve a qualitative and quantitative understanding of the structural and dynamic properties of the underlying gene regulatory networks (GRNs) by identifying all implicated nodes (TFs and regulatory elements), and map the dynamic interactions, i.e. the regulatory edges, between them. To do so, we followed two complementary strategies:

i) Gene-centered:

The first strategy is gene-centered and aims to screen adipogenic regulatory elements (e.g. promoters, enhancers) and especially those linked to TF-coding genes for interacting TFs using a high-throughput version of

the yeast one-hybrid (Y1H) assay that I developed during my post-doctoral studies (Deplancke et al., 2004; Deplancke et al., 2006). The Y1H system is conceptually similar to the better-known yeast two-hybrid system, except that a DNA fragment is used as a bait together with a single hybrid protein. The DNA bait is cloned upstream of a Y1H reporter gene (e.g. *HIS3*) and integrated into the yeast genome. Thus, regulatory elements can be tested in a "more innate" chromatinized format for their ability to associate with specific TFs. This format increases the specificity, reduces the number of false positives, and therefore provides a straightforward validation method for *in vitro* or *in silico*-derived protein-DNA interaction datasets (Deplancke et al., 2004). For Y1H assays to be most effective, regulatory elements are ideally directly screened versus a library of TFs, which requires the availability of a comprehensive TF ORF (open-reading frame) resource for your model system of interest. A first, major project in my lab was therefore the generation of a versatile mouse TF resource, which now already contains over 1,000 (out of ~1550, [Gubelmann et al., 2013]) fully sequence-verified TF ORF clones. To subsequently enable the yeast-based screening of elements of interest versus this array of mouse TFs, we developed a cross-platform pipeline to experimentally analyze these elements for interacting TFs at unprecedented throughput and resolution (**Fig. 3**). Key here is the implementation of a microfluidic approach, MARE for Microfluidics-based Analysis of Regulatory Elements, following the Y1H screen that enables the fine-grained localization of TFs of interest within specific regulatory elements (Gubelmann et al., 2013; Hens et al., 2011). The MARE technique can be compared with a series of electrophoretic mobility shift assays (EMSAs), in which a TF is tested for its ability to bind to a collection of typically small DNA sequences, and relative DNA occupancy data for each sequence can be derived. Similar to EMSA, the MARE protocol starts with small DNA elements, resulting from the fragmentation of long regulatory DNA sequences, which are tested individually for binding to a specific set of TFs (e.g., those that were identified using our Y1H screens). However, MARE accommodates >700 EMSA-like assays at once on one microfluidic chip in a relatively straightforward and cost-effective manner. This in turn enables the generation of a relative DNA occupancy landscape for each TF of interest over the length of the respective regulatory element where the regions of highest occupancy likely

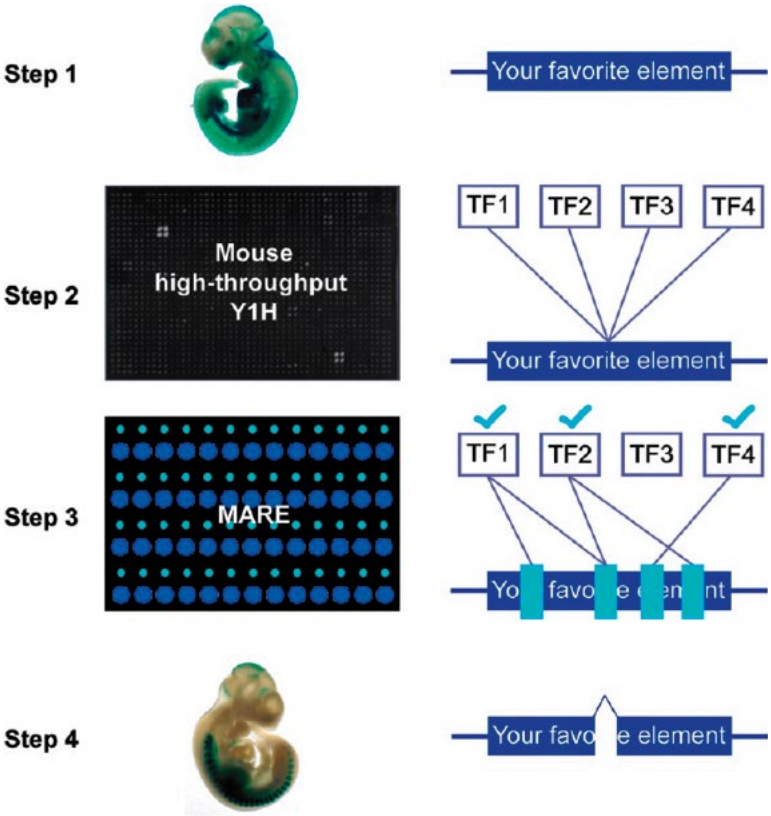

Fig. 3. Schematic overview of the pipeline employed to de-orphanize mammalian gene regulatory elements. *The regulatory element of interest is first cloned (Step 1), and then integrated into yeast to enable high-throughput Y1H screens leading to the identification of putatively interacting TFs (Step 2)(shown is a typical Y1H screening plate featuring two strongly "positive" TFs, which emerge as quadrants given that each TF is independently tested 4 times). In Step 3, MITOMI-based Analysis of Regulatory Elements (MARE, see* ***Research Aim 2*** *for more details) is performed to both validate (reflected by light blue check marks) and map the detected TF-DNA interactions (indicated by light blue boxes) within the respective regulatory element. Finally, small binding regions of interest can be deleted to examine the relevance of these DNA segments in mediating the in vivo activity of the regulatory element (Step 4). Taken from (Gubelmann et al., 2013).*

contain the respective TF binding sites. Thus, MARE allows the simultaneous validation and localization of protein-DNA interactions within regulatory elements, greatly increasing the DNA binding site resolution that Y1H screens can typically provide. As a proof-of-concept, we validated this pipeline using well-described regulatory elements and orphan enhancers, demonstrating that it enables the identification of known and novel mouse TF–DNA interactions that are relevant *in vivo* (Gubelmann et al., 2013).

ii) TF-centered:

SMiLE-seq

Our Y1H-based, gene-centered approach uniquely allows the screening of any regulatory element of interest for interacting TFs in high-throughput fashion. A clear drawback of this approach though is that, while interactions are tested *in cello* (i.e. yeast cells) in a chromatinized context, they are obviously not probed in their natural, cellular setting. In addition, having screened hundreds of regulatory elements and identified many more interacting TFs, we realized that many of these interactions could in fact be simply predicted *in silico* by computationally mapping the *in vitro-derived* DNA binding motifs of TFs to target sequences of interest. In other words, by analyzing regulatory elements of interest for the occurrence of small, often 8–12 bp long motifs that correspond to DNA binding preferences of TFs, we were able to predict the majority of TF-DNA interactions that our Y1H screen managed to pick up. Such computational analysis also suffers from disadvantages of course. For example, it is prone to false positives as such motifs are short and degenerate and it is therefore prudent to implement rather stringent motif matching parameters to avoid calling too many interactions. In addition, of the >1500 TFs that are encoded by the human or mouse genomes (making it the largest protein family), still a surprisingly high number of TFs (estimated to be >400) are still "orphan". This means that no DNA binding specificities have so far been derived for such TFs, despite the importance of characterizing these specificities to increase our understanding of the regulatory logic of a cell. The latter also rationalizes why tremendous efforts have already been invested involving various *in vitro* and *in vivo* techniques such as protein binding microarray (PBM), HT-SELEX, bacterial one-hybrid, or chromatin immunoprecipita-

tion (ChIP) coupled to sequencing (ChIP-seq) to map TF binding motifs. But despite these efforts, the current catalogue of individual, characterized TFs, let alone TF heterodimers or higher complexes, is still vastly incomplete and lacks quantitative insights. This realization led us to explore alternative protein-DNA interaction profiling strategies that would not only prove highly robust, quantitative, and superior to other, comparable assays in key DNA binding characterization parameters, but also semi-automated, fast, and thus easily implementable.

Leveraging our expertise in microfluidics and building on a clever experimental platform, MITOMI for Mechanically Induced Trapping Of Molecular Interactions that was developed by my colleague here at the EPFL, Prof. Sebastian Maerkl, while at Prof. Steve Quake's lab in Stanford, we developed a new TF DNA binding characterization technology, SMiLE-seq for Selective Microfluidic Ligand Enrichment coupled to sequencing (Isakova et al., 2017) (**Fig. 4**). We specifically engineered SMiLE-seq to complement existing TF-DNA binding profiling approaches by 1) producing binding models that tend to have superior predictive power compared to other *in vitro* models; 2) enabling the characterization of the DNA binding preferences of both monomers, homo- and heterodimers, and 3) studying the DNA binding specificities of TFs (e.g. C2H2-containing zinc fingers) that have so far consistently resisted comprehensive DNA binding characterization by other *in vitro* methods.

Given its ability to de-orphanize, difficult to probe TFs, SMiLE-seq is now being enlisted to investigate the remaining, roughly 400 uncharacterized human TFs, aiming to complete at least the "TF code". These efforts are currently part of a large consortium project led by Prof. Tim Hughes, called "Codebook", in which these 400 TFs are subjected to most available protein-DNA interaction profiling methods including protein-binding microarrays, HT-SELEX, ChIP-seq and thus SMiLE-seq. The resulting data resource, to my knowledge the largest of its kind to date, should be of great value, not only to bring the human TF motif catalogue near completion, but to also directly compare the strengths and weaknesses of the different methods themselves.

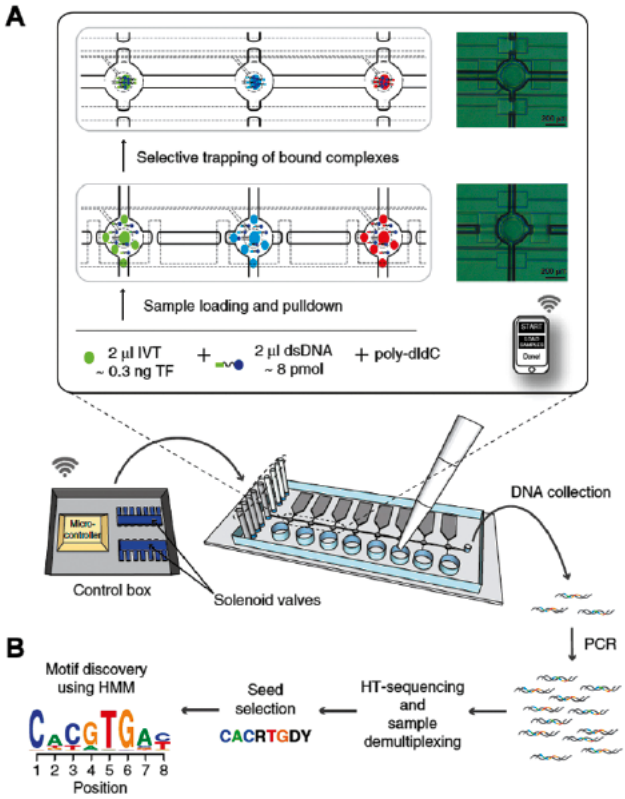

Fig. 4. *Adapted from (Isakova et al., 2017). A. Schematic representation of the experimental SMiLE-seq setup.* ***A*** *snapshot of three units of the microfluidic device is shown. In vitro transcribed and translated (IVT) bait TF, target dsDNA, and a nonspecific competitor poly-dIdC are mixed and pipetted in one of the wells of the microfluidic device. The mixtures are then passively pumped in the device for 20 min (bottom panel). Newly formed TF–DNA complexes are trapped under a flexible polydimethylsiloxane (PDMS) membrane, and unbound molecules as well as molecular complexes are washed away (upper panel). Left, schematic representation of three individual chambers. Right, corresponding snapshots of an individual chamber taken before and after mechanical trapping.* ***B.*** *TF motif discovery pipeline. The bound DNA is eluted from all the units of the device simultaneously and collected in one tube. Recovered DNA is amplified and sequenced as a 2–4% spike-in. The sequencing reads are then demultiplexed, and the seed sequence is identified for each sample (here using the algorithm MEME). This seed is then used as an input reference sequence for Hidden Markov Modeling (HMM)-based TF motif discovery.*

While the limitation of the Y1H assay is that protein-DNA interactions are probed outside their natural context, that of motif-based approaches is that any prediction is purely based on an *in silico* analysis. This is why a technology such as ChIP-seq has always remained the most popular protein-DNA interaction detection tool since it probes such interactions *in vivo* and provides thus direct read-outs of which TFs might be targeting which genes. Clear disadvantages of ChIP-seq, rationalizing the existence of other protein-DNA interaction detection tools, is the limited resolution as to where exactly a TF might be binding to DNA (although a rather novel tool such as exo-ChIP-seq managed to vastly improve this), the inability to demonstrate that the detected interaction is direct (as it could be mediated by collaborating TFs), and the slow and laborious nature of the experimental process. In my lab, we were routinely performing ChIP assays to profile the chromatin landscape in various cell types with the goal of identifying and characterizing regulatory elements or domains that may control gene expression in our biological processes of interest. Over time, we became however increasingly frustrated with the limited throughput and sensitivity of these ChIP assays as well as their high complexity. This prompted us to look for alternate solutions that would allow us to substantially simplify these ChIP-seq procedures. Building on our expertise in developing SMiLE-seq (Isakova et al., 2017), we started to investigate whether the SMiLE-seq approach used to trap and isolate DNA bound to *in vitro* expressed TFs could also be used to profile DNA bound by *in vivo* chromatin marks or TFs. Excitingly, after extensive microfluidic chip redesign and some additional tweaks and turns, we managed to develop a microfluidic ChIP-seq platform, dubbed "FloChIP" (Dainese et al., 2020), which can now be used to perform high quality epigenomic or TF binding assays on samples as small as 100 cells. In addition, the workflow is 10-20-fold faster than other available solutions, also because of its inherent modularity, for example by enabling to probe up to eight chromatin marks in parallel, starting from the same sample **(Fig. 5A)** or 8 samples in parallel, targeting one chromatin mark **(Fig. 5B)**. Finally, the platform also allows to sequentially ChIP samples, as such enabling for example to study the importance of bivalent marks in gene regulation.

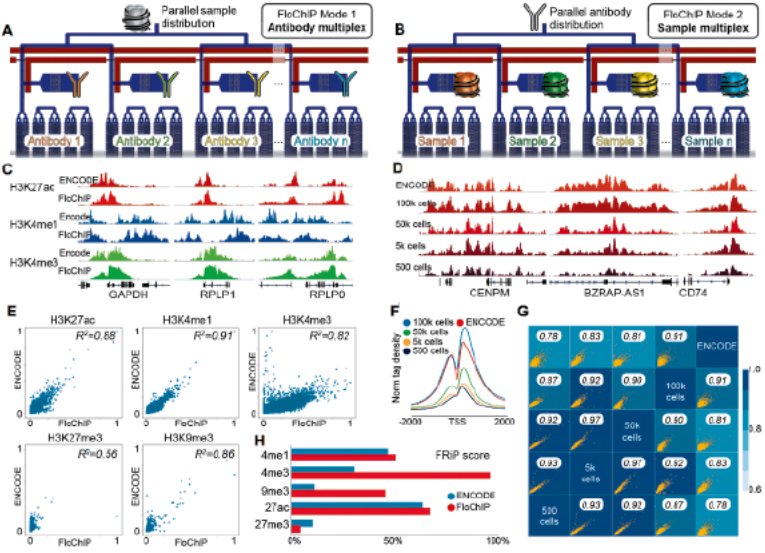

Fig. 5. A. *Schematic depiction of FloChIP's mode 1: antibody multiplex. Each IP lane is functionalized separately by introducing different antibodies through the individual inlets. During IP, one sample is introduced through the common inlet and distributed equally across all IP lanes, hence enabling multiple IPs involving distinct antibodies at once. B. Schematic depiction of FloChIP's mode 2: sample multiplex. One antibody solution is introduced through the common inlet and distributed equally across all IP lanes. During IP, each IP lane is loaded separately by introducing different samples through the individual inlets. Taken from (Dainese et al., 2020).*

Targeted, quantitative proteomic analysis of (adipogenic) TFs – TF DNA binding modeling

ChIP-seq provides valuable insights into the DNA binding landscape of focal TFs, yet how such landscapes are established remains relatively poorly understood, but are thought to at least partially reflect the DNA binding affinity and nuclear concentration of TFs. Consequently, deriving nuclear TF copy numbers has been of longstanding interest in regulatory genomics, but only few studies have so far provided estimates on the absolute *in vivo* abundance of TFs. This can be explained by the fact that TFs tend to be lowly expressed (at least compared to many other protein families), rendering them difficult to identify and specially to quan-

tify with standard shotgun liquid chromatography-mass spectrometry (LC-MS) approaches.

To alleviate this issue, we turned to a particularly sensitive MS-based technique termed Selected Reaction Monitoring (SRM), which features an excellent sensitivity by only targeting a subset of detectable peptides that are specific to the protein of interest (i.e. proteotypic peptides). Thus, for SRM to work optimally, proteotypic peptides for target proteins need to first be identified or derived. The selection of proteotypic peptides for TFs proved however challenging, mainly because of the scarcity of TF peptide data in public repositories and the difficulty of detecting TF-specific tryptic peptides in discovery experiments. We therefore decided to adopt an *in vitro* full-length protein expression-based strategy to initiate the construction of a mammalian TF-specific proteotypic peptide atlas (Simicevic et al., 2015), capitalizing on the availability of a comprehensive TF ORF clone library in the lab, as described above (Gubelmann et al., 2013). Building on these efforts, we then developed a state-of-the-art, targeted SRM-based assay, which combines high sensitivity and technological innovation to enable the monitoring of absolute copy number changes of TFs of interest during specific biological processes using *in vitro*-expressed, isotopically-labeled protein standards (**Fig. 6**).

As a proof-of-concept, we quantified in absolute amounts the levels of the adipogenesis master regulators PPARγ and RXRα in the nucleus of 3T3-L1 cells at six time-points during adipogenesis, and subsequently inferred their nuclear copy number per cell. We then used these data to build a quantitative model of genome-wide TF DNA binding in collaboration with our EPFL colleague Prof. Felix Naef. The goal here was to extend already available GRN models by incorporating our rather unique TF abundance data, allowing for better model calibration and thus prediction of dynamic network changes. Next to our microfluidics (MITOMI)-based biophysical characterization of PPARg's protein and DNA binding properties (Isakova et al., 2016) the ensuing model provided unique, quantitative insights into *in vivo* PPARg DNA binding. Specifically, we revealed that PPARγ's DNA binding profile can be faithfully modeled by considering its own copy number, thermodynamic principles, and chromatin accessibility. The functional consequence of our findings is that the chromatin state appears to constitute a "landing map" for

PPARg DNA binding, thus emphasizing the importance of both protein copy number and chromatin remodeling in dictating TF DNA binding behavior during adipogenesis (Simicevic et al., 2013).

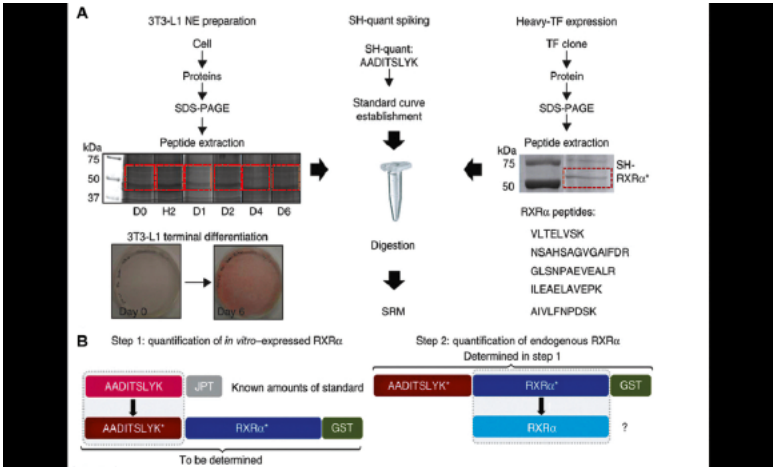

Fig. 6. A. *Left, preparation of 3T3-L1 total nuclear protein extract (NE). Cells are lysed at the indicated differentiation time point (D0–D6; D, day; H, hour), after which nuclear proteins are extracted. The resulting protein mixture is separated by SDS-PAGE, and TF bands are excised from the gel. Right, preparation of in vitro-expressed SH-tagged TFs. The constructs are expressed as heavy-labeled versions (*), purified by glutathione S-transferase (GST) affinity and separated by SDS-PAGE. Bands containing the heavy-labeled constructs, here SH-RXRα-GST*, are excised from the gel. Center, each nuclear extract band to be quantified is mixed with a gel slice of the in vitro-expressed TF construct, spiked with known amounts of light SH-quant tag and digested in gel. SH-quant features a C-terminal trypsin-cleavable fluorescent tag (here termed JPT) that is used to quantify this quantotypic peptide. The resulting peptide mixtures are quantified by SRM using proteotypic peptides selected by performing shotgun mass spectrometry analyses on each in vitro-expressed TF. Quantification of each TF requires a separate experiment in this configuration.* ***B.*** *Schematic of the quantification approach as outlined in* ***A.*** *Taken from (Simicevic et al., 2013).*

TF overexpression screen

The TF-centric approaches introduced above enable the characterization or quantification of TFs of interest, yet are unable to provide comprehensive insights into the TFs and underlying GRNs that mediate a specific biological process such as adipogenesis. To address this, we devised a high-throughput screening strategy involving both our mouse TF ORF collection which was transferred to a lentiviral overexpression vector and a robotic screening and imaging platform to systematically evaluate the functional involvement of TFs in fat cell differentiation. These experiments, performed in collaboration with the labs of Profs. Didier Trono (EPFL) and Christian Wolfrum (ETHZ), revealed 26 (3.5%) and 39 (5%) TFs that significantly enhance or repress adipogenesis, respectively, constituting a rich catalog to further dissect the adipogenic GRN. Intriguingly, while PPARγ was identified within the top 10 pro-adipogenic TFs, validating our screen, very little was known about the top three enhancing TFs: ZEB1, ZFP30, and ZFP277.

Follow-up molecular validation experiments revealed that the latter top pro-adipogenic TFs are indeed required in adipogenesis. Specifically, for ZEB1, we found that this well-known EMT TF directly targets virtually every TF that has so far been mapped within the adipogenic GRN and the majority of these TF-coding genes decrease in expression upon ZEB1 knockdown ([Gubelmann et al., 2014]; **Fig. 7**). This identifies ZEB1 as a new adipogenic master regulator with likely involvement in both the commitment and terminal differentiation phases given its high expression at these time points. Consistent with the latter hypothesis is the observation that knockdown of ZEB1 significantly reduces the expression of commitment markers and also inhibits mesenchymal stem cell differentiation to adipocytes. We further found evidence that ZEB1 may mostly act as a co-activator of gene expression through its association with the early adipogenic TF C/EBPb, which we had previously revealed primes adipogenic regulatory elements for activation through its association with the co-repressor and gatekeeper Nuclear Receptor Co-Repressor 2 (Raghav et al., 2012).

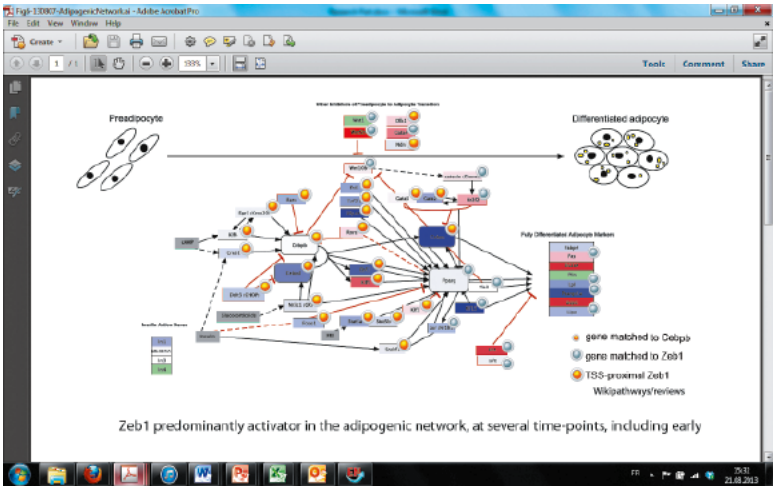

Fig. 7. I*ntegration of ZEB1 within the established core adipogenic GRN through RNA-seq and ChIP-seq-based analyses. Genes that are up – or downregulated upon ZEB1 knock-down are highlighted in respectively red and blue (with the extent of change scaling with color intensity). The data shown are retrieved at D0 of differentiation.*

For ZFP30, we had to start from scratch since virtually nothing was known at the time about the function of this TF. Domain analysis revealed however that ZFP30 belongs to the family of KRAB domain containing zinc finger proteins (KZFPs). Despite their abundance, most of the KZFPs have not been functionally characterized, although they are generally thought to be involved in the repression of transposable elements (TEs) through their association with the co-repressor KAP1. Thus, by studying the transcriptional function of ZFP30, we could contribute both to dissecting the adipogenic regulatory network and to elucidating the role of co-repressors within this system. Using *in vitro* and *in vivo* assays in mouse and human, we were able to define the mechanism of ZFP30-mediated adipogenic activation**,** revealing that the expression of the master adipogenic regulator *Pparg2* is controlled by an ancient retrotransposon-derived enhancer targeted by ZFP30 (**Fig. 8**). This potent regulatory sequence is over 100 million years old and derived from an L1 retrotransposon that was likely under repressive control of ZFP30 in the ancient past. ZFP30 was thus co-opted into the local eutherian adipogenic regu-

latory network, influencing the expression of its very master regulator, *Pparg*2. Intriguingly, however, unlike the canonical role of KZFPs as repressors of retrotransposon elements, we found that ZFP30 activates the *Pparg2* enhancer by maintaining its KRAB-mediated interaction with KAP1, but Ser-473 phosphorylation of KAP1 appears to mediate a switch from the canonical co-repressor to a co-activator function. Consistently, we demonstrate that loss of KAP1/phosphorylation negatively affects adipogenesis, revealing a context-specific regulatory function for KAP1 in driving fat cell differentiation.

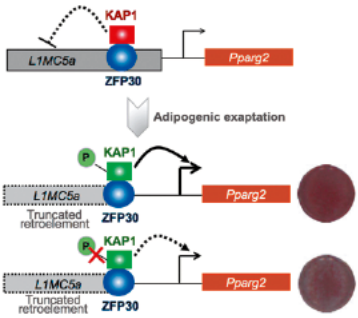

Fig. 8. *Graphical abstract illustrating the proposed molecular mechanism underlying the pro-adipogenic function of ZFP30. The latter TF was likely originally recruited to the Pparg locus to repress the activity of the L1MC5a retroelement. The subsequent insertion of a murine-specific retroelement (B3A, not shown) truncated the first retroelement, rendering the repressive function of ZFP30 obsolete. Subsequently, we hypothesize that ZFP30 was adopted into the local adipogenic regulatory network, as mediated by the phosphorylation of its co-regulator KAP1 which turned this complex into an activating rather than repressing entity. Consequently, loss of KAP1 phosphorylation abrogates its activating capacity, resulting in reduced Pparg2 expression and less adipogenesis (as visualized by Oil-red-O staining of differentiation-induced 3T3-L1 cells).*

Leveraging the resolving power of single cell transcriptomics to study adipogenesis

Our work on ZEB1 revealed that, while great advances were made to understand the terminal phase of adipogenesis, much less was known about the molecular drivers underlying adipogenic commitment. This in part reflected the lack of universally accepted markers that are either specific for one adipocyte precursor type or uniformly label a particular precursor population, rendering the identity of adipocyte stem and precursor cells (ASPCs) still enigmatic. We realized that this is exactly the type of problem that could be very effectively addressed by single cell transcriptomics (scRNA-seq) given that it has enormous potential to contribute to

our understanding of cell type diversity, tissue structure and homeostasis, development, and pathology. In collaboration with our collaborator Prof. Wolfrum (ETHZ), we therefore set out to acquire for the first time an scRNA-seq-based, high-resolution view of cellular heterogeneity within the stromal vascular fraction (SVF) of fat depots. To do so, we profiled and characterized FACS-isolated CD34+, CD29+, SCA1+, Lin- C56BL/6J mouse subcutaneous fat SVF cells, which are widely regarded as most closely resembling ASPCs. Our initial analysis involved 191 mouse ASPCs from three independent experiments using the Fluidigm C1 system, revealing ~5,000 genes that were expressed on average in each cell ([Schwalie et al., 2018]; **Fig. 9A**). To examine whether these ASPCs constituted one large or several subpopulations, we performed *de novo*, unsupervised clustering based on cell-derived gene expression profiles. Interestingly, this analysis revealed three main subpopulations. These were not only largely validated using another independent analysis involving now more than 1,804 Lin-cells that were processed using the 10X Genomics Chromium dropleting system, but they were also discovered in subsequent analyses by independent research groups, as shown through a meta-analysis by our group in (Ferrero et al., 2020).

The identification of these scRNA-seq-based subpopulations suggested functional differences among ASPCs, prompting us to phenotype the different cell types *in vitro* and *in vivo*. We analyzed several surface markers that are (relatively) specific to one of the three subpopulations and that thus could allow subpopulation-specific cell isolation and characterization. Using the marker CD142, encoded by the gene *F3*, we were able to highly enrich for the blue population (**Fig. 9A**). Intriguingly, and contrary to their presumed multipotent nature, subsequent differentiation analyses revealed that these CD142+ cells are largely refractory to adipogenesis (**Fig. 9B–C**). Moreover, depletion of these cells from the initial pool of ASPC-enriched SVF appeared to highly enhance adipogenesis, suggesting that CD142+ASPCs have inhibitory properties (**Fig. 9B–C**), an effect that could be replicated *in vivo* (**Fig. 9D-E**).

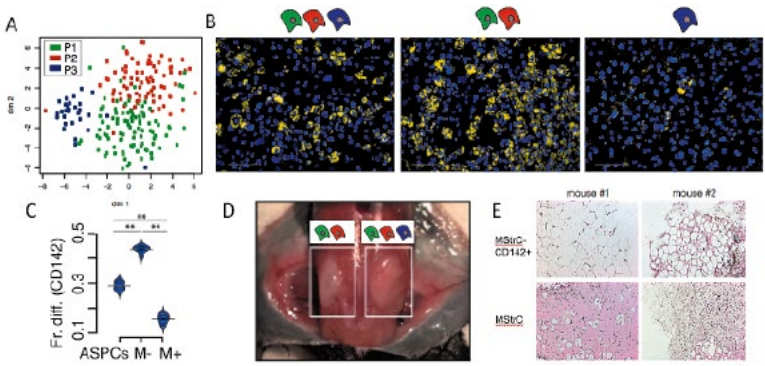

Fig. 9. A. *Unsupervised clustering of 191 ASPCs from C57BL/6J mouse subcutaneous fat SVF using tSNE revealed three major subpopulations (P) (represented as different colors) that exhibit significant differences in gene expression.* ***B.*** *Microscopic images of distinct ASPC fractions (total ASPCs, left; CD142- ASPCs, middle; CD142+ ASPCs, right) after adipogenic differentiation. Nuclei are stained with DAPI (blue) and lipids with Bodipy (yellow).* ***C.*** *Beanplots showing the distribution of the fraction of differentiated cells per each ASPC fraction (enriched or depleted in cells that feature the CD142 marker (M)) shown in* ***B.*** *(* p-value <=0.05, ** p-value <=0.01, t-test).* ***D–E.*** *Histological images of matrigel implant plugs composed of either total ASPCs or CD142-ASPCs after three weeks of high-fat diet feeding (adapted from (Schwalie et al., 2018)).*

To our knowledge, the existence of such a cell type within a specific population of cells (in this case: Lin- CD29+ CD34+ SCA1+) had so far never been reported, except for T regulatory cells (Tregs). In the immune system, Tregs use their immunosuppressive capacities to maintain immune homeostasis and mediate peripheral tolerance. We therefore proposed to name this newly uncovered cell population “Aregs” (for Adipogenesis Regulators) and provided in our study its first extensive anatomical and molecular characterization: we show that Aregs reside around the vasculature and are typified by the expression of coagulation and complement cascade factors that are recognized modulators of adipose tissue. These collective findings have great biomedical implications in metabolism and beyond because: 1) adipose tissue mass can expand both by hyperplasia (increase in cell number) and hypertrophy (increase in cell mass). Since the former is widely regarded as metabolically “healthier obesity” compared to the latter, it is of great interest to understand why obese individuals frequently experience overtime this mode shift in fat mass expan-

sion. One recurrent hypothesis is that hypertrophy may be triggered or enhanced upon depletion of the ASPC pool after chronic overfeeding. However, our findings now suggest that this mode shift might not be solely due to alterations in the number of ASPCs, but also to the amount of Areg cells, as these could control the tissue's *de novo* adipogenic capacity; 2) mesenchymal stromal cell-driven adipogenesis is routinely performed in thousands of labs around the world, yet it remains entirely unclear why overall fat cell formation is relatively inefficient, rarely reaching >50% cell differentiation. Our results now indicate that there is a thus far unrecognized cell population among these isolated cells that actively blocks this differentiation process. Whether a similar concept could be applicable to other differentiation systems is an exciting prospect; 3) it is well established that adipocytes can arise in other tissues such as bone marrow and muscle, albeit it remains unclear why these cells only form under specific (patho)physiological conditions. Based on our data, it is tempting to speculate that in these tissues, *de novo* adipogenesis is controlled not only through the presence or absence of adipocyte precursors, but also through the presence of Aregs. Thus, our findings point to a critical role for Aregs in modulating the plasticity and metabolic signature of distinct fat-cell containing systems, where they may constitute essential components of the elusive adipogenic precursor niche.

At this point, we still have a very poor understanding of the molecular nature and function of these enigmatic Lin-CD34+SCA1+CD142+ cells and whether they are functionally conserved in humans. In a recent, follow-up study (Zachara et al., 2021), we therefore aimed to provide multi-omics- and experiment-based insights into the molecular mechanisms that control the developmental emergence and function of at least mouse Aregs. As summarized in **Fig. 10**, we found that Aregs constitute a clearly distinct and stable CD142+ ASPC subpopulation in adult mice, which remains phenotypically robust regardless of experimental conditions, such as the source of anti-CD142 antibodies, various cell sorting gating strategies, the strength of adipogenic differentiation cues, or even sex of the animals. In addition, we uncovered unexpected developmental dynamics since, contrary to adult Aregs, pre-weaning CD142+ ASPCs exhibit a high adipogenic propensity. Indeed, they acquire *bona fide* Ar-

eg-like molecular and functional properties only during the third post-natal week, an event possibly triggered by weaning or sexual maturation. Finally, using a multi-omic data integration workflow supported by experimental validation, we show that the inhibitory nature of these cells is driven by specifically expressed secretory factors that cooperate with the retinoic acid signaling pathway to transform the adipogenic state of CD142– ASPCs into a non-adipogenic, Areg-like one.

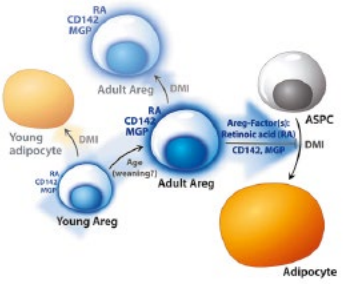

Fig. 10. *Graphical summary of the multi-omic and experimental characterization of mouse Areg-like cells (Zachara et al., 2021).*

Nevertheless, many questions remain. In the years to come, we therefore plan to invest substantial efforts to for example assess the physiological impact of Aregs on adipose biology, ideally by developing an Areg-specific ablation approach and monitoring the effect of eliminating these cells on adipose tissue growth and homeostasis as well as metabolic health in general under a control and high fat diet feeding regime. In addition, we would like to extend our studies to human adipose tissue, since the jury is still out with respect to whether Aregs are functionally conserved. Our initial findings pointed to the existence of a human CD142+ ASPC population with comparable molecular and cellular phenotypes to its mouse counterpart (Schwalie et al., 2018). Yet, while scRNA-seq-based analyses have revealed the existence of the “green” and “red” populations in human adipose stromal cells, these same analyses failed to resolve a clearly distinct transcriptomic signature that resembled that of mouse Aregs. Further analyses, involving more individuals / patients, also in different metabolic contexts, will however be required before firm conclusions can be drawn.

Engineering next-generation single cell phenomic technologies

DisCo: Deterministic scRNA-seq

Our scRNA-seq-based resolution of ASPC heterogeneity truly exposed us to the analytical power of single cell transcriptomics studies. However, it also revealed opportunities for technological improvements. For example, Aregs constitute a relatively minor proportion of all ASPCs (on average around 10%) and would as such be difficult to process as a standalone cell population with conventional scRNA-seq approaches, since these are all geared toward highest possible throughput. Indeed, little progress has so far been made to enable studies on small samples comprising <1,000 cells. To date, the Fluidigm C1 system, and other hydrodynamic trap-based methodologies, are the only mainstream systems able to process low input samples. Yet, cell size and shape capture biases have rendered these systems unpreferred for the processing of small heterogeneous samples, and thus of virtually any tissue. Hence, a wide array of precious biopsy samples and small cell populations or tissues are currently difficult to access by scRNA-seq. In response, such samples tend to be massively pooled (for example Zebrafish embryos, whole organism *C. elegans*, intestinal organoids etc.). This is rather counterproductive given that, despite having single cell-resolved transcriptomes, such sample pooling generally averages cell-type abundances and correlations, making the exploration of interindividual heterogeneity, particularly important in the context of developmental or clinical studies, impossible.

To address this technological gap, we developed a "no cell left behind" scRNA-seq platform, leveraging our expertise in multilayer microfluidics to engineer a DeterminIStic CO-encapsulation (DisCo) system for droplet-based scRNA-seq. Conventional Drop-seq allows for the straightforward processing of thousands of cells, which is why it is also so widely used. Its clear drawback is that the co-encapsulation process of one cell with one mRNA capturing bead in a droplet is uncontrolled, i.e. stochastic. This results in the generation of many droplets that either contain a cell or a bead but not both, making the process highly inefficient and incompatible with small samples since <20% of processed cells tend to be captured. In other words, >80% of the cells tend to be inevitably lost, which necessitates inputs of at least several thousands of cells. To address this im-

portant issue and thus to make Drop-seq compatible with small cell input samples, we supplemented the canonical Drop-seq chip with a control layer using multilayer microfluidics. This control layer is used to coordinate cells and beads at the co-encapsulation point (**Fig. 11**). The coordination is controlled by a machine-vision system, operating on bright-field (BF) images obtained from a microscope camera (**Fig. 11A**). Thus, by combining machine-vision and multilayer microfluidics and building on earlier advances by our lab to optimize mRNA capturing bead collection (Biočanin et al., 2019) we now achieved for the first time, to our knowledge, full control of a two-particle co-encapsulation process: precise placement of one cell and one bead, encapsulation by dropleting on demand, and on-chip sorting of dedicated droplets. Importantly, by performing precise cell-capture efficiency measures, we were able to demonstrate that our DisCo platform now routinely achieves >80% cell processing efficiency on 100 cells and less. This is a significant improvement over all currently available scRNA-seq technologies, and assured that the technology itself is no longer a limiting factor when processing low cell input samples. Rather, we believe that the most inefficient step in any experimental scRNA-seq setup is now the dissociation efficiency, which is unfortunately all too often ignored by the field as a whole. Much more efforts should therefore be devoted to improve the robust and comprehensive isolation of individual cells and assure a correct representation of all cell types in scRNA-seq samples.

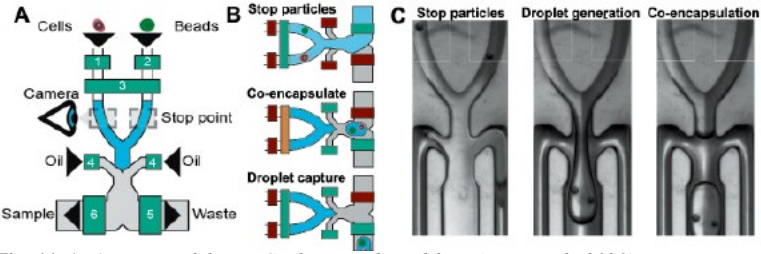

Fig. 11. A. *Overview of the DisCo device (adapted from (Bues et al., 2020)current microfluidics-based scRNA-seq technologies are limited to samples with large amounts of cells (> 1,000 cells), showing three inlet channels for cells, beads, and oil, and two outlets for waste and sample liquids. All inlets and outlets are augmented with "Quake valves" (green boxes): 1. cell, 2. bead, 3. dropleting, 4. oil, 5. waste, and 6. sample valves. The device is continuously monitored by a high-speed microscopy camera to detect and place particles at the Stop point. B. Illustration of the particle co-encapsulation process on the DisCo device (red: closed valve, green: open valve, orange: actuation for dropleting). C. The co-encapsulation process of two beads and droplet generation as observed on chip.*

Live-seq: transcriptomic recording of live cells

As exemplified by our studies on adipogenesis, most biological processes are inherently transient and dynamic, with cell states changing according to internal programs and/or external stimuli. It is thus critical to not only understand a cell's current state, but also how a cell arrived at that state, i.e. its molecular history. For example, ASPCs are able to differentiate in adipocytes, but as shown in **Fig. 9**, not all cells do so. Why this is the case remains a grand, outstanding question, but one of the hypotheses is that the pre-differentiation cell state may have been intrinsically refractory to, in this case, adipogenic stimuli. To test this hypothesis, one would ideally probe a cell's molecular state pre-differentiation and then track this same cell throughout adipogenesis with the aim of identifying factors that reflect either a cell's pro- or anti-adipogenic state. However, despite its obvious importance, revealing a cell's molecular history remains a great, outstanding technological challenge. Several approaches, from recombinase- and Crispr-based DNA editors, over live cell imaging, to single cell transcriptomics (scRNA-seq)-based trajectory inference have therefore been developed with the intent of exploring a cell's past. While constituting exciting advances, these methods have intrinsic limitations, including their ability to record only a couple of events per single cell rather than the whole transcriptome and thus by their reliance on prior knowledge of informative target genes or pathways. In addition, scRNA-seq-based trajectory models still need to be interpreted as statistical expectations rather than the real transition path of cells. This is because all current scRNA-seq assays depend on cell lysing to retrieve the respective transcriptome, which makes it impossible to link the individual cell to downstream molecular and phenotypic states.

To overcome the issue of lack of baseline data to predict cell responses or trajectories at the single cell level, in collaboration with Prof. Julia Vorholt's lab (ETHZ), we have recently established Live-seq, a single-cell transcriptome profiling approach that preserves cell viability during the extraction of RNA (Chen et al., 2021). Live-seq relies on fluidic force microscopy (FluidFM) to extract a cytoplasmic sample, coupled to a sensitive low-input RNA-seq strategy that our lab developed (**Fig. 12A-B**). Remarkably, benchmarking experiments evaluating the molecular impact of extracting up to 1 picoliter (pL) of cytoplasm from pre-adipocytes (IBA

cells) revealed few gene expression differences between unprobed cells (control IBA cells) and those 1h or 4h post Live-seq extraction (**Fig. 12C**). These observations suggest that Live-seq does not impose major perturbations on cellular function, suggesting, perhaps provocatively, that mammalian cells do not possess built-in defense mechanisms against such cytoplasmic sampling. This conclusion is further supported by the fact that probed cells generally show high post-extraction cell viability (>80%) and that they behave functionally similar to their untreated counterparts, as we, for example, have shown for LPS-treated RAW-G9-like macrophages (**Fig. 12D**). Thus, we believe that Live-seq opens a new avenue to link a cell's molecular state directly to its present and future phenotypic properties (**Fig. 12E**), providing an opportunity to acquire direct rather than inferred cell dynamics read-outs. To further explore Live-seq's capacity and provide a first proof-of-concept, we sampled 14 RAW-G9 cells a first time, then stimulated them with LPS, after which we sampled the same cells a second time (**Fig. 12E**), yielding two cells that passed the filtering criteria at both sampling points. While clearly limited in number, these two cells, each constituting two distinct points in the same t-SNE map (**Fig. 12F**), provide to our knowledge the first empirically determined, transcriptome-wide read-out of a cell's trajectory, transitioning from a pre-treatment to post-treatment (LPS) state (**Fig. 12B & F**). Moreover, we found that Live-seq and conventional scRNA-seq data of control and LPS-treated RAW-G9 cells could be properly integrated (**Fig. 12F**), which allowed us to unambiguously establish the correct trajectory of cells that were processed by conventional scRNA-seq.

In sum, we believe that Live-seq is orthogonal to any other scRNA-seq approach today in that it keeps cells alive while all other approaches do not. This, in turn, enables the transcriptome of the cell to be recorded prior to phenotyping and allows questions to be addressed that no other scRNA-seq method *directly* can. These include, as illustrated in (Chen et al., 2021), how molecular and cellular heterogeneity is established and what the actual (and not statistical) trajectory of cells is. We therefore anticipate that Live-seq has the potential to transform scRNA-seq from the current, end-point type assay into a real-time analysis workflow. For Live-seq to be widely relevant and transferable though, we will need to substantially increase its throughput and efficiency and reduce its overall experimental complexity. These constitute exciting technological challenges that we hope we will be able to address in my lab in the coming years.

Beyond cellular heterogeneity: understanding how regulatory variation induces phenotypic diversity

The genome is a remarkable molecular entity since it contains all the instructions to generate a multitude of different cell types that are derived from a single zygote. To understand development, it is therefore imperative to be able to decode these instructions, as I have explained above. Deciphering these same instructions is however also crucial to understand phenotypic variation, since it is now widely accepted that seemingly small divergences in this "regulatory grammar" are for a large part responsible for interindividual differences in complex traits such as height, but also in disease susceptibility. Indeed, well over 10 years of genome-wide studies have revealed that the majority of common trait or disease-associated genetic variants fall into non-coding, likely regulatory regions and affect transcriptional programs. Consequently, resolving how variation in regulatory sequences is translated into phenotypic variation at the molecular, cellular, or organismal level is of great biomedical importance. However, few studies have so far been able to mechanistically disentangle how regulatory variants contribute to human inter-individual variability. This is further illustrated by the newly launched International Common Disease Alliance (ICDA, http://icda.bio), aiming to address this "variant to function" challenge head-on. The aim here is to develop novel method-

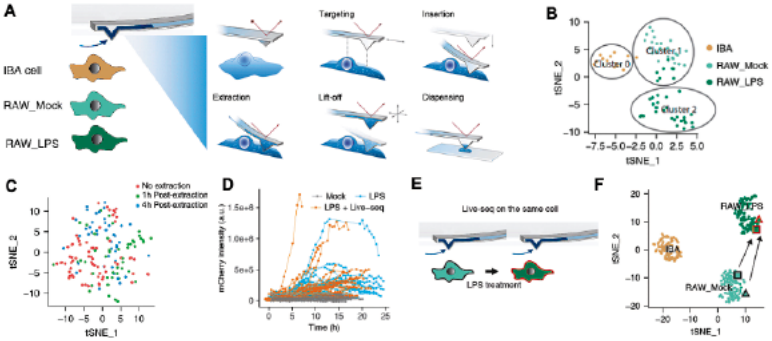

Fig. 12. A. *Live-seq workflow involving FluidFM and sensitive scRNA-seq to determine the molecular state of distinct live cell types and states (here, pre-adipocytes (IBA) versus mock- or LPS-treated macrophages* [*RAW-G9*]*).* ***B.*** *Unsupervised clustering of IBA and RAW-G9 Live-seq samples based on the top 500 variable genes as visualized in a tSNE plot.* ***C.*** *Unsupervised clustering of conventional scRNA-seq samples based on the top 500 variable genes as visualized in a tSNE plot. Shown in the plot are IBA control cells or IBA cells 1h, or 4h post-Live-seq extraction. The latter IBA cells do not show clearly distinct clustering, suggestive of high transcriptional similarity.* ***D.*** *RAW-G9 cells containing an mCherry reporter under the control of the* ***Tnf*** *promoter and GFP-tagged RELA (NFkB). mCherry intensity profiles of LPS-treated control RAW-G9 cells (blue) or those subjected to Live-seq sampling (orange). Mock-treated cells were used as negative control (grey). Such profiles can be derived since the FluidFM system is mounted on an optical microscope, which allows for monitoring cells in real time or in a time-lapse manner. Importantly, no striking behavioral differences were observed between the control and Live-seq-probed cell profiles.* ***E****. Sequential sampling procedure. The shape outline represents unstimulated (black) or LPS-stimulated cells (red).* ***F.*** *tSNE-based visualization of integrated scRNA-seq and Live-seq data, highlighting the transition of two sequentially sampled cells (triangle and square) from one state (ground) to another (LPS). The annotation of these cells is as described in (****B & E****). (Adapted from* [*Chen et al., 2021*]*).*

ologies and concepts that go well beyond *a priori* or even intuition-based knowledge of the gene(s) that likely mediate(s) the observed phenotypic variation, which, so far, is still the most common approach. While such gene-centred strategies greatly reduce the search space for causal variants, they also restrict the research scope to mostly gene-proximal regulatory elements (REs) such as promoters or introns.

To close this mechanistic gap, additional molecular traits, including TF binding, chromatin accessibility and state, are increasingly being assayed, again aiming to find causal variants by prioritizing variants in function of their location in functional genomic regions. While the integration of these additional molecular layers is challenging, it has nevertheless propelled the elucidation of rather convoluted molecular scenarios. A prime example involves obesity-associated variants that are located in the FTO gene. While initially hypothesized to affect the FTO gene itself, a whole palette of experimental and computational approaches has since demonstrated that the causal variant is indeed positioned in an FTO intron but affects the expression of two TF-coding genes (*IRX3* and *IRX5*). The latter genes are located more than 1 Mb downstream of the focal variant that disrupts the binding ability of yet another TF, ARID5B (Claussnitzer et al., 2014, 2015; Smemo et al., 2014). This appears to influence white fat cell function, providing a plausible link to excessive fat accumulation and obesity. Thus, this labour-intensive case study clearly emphasizes the need for novel approaches or concepts that could facilitate our ability to disentangle the role of regulatory variation in complex traits and disease susceptibility.

Another striking finding that emerged from recent, functional genomics studies is that the manner by which genetic variation impacts supposedly less convoluted phenotypes, such as gene regulatory processes, is also more complex than anticipated. We contributed to this important realization through a large-scale, integrative genomics study that my lab undertook in collaboration with Profs. Manolis Dermitzakis (University of Geneva), Alex Reymond and Nouria Hernandez (both at the University of Lausanne) (Kilpinen et al., 2013). The goal of our study was to quantify the allelic coordination among different molecular phenotypes to increase our knowledge of the chain of regulatory events leading to the transcriptional readout of a gene. We thereby aimed to also improve our under-

standing of to what extent genetic variation affects the chromatin landscape including TF binding profiles and chromatin mark enrichment. For this purpose, we performed ChIP of five chromatin marks (H3K4me1, H3K4me3, H3K27ac, H3K27me3, and H4K20me1), three transcription factors (TFs) (TFIIB, PU.1, and MYC), and the second largest RNA polymerase II subunit RPB2 in lymphoblastoid cell lines (LCLs) from two parent-offspring trios. A subset of the ChIP assays was additionally performed in eight additional unrelated individuals. In addition, all 14 individuals were profiled for gene expression.

Using these data, we then investigated the allele-specificity and inter-assay coordination among different molecular phenotypes. We observed abundant allele-specific effects across all probed molecular phenotypes, with the proportion of significantly biased sites ranging from 5% in mRNA to 11-12% in TF data and 6-30% in chromatin marks. We assessed the degree of parental transmission of the allelic effects, a proxy for genetic influence on the assays, and discovered that effects of DNA sequence variation are largely transmitted from parents to children from TF binding through chromatin marks to transcription. Transmission of allelic chromatin mark effects appeared much more sensitive to context-dependent effects compared to TFs though, with strongest transmission seen at promoters (for H3K4me3) and known chromatin accessibility-affecting variants (for H3K4me1 and H3K27ac). Interestingly, we also observed highly coordinated allelic (local) and haplotypic (short- and long-range) behavior among molecular phenotypes at different functional elements of the genome, suggesting that TF binding, presence of histone modifications, and the transcriptional readout at these regions all operate within the same allelic framework. Therefore, genetic effects on chromatin marks are probably closely tied to the sequence context of a given functional element and thus manifested indirectly through TF binding, which we observed to be causatively affected by DNA sequence variants within binding motifs of the same or other TFs.

The latter observation is nicely illustrated in **Fig. 13A**, which shows that the impact (in terms of DNA binding affinity) of SNPs on TF motifs (here, that of PU.1) scales with the likelihood to observe significant allele-specific effects, suggesting that the SNP-mediated disruption of the TF motif is directly causal to the observed allele-specific binding of the respective

TF. Nevertheless, a striking finding that emerged from these analyses is that despite PU.1's strong binding preference for its own motif (**Fig. 13A**), only a minority of variable PU.1 DNA binding events could be attributed to variants that disrupted the PU.1 motif (**Fig. 13B**). In other words, for the majority of PU.1 binding (70%) events that exhibit allelic DNA binding bias, the PU.1 motif is intact, suggesting that other factors / mechanisms are influencing PU.1 DNA binding. In our search to uncover these mechanisms, we found that for a small portion of these focal motif-independent variable PU.1 DNA binding events, disruptions in other TF motifs may also be responsible for changes in PU.1 DNA binding, implying cooperative effects between TFs. Specifically, a scan for allelic binding cooperativity within individuals identified four motifs that show a significant correlation between motif covariance and allele-specific PU.1 binding (**Fig. 13C**), collectively explaining another portion (7.5%) of all detected significant allele-specific PU.1 binding sites.

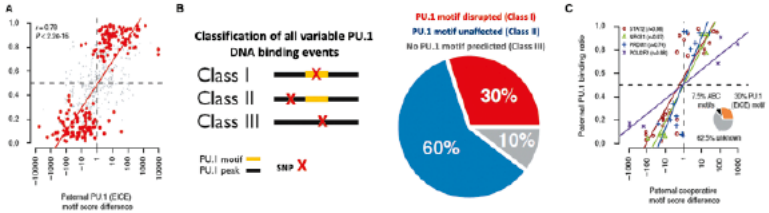

Fig. 13. *Genome-wide analyses of allele-specific PU.1 binding.* ***(A)*** *PU.1 motif score changes are predictive of allele-specific PU.1 binding. Ratio between paternal and maternal PU.1 PWM scores (x-axis) and fraction of reads mapping to the paternal allele (y-axis) (Red, significant sites; gray, non-significant).* ***(B)*** *Classification and proportion of all variable PU.1 DNA binding events according to three listed scenarios.* ***(C)*** *SNPs in co-associated TF binding sites are predictive of allele-specific PU.1 binding (5% FDR). (Adapted from [Kilpinen et al., 2013]).*

Together, our analyses support the notion of TFs being the primary mediators of sequence-specific regulation of gene expression programs, while chromatin marks are more prone to stochastic, possibly transient effects (e.g. due to environmental triggers) and likely reflect, rather than define, coordinated regulatory interactions. Our study also raised important, fundamental questions including how the uncovered, often long-range molecular coordination is genomically organized and specifically,

why and how only the minority of genetically variable TF binding events can be explained by sequence differences in the respective binding sites. To address these questions, we expanded our initial study by performing genome-wide profiling of PU.1 and three chromatin marks (H3K4me3, H3K4me1 and H3K27ac) as well as gene expression (RNA-seq) in lymphoblastoid cell lines of 47 individuals whose genomes were well characterized. Integrating these data, we observed strong quantitative co-variation between TF binding and chromatin mark levels at distinct regulatory regions, revealing a fine-grained molecular modularity of the genome that we newly defined as sub-megabase scale "variable chromatin modules (VCMs)" (**Fig. 14**) (Waszak et al., 2015). In addition, we found that the activity level of most VCMs can be captured by a single quantity value, which suggests that the molecular processes within each VCM (TF binding, chromatin mark enrichment, and gene expression) are all subject to one or few highly penetrant causal events. As such, VCMs may provide a conceptual framework as to why most regulatory variation is independent of local genetic variation. Indeed, we now hypothe-

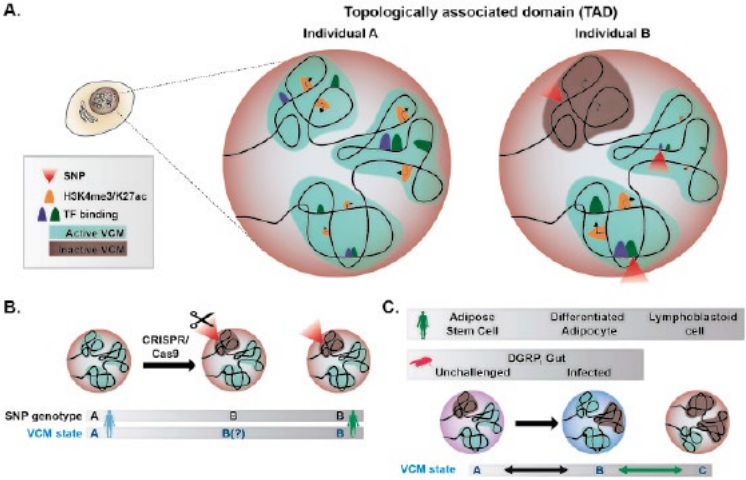

Fig. 14. *VCMs are genomic modules at sub-TAD, i.e. sub-Mb scale that capture coordinated variation in TF binding, chromatin state, and gene expression. We hypothesize that VCM activity is driven by TF-DNA interactions and that the perturbation of a single or few TF binding events may influence the molecular state of the entire VCM (taken from* [*Deplancke et al., 2016*]*).*

size that such regulatory variation is in large part driven by the activity state of the VCM in which the respective molecular phenotypes such as TF binding or chromatin mark enrichment are embedded.

This in turn shifts the question toward which genetic or molecular factors control the activity state of a VCM? This fundamental question is central to the studies that are currently ongoing in my lab and that leverage several important clues that have recently been reported. These include i) that genetic variation can influence chromatin accessibility independent of expression change and ii) that genetic variation in some, but not all regulatory elements within a certain locus influences the activity profile of all other molecular phenotypes in that locus. The latter findings indicate that regulatory elements not only undergo genotype-specific changes in accessibility, as presumably mediated by the gain or loss of cell type-specific TF binding, but that they may also be subjected to a certain regulatory hierarchy, involving both "lead" and "dependent" regulatory elements. It is tempting to speculate that this regulatory hierarchy is conceptually linked to the VCM principle. Deciphering which molecular features distinguish lead from dependent regulatory elements and thus how regulatory hierarchies are established across cell types and states are therefore among the main research themes in my lab. This is because resolving these hierarchies may provide us with key insights into how VCMs are established across systems, which in turn may prove instrumental to understand the contribution of genomic variation to molecular, cellular, and organismal diversity. The underlying rationale thereby is that we hypothesize that genetic variants that affect VCM activity or underlying regulatory hierarchies tend to have long-range impact on surrounding molecular phenotypes, increasing the likelihood that they induce downstream molecular and cellular effects, especially if they overlap with variants that impact gene expression. Thus, among the many variants that have so far been associated with specific traits or diseases, we would like to argue that those that affect VCM activity should be prioritized for further characterization given their anticipated, significant impact on local regulatory networks, which renders them attractive candidate drivers of phenotypic variation. We would thereby be able to truly exploit the coordinated molecular nature of a VCM of interest (e.g. linked to a variant with clear phenotypic / disease impact) for uncovering the flow of regu-

latory information, from causal nucleotides over gene(s) to phenotype, thus providing unprecedented insights into the molecular mechanisms driving phenotypic variation.

Conclusions and outlook

This year (2021) marked the 20th anniversary of the release of the human genome sequence. In these two decades and aided by immense technological and computational advances to which my lab also made a few contributions, tremendous progress has been made in our understanding of how our genome generates this stunning cellular and phenotypic diversity. The real challenge ahead will now be to move beyond the type of descriptive work that allowed us to catalogue the principal elements in our genome (genes, regulatory sequences etc.) to a mechanistic understanding of how all these elements work together to create a functional cell or organism. As such, I believe that the challenge is no longer in generating omic data, but in the way such data will be analysed and integrated with other datasets to decipher and even reverse engineer complex biological processes or systems.

For example, thanks to large-scale efforts such as ENCODE and contributions by numerous other groups, we now have great oversight of which TFs are encoded by a genome, which are the regulatory elements at which these TFs are active and in which chromatin context these elements are embedded in function of cell state or type. However, this does not suffice to understand the underlying regulatory code, as we are still unable today to robustly engineer from scratch a synthetic DNA sequence that allows us to induce a regulatory activity in a specified cell type and at a pre-defined time and level. Consequently, we also still have great trouble in predicting the effects of regulatory variants on gene expression, let alone at the cellular or organismal level. This is further exacerbated by the fact that our work on VCMs has taught us that regulatory elements operate in coordinated fashion and abide to certain hierarchies. This is why we need to not only better understand the impact of genetic perturbations locally, but also from a more integrative, network or regulatory module perspective. Only then do I believe will we be able to truly grasp how regulatory networks operate to define cell states and to enact cell fate transitions

such as from a mesenchymal stromal cell to a mature adipocyte, and what might be the molecular and phenotypic consequences when such networks are genetically perturbed. With the truly exciting advances in single cell omics, whose contributions, I anticipate, will reach well beyond the field of regulatory genomics into practically every aspect of biology and medicine, we now have an unprecedented opportunity to define these cell states / types with very high precision. This is an absolute pre-requisite in our quest to link regulatory networks to cell identities. In addition, novel deep learning approaches that now even consider genomic architecture are increasingly emerging and appear to rapidly improve our ability to infer gene expression from DNA sequence. These advances offer hope that we will be able to extract more general regulatory rules that then can be tested, for example using forward genetics approaches such as CRISPR-based sequence interrogations. The ultimate goal is then to apply these rules on each person's genome, catalysing the long-anticipated transition of clinical practice toward precision medicine.

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INSTITUTE OF MOLECULAR CANCER RESEARCH
UNIVERSITY OF ZURICH

FOR HER GROUND-BREAKING CONTRIBUTIONS
TO RESEARCH IN HELICOBACTER PYLORI
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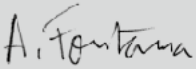

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SNF project grant 310030_192490 "Transcription-coupled DNA damage as a driver of infection-induced gastric carcinogenesis" CHF 904 000	2020–2024
Cancer Research Center Zurich "Mutations in R-loop metabolism genes as drivers of gastric carcinogenesis" CHF 280 000 (with Prof. Dr. Achim Weber, Institute of Pathology)	2020–2022
Clinical Research Priority Program "Precision Hematology/Oncology" CHF 70 000 per year	2019–2021
Cancer Research Center Zurich "Targeting the tumor microenvironment in B-cell malignancies: exploiting patient-derived xenografts for the rational pre-clinical testing of interventions that result from drug and genetic screening" CHF 280 000 (with Prof. Dr. Thorsten Zenz, Experimental Hematology USZ)	2019–2020
Swiss Cancer League KFS-4120-02-2017 "The sphingosine-1-receptor 2 is a novel tumor suppressor in diffuse large B-cell lymphoma: investigating its regulation, mode of action and clinical relevance" CHF 219 850	2017–2019
Clinical Research Priority Program "Human Hemato lymphatic Diseases" CHF 130 000 per year	2016–2018

Swiss Cancer League KLS-3612-02-2015 2015–2017
"The hematopoietic oncoprotein FoxP1 promotes tumor cell survival in DLBCL: identification of FoxP1 target genes and their relevance for patient stratification and prognostication" CHF 165 000

SNF Temporary Backup Schemes Consolidator Grant 2015–2020
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Reviewing for funding agencies: SNF (Switzerland), Wellcome Trust (UK), MRC (UK) DFG (Germany), Academy of Science (Finland), Swiss Cancer League (Switzerland), FWF and others

Patents (Granted only)

Daniela Engler, Christian Taube, **Anne Müller**. Helicobacter-specific vaccination for the treatment of asthma. Application submitted in Jan. 2014. EP 14153365.3; University of Zurich and University of Leiden. Licensed to Geneva Biotech Center Inc., Geneva, Switzerland, 2016.

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THE TWO FACES OF HELICOBACTER PYLORI

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Summary

What does it take to be a bacterial pathogen? Why can some bacteria efficiently infect their hosts and other, closely related ones not? How do bacteria persist in their host, with their presence often going unnoticed? Is it because of the genetics of the bacteria, or of their host? Or do the circumstances of infection – age of the host, infectious dose, site of infection – play a role? Only very few bacterial pathogens of humans are suited to address such a diverse set of questions. One of them, and perhaps the most appropriate of all, is the stomach-colonizing bacterium *Helicobacter pylori,* the dominant cause of and risk factor for chronic gastritis and gastric ulcer, gastric adenocarcinoma and gastric lymphoma. At the same time, *H. pylori* is an ancient companion of humans and normal constituent of a healthy gastric microbiota of half of the world's population. Thus, from an evolutionary perspective it is likely that the co-existence of *H. pylori* and humans benefits both, maybe at the expense of detrimental effects in some individuals.

I have studied many aspects of *H. pylori* biology in the last 20 years, but it sometimes feels like we are still only beginning to understand the intricacies of the interaction of *H. pylori* with its host. Being able to manipulate both the host (i.e. most often the surrogate murine host) and the bacterium has helped in the past, but linking specific persistence or virulence factors to target cell types and molecular mechanisms has remained a challenge. Some of our insights into the features – of both host and bacteria – that tip the balance in favor of gastric disease, or of peaceful and maybe even mutually beneficial co-existence, are shared below.

1. Introduction

Bacteria colonize all mucosal surfaces of the human body and are numerically roughly as abundant as our own 10^{13} to 10^{14} human cells.[1] The human gastrointestinal tract harbours the densest bacterial communities, with a maximum of 10^{11} bacteria/g reached in colonic content.[2] Whereas many bacteria temporarily pass through the human alimentary tract and other sites of bacterial colonization, and can be identified there only transiently, others have adapted to permanently live in, on and with their human hosts. Bacteria have evolved complex adaptations to new environments, and some species effectively deploy these skills as pathogens during colonization within human hosts; examples include *Pseudomonas aeruginosa,* which has made the transition from life in the environment to persistent colonization of the airways of human cystic fibrosis patients,[3, 4] *Mycobacterium tuberculosis,* which can colonize the lungs of their latently infected hosts for decades,[5] and typhoidal and nontyphoidal serovars of the species *Salmonella enterica* that can cause persistent infection in humans and serve as a reservoir for human-to-human transmission.[6] Probably the most extreme case of a persistent bacterial infectious agent is *Helicobacter pylori,* a gram-negative spiral-shaped bacterium that infects one half of the human population.[7] In endemic regions with a high prevalence of *H. pylori* (Figure 1A), the bacteria are acquired already in early childhood, and typically from the mother.[8] Studies that have monitored the same human host over time indicate that the exact same strain may be present – in the absence of treatment – for at least 6 years, diversifying and adapting to micro-niches as it co-exists with its host over time.[9]

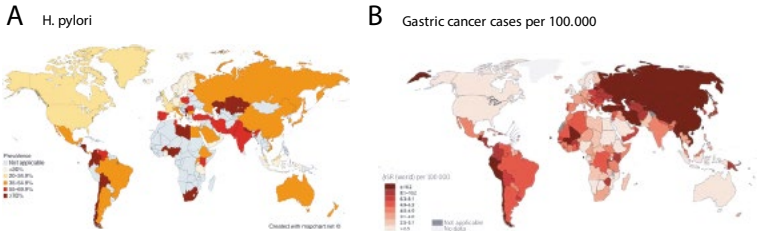

Figure 1. Global prevalence of H. pylori and of gastric cancer. *A, Prevalence of H. pylori, as estimated by Zamani et al.*[12] *B, Gastric cancer cases per 100 000 residents, as determined by Rawla et al.*[13]

It is assumed, but not proven, that in the absence of intervention, we are colonized with the same *H. pylori* strain from the cradle to the grave. *H. pylori* is estimated to have colonized humans at least since they migrated out of Africa over 58 000 years ago.[10] The long-term co-existence, and co-evolution of humans and their *H. pylori* strains have allowed the tracing of human migration patterns, for instance those leading to the colonization of the Americas.[11]

Whereas it is well-accepted that *H. pylori* is among, if not THE most successful bacterial companion of humans, much less is known about why it causes disease in "only" a subset of infected individuals. Approximately 20% of infected individuals will develop gastric disease symptoms that range from chronic gastritis, to gastric or duodenal ulcers, to gastric lymphoma and gastric adenocarcinoma.[14] Gastric adenocarcinoma develops in roughly 1% of the *H. pylori*-infected population and chronic *H. pylori* infection is recognized as the main risk factor for gastric cancer development.[15] *H. pylori*-associated gastric cancer is one of three common infection-induced cancer entities; the others are human papilloma virus-associated cervical cancer and hepatitis virus B and C-associated liver cancer.[16] Over 800 000 newly diagnosed gastric cancer cases per year are directly attributable to *H. pylori*.[16] Gastric cancer is a huge worldwide public health problem, but most common in East Asia and parts of South America (Figure 1B). Almost all cases of gastric cancer are detected when it is too late for disease-modifying treatment, making gastric cancer the third most common cause of cancer-related deaths (768,793 deaths in 2020; which is 7.7% of all cancer-related deaths), surpassed only by lung and liver cancer.[17]

I have invested the last 20 years, of which 15 were spent as an independent PI, studying various aspects of *H. pylori* biology, pathogenesis, infection immunology and immunomodulation. We mostly use mouse and cell culture models, and more recently also organoid models in the lab. We manipulate both the host and the bacteria to comprehensively study various aspects of the host/pathogen interface. Our research interests have evolved quite a bit over the years but can be summarized under the three following broader topics: (1) direct and indirect pathogenic mechanisms of *H. pylori* that drive gastric carcinogenesis, (2) beneficial effects of

H. pylori on its host, especially in models of chronic inflammatory and allergic diseases and (3) the pathogenesis of bacterially induced and of non-infection-associated aggressive lymphomas. Each topic will be covered in depth below.

2. H. pylori is a gastric carcinogen

Both direct and indirect detrimental effects of *H. pylori* infection on gastric homeostasis have been reported. It is now clear from work by my lab and others that the immune response to the infection is at least partly to blame for the development of gastric cancer and its precursor lesions.[18–21] Mice that lack all lymphocytes, or T-cells only, are protected against *H. pylori*-induced preneoplastic lesions; conversely, immunization prior to experimental infection, which enhances anti-*Helicobacter* immunity but does not clear the infection, accelerates and dramatically aggravates gastric preneoplasia.[18–21] The main culprit in experimental infection scenarios appears to be the T-helper 1 (Th1)- polarized $CD4^+$ T-cell population which, by virtue of its IFN-γ production, directly compromises the integrity of the gastric epithelium.[18, 21] Evidence towards this end comes from adoptive T-cell transfer experiments, and from a transgenic mouse model of IFN-γ overproduction in gastric epithelial cells, which phenocopies the immunopathological effects of Th1 cells.[18, 22, 23] In humans, polymorphisms affecting the strength of pro-inflammatory and adaptive immune responses to bacterial and viral infectious agents have been linked to gastric cancer risk in large epidemiological surveys conducted in populations of both the Western and Eastern hemisphere. Examples of loci known to be subject to polymorphism in this context are *IFNGR1*, *IL1B*, *IL1BR*, *TNFR*, *IL10R*, *TLR1*, *TLR6* and *TLR10.*[24–28] In cases where the effects of the "tumor-promoting" allele have been investigated, its expression is associated with a more pronounced inflammatory response at steady state and during bacterial challenge.[25–27] In humans as in experimentally infected mice, the polarization of the anti-*Helicobacter* T-cell response is a major determinant of gastric cancer risk.[29] Regulatory T-cells in particular, which are known to be induced in the wake of *H. pylori* infection in humans as well as mice, appear to play a key role in balancing immunity and tissue homeostasis; their depletion leads to a severe dysregulation of Th1 responses and the concomitant

acceleration and aggravation of gastric preneoplasia.[21, 30] In humans, a Treg-dominated (as opposed to T-effector cell-dominated) anti-*Helicobacter* T-cell response is linked to asymptomatic carriage of the bacteria and a relatively benign host/bacterium interaction and well-balanced equilibrium that benefits both host and bacteria.[29]

In addition to the strength and polarization of the host immune response to *H. pylori*, the genetic makeup of the infecting strain has emerged as an important determinant of gastric cancer risk. Strains harboring the Cag pathogenicity island (Cag-PAI), which encodes a type IV secretion system (T4SS),[31] are much more tightly associated with gastric cancer than strains lacking the ability to assemble a functional Cag-PAI-encoded T4SS.[32] The only known protein substrate of the T4SS, CagA, has received much attention as a possible bacterial oncoprotein, but the direct evidence from a transgenic mouse ectopically expressing CagA has been disappointing, with less than 10% of mice developing CagA-driven gastric cancer.[33] CagA may contribute to the carcinogenic effects of T4SS-positive *H. pylori*, but it is unlikely to be the only culprit. Rather, recent work by several groups has assigned a second function to the T4SS that may be just as important as CagA delivery in promoting gastric carcinogenesis.[34–36] According to these combined studies, T4SS-positive strains have the ability to transfer an intermediate of inner core LPS biosynthesis, ADP-beta-D-manno-heptose (β-ADP-heptose), into the cytoplasm of target cells, where it binds to a newly described innate immune sensor, the alpha kinase 1 (ALPK1). Binding of β-ADP-heptose to ALPK1 stimulates its kinase domain to phosphorylate and activate TIFA,[37] which forms large complexes (called TIFAsomes) that also include interactors such as TRAF2.[35] *H. pylori* mutants that lack the ability to produce β-ADP-heptose are incapable of activating the ALPK1/TIFA pathway.[35, 36] Activation of the ALPK1/TIFA signaling axis leads to NF-κB activation and the subsequent production of pro-inflammatory cytokines and other NF-κB target gene products. NF-κB signaling has long been considered a lynchpin linking chronic inflammation and cancer, and it is plausible that NF-κB activation by *H. pylori* via the T4SS/β-ADP-heptose/ALPK1/TIFA signaling axis contributes to malignant transformation of gastric epithelial cells, for example by driving the expression of anti-apoptotic and survival/proliferation-promoting genes.[38–40]

In addition to the direct (via the T4SS, NF-κB and CagA) and indirect (via pathological immune activation) effects of *H. pylori* on gastric homeostasis, we and others have put forward the hypothesis that *H. pylori* possesses DNA-damaging properties that cause DNA double strand breaks (DNA DSBs) in *H. pylori*-exposed gastric epithelial cells (Figure 2A-C).[41–43] We and others found DNA DSB induction to be dependent on a functional T4SS[42–44] and to preferentially occur in transcribed regions of the genome.[43] Whereas translocation of CagA does not contribute to DNA DSB induction, we showed that active transcription of NF-κB target genes critically drives this form of DNA damage.[44] The specific depletion of NF-κB subunits strongly reduced DNA DSBs; interestingly, similar effects were observed upon depletion of the nucleotide excision repair (NER) endonucleases XPG and XPF (Figure 2C).[44]

As NF-κB signaling appeared to be involved in *H. pylori*-induced DNA damage, we asked whether the upstream elements of the ALPK1/TIFA/NF-κB signaling axis contribute to 53BP1 and γH2AX foci formation as a well-accepted quantitative readout of DNA damage that identifies sites of DNA DSBs. Indeed, the genetic ablation of *ALPK1* or *TIFA* in AGS cells (a gastric epithelial cancer cell line) strongly reduced *H. pylori*-induced DNA DSBs. Importantly, we found DNA damage to be limited to cells in S-phase, which were identified by PCNA staining or EdU incorporation. Interestingly, mutants of *H. pylori* that lack the gene *rfaE* (also called *hldE*; HP0858), which encodes a bifunctional enzyme involved in the synthesis of β-ADP-heptose, showed a strong defect in inducing DNA damage that was comparable to the consequences of Cag-PAI deficiency. Conversely, we found the product of RfaE activity, β-ADP-heptose to be sufficient to induce DNA damage in S-phase cells when administered in synthetic form. These combined results indicated that RfaE activity is required, and its product β-ADP-heptose is sufficient, to induce the ALPK1/TIFA-dependent DNA damage observed upon live *H. pylori* infection.

Active replication and transcription that co-occur in the same regions of the genome typically result in replication stress and DNA damage at sites where both machineries collide. In particular, nucleic acid structures known as R-loops – consisting of an RNA/DNA hybrid and displaced single-stranded DNA –[45] are known to preferentially form at sites where replication forks and actively transcribing RNA polymerases collide.[46]

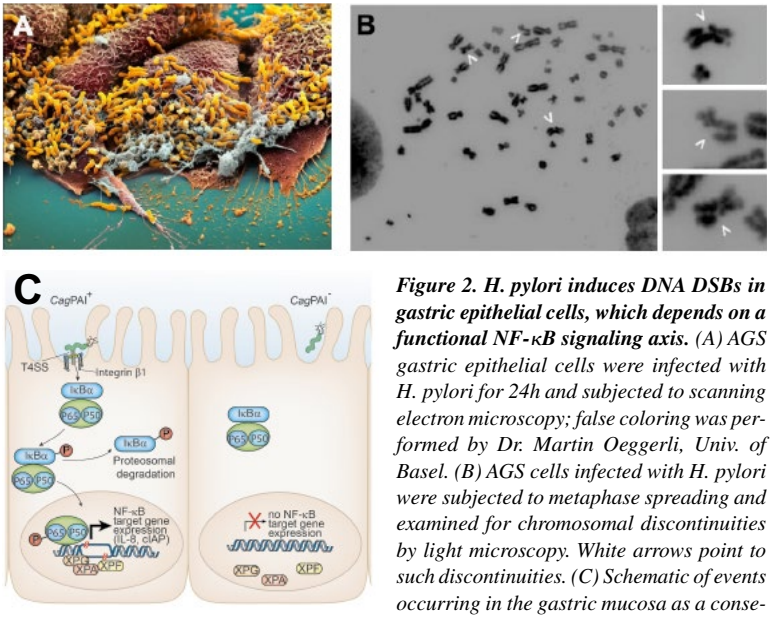

Figure 2. H. pylori induces DNA DSBs in gastric epithelial cells, which depends on a functional NF-κB signaling axis. *(A) AGS gastric epithelial cells were infected with H. pylori for 24h and subjected to scanning electron microscopy; false coloring was performed by Dr. Martin Oeggerli, Univ. of Basel. (B) AGS cells infected with H. pylori were subjected to metaphase spreading and examined for chromosomal discontinuities by light microscopy. White arrows point to such discontinuities. (C) Schematic of events occurring in the gastric mucosa as a consequence of exposure to Cag-PAI⁺ and Cag-PAI- H. pylori. Upon attachment to the cell surface, the former use their Cag-PAI-encoded T4SS to activate the canonical NF-κB- signaling pathway; nuclear translocation of the p50/p65 heterodimer results in transactivation of NF-κB target genes (IL-8 and others). The XP endonucleases XPF and XPG, along with other factors of the nucleotide excision repair machinery, are recruited to the chromatin of H. pylori-infected cells, where they introduce DNA DSBs in transcribed regions of the genome. The depletion of NF-κB subunits, or of XP endonucleases, prevents the DNA damage induced by H. pylori.*

To address whether R-loops are required for the DNA damage induced by *H. pylori*, we took advantage of a cell line that inducibly expresses human RNase H1, an enzyme that cleaves the RNA strand in RNA/DNA hybrids and thereby resolves and eliminates R-loops. Interestingly, the induction of RNase H1 expression by doxycycline abrogated both 53BP1 foci formation upon *H. pylori* infection, and also upon β-ADP-heptose treatment (Figure 3A-C). A mutant version of RNase H1 that binds to, but does not resolve R-loops and therefore serves as useful R-loop "reporter tool", allowed us to show that R-loops indeed form upon *H. pylori* infection and upon β-ADP-heptose treatment in S-phase cells (Figure

3D-F). Similar findings were obtained with an antibody (clone S9.6) that specifically recognizes RNA/DNA hybrids (data not shown). The combined results implicate replication-associated R-loops in the DNA damage associated with *H. pylori*. This work was recently published.[47]

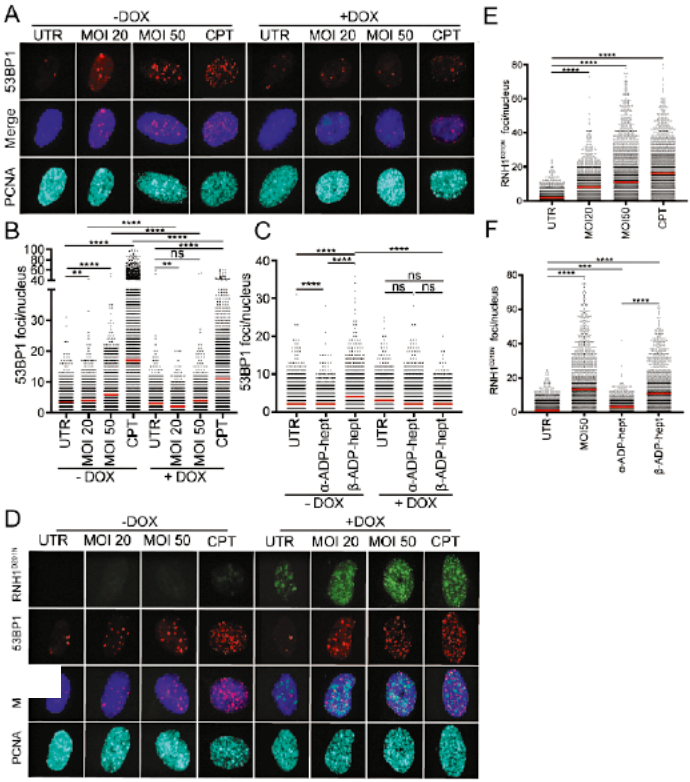

Figure 3. H. pylori-induced DNA damage and replication stress is prevented by over-expression of RNAse H1. *(A,B) U2OS cells were either infected for 6 hours with H. pylori P12 (MOI of 20 or 50), or treated with 100nM camptothecin (CPT), and were treated or not with doxycycline (-/+ DOX) to induce the expression of RNAse H1. Cells were subjected to immunofluorescence staining for 53BP1 and PCNA as well as DAPI. Representative images are shown in A alongside scatter dot plots of >1382 and up to 1752 cells per condition in B. (C) U2OS cells were exposed to* α- *or* β*-ADP-heptose at 0.5mM final concentra-*

tion for 6 hours and treated or not with doxycycline (-/+ DOX) as described in A to induce the expression of RNAse H1. (D-F) U2OS cells were either infected or treated with campthothecin or both versions of ADP-heptose as described in A-C and treated with doxycycline (-/+ DOX) to induce the expression of a (D210N) mutant version of RNAse H1 fused to GFP. Representative images are shown in D of RNAse H1 (RNH1)^{D210N}/GFP foci and 53BP1 foci, alongside scatter dot plots of RNH1^{D210N}/GFP foci of >1468 and up to 1661 cells per condition in E and F. Data are pooled from three independent experiments.

3. H. pylori is a beneficial symbiont with strong immunoregulatory properties in a majority of infected individuals.

The stomach was considered a sterile organ until Barry Marshall and Robin Warren described, in 1982, its colonization with spiral-shaped bacilli (later called *H. pylori*). Even if *H. pylori* is present, bacterial numbers in the stomach are very low at only ~10^4 bacteria/g of tissue. *H. pylori* is the only bacterium known so far to persistently colonize the adult stomach. Numerous epidemiological studies now suggest that the presence of *H. pylori*, especially from early life onwards, makes a tremendous difference in terms of the health of the host. Allergies, chronic inflammatory conditions and possibly autoimmune diseases are less common in children and young adults who are colonized with the bacteria. This inverse association has been shown for both rural and urban populations, and for the following diseases: childhood allergic asthma and allergic rhinitis, atopic dermatitis and eczema,[48–51] celiac disease,[52] ulcerative colitis[53] and Crohn's disease[54], and multiple sclerosis.[55] My lab has made a considerable effort to prove a direct protective effect of *H. pylori* on a subset of the listed diseases in state-of-the-art animal models. In particular, we showed that early life colonization with *H. pylori* in mice protects against allergic asthma[56–58] and chronic intestinal inflammation[59] (Figure 4) in a manner that depends on its ability to interact with, and reprogram dendritic cells so that these cells acquire tolerogenic properties. The realization that *H. pylori* interacts with dendritic and other myeloid cell types in the stomach in a way that favors immune tolerance, not only directed against itself, but also against other antigens, prompted us to examine this interaction more closely. Much of the recent work in the lab has been dedicated to better understanding the bacterial and host factors that affect this interaction, and that derive the differentiation of tissue-protective Tregs.

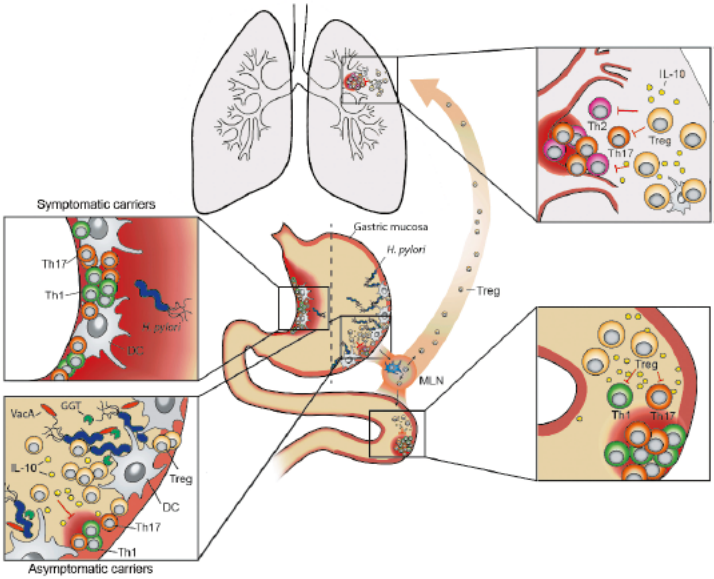

Figure 4. Dual role of the gastric pathobiont H. pylori. *H. pylori exclusively inhabits the gastric mucosa of humans. 10–20% of infected individuals will develop one of several gastric infection-associated diseases, such as chronic gastritis and gastric ulcers (shown in the upper left inset), that are driven by pathogenic T-cells polarized to express Th1 and Th17 cytokines. The majority (greater than 80% of the infected population) will remain asymptomatic throughout life despite harboring high levels of H. pylori (lower left inset). Both outcomes can be mimicked in experimentally infected mice. The H. pylori persistence factors γ-glutamyl-transpeptidase (GGT) and vacuolating cytotoxin (VacA) promote chronic infection by tolerizing DCs and thereby promoting Treg differentiation. H. pylori-induced Tregs are required for the suppression of allergen-specific immune responses in the lung and for the alleviation of colitis symptoms in models of inflammatory bowel disease (upper and lower right insets). Treg- and DC-derived IL-10 contributes to H. pylori-specific immunomodulation. Children and young adults are more likely than older hosts of H. pylori to benefit from the infection in terms of their individual allergy and IBD risk.*

As a consequence of its very low or virtually absent resident microbial community, the stomach lacks a well-developed mucosal immune system at steady state, especially in direct comparison to the small and large intestine. There is also surprisingly little literature on the gastric mucosal immune system. Therefore, when we decided to study immune cell recruitment to the infected stomach and its consequences for *H. pylori*-specific immunity and immune tolerance, we first had to establish multi-color flow cytometry panels that would allow us to capture the diversity of cells that were recruited upon infection. The following findings summarize several years of work on the topic.[60–62] First, we found that the immune cell compartment of the stomach, quite surprisingly, bears more similarities to the colon than to the small intestine. This is especially true for the myeloid compartment.[61] Second, we found that at least six distinct myeloid populations with quite diverse functions appear in the infected stomach but are virtually absent in the steady state stomach; of these, three are considered bona fide dendritic cells (DCs), as they express CD11c and depend on the growth factor FLT3 ligand for their differentiation from bone marrow precursors.[61] The others are macrophages and monocytes expressing the respective lineage markers F4/80, CD64 and Ly6C, among others. The use of RFP^+ bacteria has allowed us to show that all macrophage and monocyte, and some, but not all DC lineages come in direct contact with live bacteria in the gastric lamina propria (Figure 5A-C).[61] We also found RFP^+ bacteria to be in direct contact with human monocytes, macrophages and eosinophils in mice that we had "humanized" at birth by reconstitution with cord blood hematopoietic stem and progenitor cells.[61, 62]

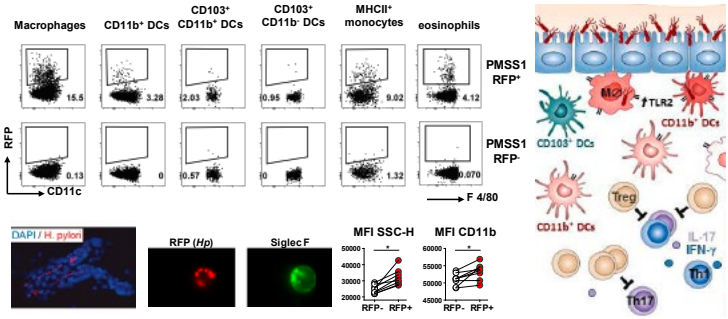

Figure 5. RFP⁺ H. pylori is sampled in the gastric lamina propria by monocytes, macrophages, DCs and eosinophils. *(A-E) Mice were infected with RFP⁺ or WT (RFP⁻) H. pylori for three months prior to the flow cytometric analysis of gastric lamina propria leukocyte populations. (A) Frequency of RFP⁺ cells among F4/80⁺ CXCR3^{hi} macrophages, CXCR3^{int} F4/80⁻ CD11b⁺ DCs, CD11b⁺ CD103⁺ DCs, CD11b⁻ CD103⁺ DCs, MHCII⁺ monocytes and SiglecF⁺ eosinophils. Please refer to Arnold et al 2017[61] and 2018[62] for the gating strategies we use to identify these populations among all gastric lamina propria leukocytes. (B) RFP⁺ H. pylori residing inside a gastric gland, prepared by collagenase digestion and mechanical disruption of the tissue. (C) Schematic representation of the events occurring at the gastric mucosa. RFP⁺ H. pylori are sampled by macrophages and dendritic cells that presumably extend dendrites across the epithelial layer; this interaction induces a distinct transcriptional program (such as upregulation of TLR2 and IL-10 production) in these cells. Both Tregs and effector T-cells (Th17, Th1) are recruited as a consequence of myeloid cell interactions with H. pylori, and the strength of one response over the other determines colonization levels. (D) Image stream analysis of a SiglecF⁺ eosinophil that has engulfed four or five RFP⁺ H. pylori. (E) Activation of eosinophils, as assessed by their surface expression of CD11b and their side scatter, that either have (RFP⁺) or have not (RFP⁻) come in direct contact with H. pylori and are from the same stomach.*

In addition to murine DCs and macrophages/monocytes encountering live fluorescent *H. pylori*, we also found eosinophils to be in direct contact with the bacteria, in some cases up to five bacteria per cell (Figure 5 A, D, E).[62] Subsequent extensive work on the role of eosinophils in immunity to *H. pylori* revealed that (1) eosinophils are recruited to the infected stomach in large numbers, (2) eosinophils have homeostatic properties (suppressing excessive Th1 responses to the infection and thereby preventing tissue damage), that (3) eosinophils have bactericidal activities against other bacteria (e.g. *Citrobacter rodentium*) that are however efficiently evaded by *H. pylori* and that (4) the Th1 cytokine IFN-γ conditions eosin-

ophils to exhibit both bactericidal and immunomodulatory properties.[62] We have more recently also investigated the role of eosinophils also in models of gastrointestinal carcinogenesis.[63] The interaction of *H. pylori* with these diverse myeloid cells has distinct, and in some cases opposing consequences for the host. This is best understood for $CD103^{+}CD11b^{-}$ DCs, for which we had a very selective knock out mouse available. $BATF3^{-/-}$ (basic leucine zipper transcriptional factor ATF-like 3) mice lack $CD103^{+}$ DCs completely;[64] we find that these mice are incapable of controlling an experimental *H. pylori* infection due to their inability to launch proper Th1 responses.[60] We observed a similar defect in Th1 immunity of $BATF3^{-/-}$ mice in a tumor model and another bacterial infection model using *Mycobacterium bovis* BCG.[60] More detailed mechanistic studies showed that, while T-cell priming and Th1 differentiation in the draining lymph nodes was not impaired due to BATF3 deficiency, these cells lacked expression of the surface receptor CXCR3 and therefore failed to home to infected tissues in response to gradients of the chemokines and CXCR3 ligands CXCL9, CXCL10 and CXCL11 (Figure 6). The same problem applied to regulatory T-cells, which differentiated normally, but failed to upregulate CXCR3 in the absence of $CD103^{+}$ DCs (Figure 6).[60]

$BATF3^{-/-}$ mice thus lack both Th1 cells and Tregs at sites of infection and tumorigenesis. One type of Tregs arises in the thymus (tTregs), where they are selected based on the affinity of their interaction with self-peptide in conjunction with MHC complex, and acquire the ability to suppress pathological self-reactivity, i.e. autoimmune disease.[65, 66] The other major subtype of Tregs differentiates in the periphery (so-called pTregs) from naïve $CD4^{+}$ T-cells that have been exposed to a TCR signal in conjunction with high levels of TGF-β, retinoic acid, and other DC-derived factors associated with peripheral immune tolerance; this Treg subtype establishes and maintains tolerance to harmless dietary, environmental and commensal antigens and promotes immune homeostasis, especially in the GI tract.[65, 66]

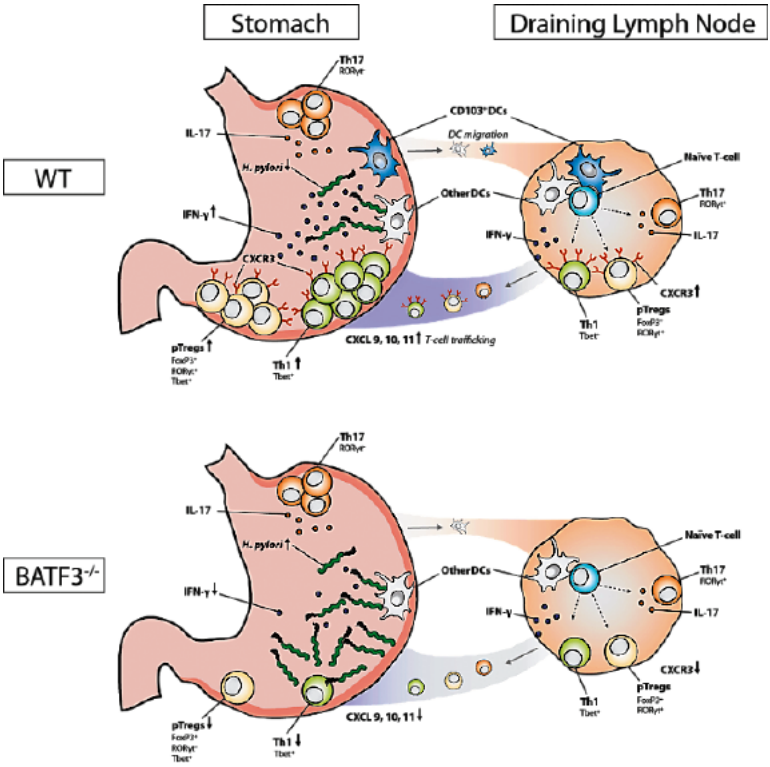

Figure 6. BATF3-dependent DCs drive immune control of H. pylori by producing chemokines and priming Th cells to express CXCR3. *In the gastric mucosa of wild type mice, BATF3-dependent DCs and several other myeloid lineages sample H. pylori and trigger a vigorous mixed Th1/Th17 response, which nevertheless is incapable of completely clearing H. pylori. H. pylori-specific Th responses are primed in the draining mesenteric lymph nodes. Th1 cells, but not Th17 cells, home to infected tissue following a gradient of CXCL-9, -10, -11 and probably other chemokines. In the absence of BATF3-dependent DCs, Th1 differentiation (evidenced by Tbet expression and IFN-γ production) occurs normally; however, Th1 cells primed in the absence of this DC lineage fail to upregulate the chemokine receptor CXCR3 and therefore fail to follow CXCL-9/10/11 gradients and to traffic to the H. pylori-infected gastric mucosa. The same mechanism also explains the deficiency of BATF3-/- mice in controlling other bacterial infections and also tumors, and further accounts for defective H. pylori-specific Treg responses in BATF3-/- mice.*

H. pylori infection induces pTregs (identified as neuropilin-negative, Helios-negative) that can be found in the infected stomach in large numbers, whereas tTregs remain unchanged.[60] Interestingly, we recently found that eosinophils are required for pTreg expansion in bacterially infected tissues. The ability of eosinophils to support Treg proliferation in tissues requires TGF-β; a mouse strain specifically lacking TGF-β in the eosinophil compartment is defective for pTregs in tissues; such mice have fewer tissue Tregs upon infection with various gastrointestinal pathogens (we tested *H. pylori*, *H. hepaticus* and *Citrobacter rodentium*), and the characteristic proximity of eosinophils and Tregs in tissues is not observed (Figure 7).

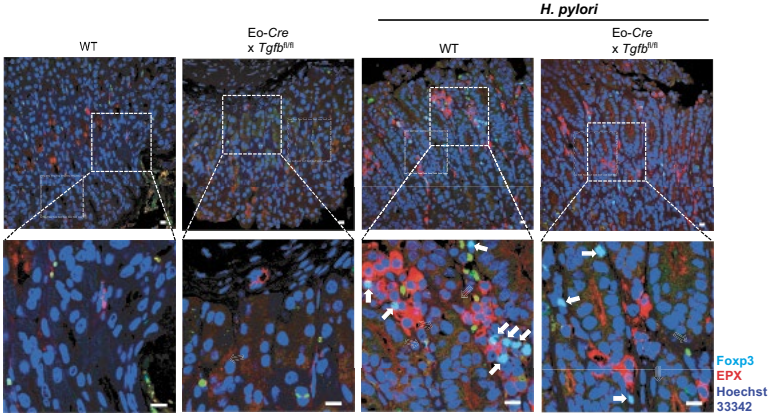

Figure 7. Tregs reside in close proximity to Tregs in the gastric mucosa of wild-type, but not Eo-Cre x Tgf$b^{fl/fl}$ mice. *Eo-Cre x Tgf$b^{fl/fl}$ mice and their wild-type littermates were infected with H. pylori strain PMSS1 for six weeks, or remained uninfected. EPX-positive eosinophils (red) and of Foxp3^{+} Tregs (turquoise) in the gastric mucosa were visualized by immunofluorescence microscopy (scale bar: 10 mm). White arrows point to Foxp3^{+} Tregs. Pictures courtesy of Hans-Uwe Simon, Univ. of Bern.*

Our next plans along the described avenues of research are to characterize the *H. pylori*-induced Tregs more comprehensively by single cell RNA sequencing and spectral flow cytometry approaches, and to study in suitable mouse strains that lack the ability to induce Tregs upon *H. pylori* infection how these cells prevent allergic asthma, intestinal inflammation and other disorders that are inversely associated with *H. pylori* infection. In more practical terms, we are also exploring how an important immunomodulator produced by *H. pylori*, the VacA protein, can be exploited for preventive and even therapeutic purposes in patients suffering from, or at risk of developing severe asthma and other allergic manifestations. Preclinical studies in mouse models suggest that regular injections (oral or intraperitoneal) of VacA effectively prevent allergic asthma, especially if administered early in life, and can even reduce the severity of allergic asthma in a therapeutic setting. We are conducting this work in collaboration with a Biotech company that is developing recombinant VacA as a possible intervention in allergy and autoimmunity (http://www.gbc-hpvac.com/).

3. The pathogenesis of bacterially induced and of non-infection-associated aggressive lymphomas

In addition to the work we have pursued over the years on *H. pylori* as a pathogen, and as a symbiont of humans, we have maintained a long-standing interest in understanding the pathogenesis of B-cell lymphoma, initially focusing on lymphoma subtypes with underlying bacterial causes.[67–69] We have more recently expanded our work to also include lymphoma entities of other pathogenetic origins, especially focusing on the very common and aggressive diffuse large B-cell lymphoma (DLBCL). In DLBCL, we have elucidated a cascade of events driving malignant transformation that is initiated by the aberrant silencing of a microRNA, miR-34a, due to over-expression of MYC,[70] which in this scenario acts as a repressor of non-coding microRNA genes. Loss of miR-34a expression in turn favors abnormally high levels of the transcription factor FOXP1, which in normal B-cells is post-transcriptionally silenced by this microRNA. FOXP1 functions as a repressor of the G-protein-coupled receptor S1PR2; as a consequence, S1PR2^{lo} DLBCL cells escape apoptosis induction.[71] We have more recently discovered that DLBCL cells survive not only because they

exploit the miR-34a/FOXP1/S1PR2 axis, but by additionally silencing a second signaling pathway (involving TGFβ receptor II and its downstream target SMAD1) that also converges on S1PR2.[72] This negative regulation is initiated by SMAD1 silencing by promoter hypermethylation,[73] which is common in DLBCL and affects various important tumor suppressors,[74] and likely is driven by mutations in epigenetic modifiers.[75]

Our described work on DLBCL was sparked by observations made in human samples, and was continued and validated using various cell culture and mouse model systems (spontaneous and serial transplantation models, as well as orthotopic xenotransplantation and patient-derived xenograft models). To close the circle and move back to patients, we patented our discovery of the tumor-suppressive properties of miR-34a ("Treatment of B-cell lymphoma with microRNA" EP10182950.5, University of Zurich) and licensed the patent to MIRNA Therapeutics, Austin, Texas. The licensing prompted the company to add a cohort of lymphoma patients to their ongoing phase I dose escalation trial. Sadly, despite promising initial reports of on-target effects of the microRNA on tumor cells and partial clinical responses in a subset of the patients, the trial had to be terminated in 2016 due to toxicities at the higher, effective doses. Despite this setback, we continue to be actively engaged in identifying new treatment modalities using the cell lines, primary samples and mouse models we have at our disposal. Most recently, in research towards this end, we have conducted a screen using 20 DLBCL and other lymphoma cell lines, of drug susceptibility to a selection of 126 compounds that are approved for clinical use (Figure 8A). One of the promising compounds emerging from the screen, venetoclax, was further investigated in both *in vitro* and *in vivo* settings; specifically, we were able to confirm in individual viability assays that our cell lines are differentially susceptible to venetoclax (Figure 8B). Only cell lines with strong BCL-2 expression due to BCL2 amplification, but not translocations affecting the BCL2 locus, were found to potently be killed with venetoclax (Figure 8C). Only sensitive (RIVA), but not resistant (U2932) cell lines were efficiently targeted also *in vivo* with venetoclax in an orthotopic xenotransplantation model (Figure 8D). Our subsequent analysis of cancer genome atlas (TCGA) data revealed that high BCL-2 expression is a hallmark of the particularly aggressive MCD and EZB subtypes, in which it is caused by BCL2 gains (MCD), and by BCL2 translocations (EZB, Figure 8E), respectively.

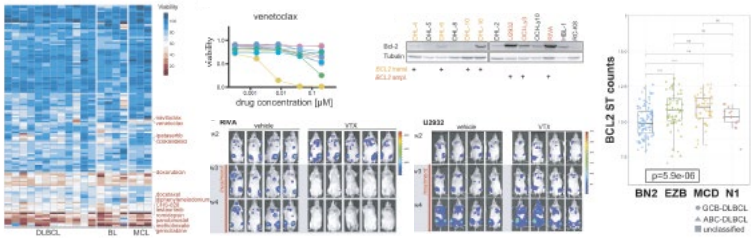

Figure 8. Venetoclax is effective in killing BCL-2^{hi} DLBCL cells in vitro and in vivo. *A, Heat map displaying the viability (calculated as the mean of the five concentrations assessed per drug) of 20 cell lines of the indicated entities, as assessed by CellTiter-Glo viability assay, after 48 hours of exposure to 126 manually selected compounds targeting deregulated pathways in hematological malignancies. Select compounds with differential effects on viability are indicated. B, Validation of viability after 48 hours of exposure to venetoclax. The yellow curve represents the RIVA cell line. C, Bcl-2 expression of the indicated cell lines as determined by Western blotting; tubulin expression served as loading control. The color code indicates the BCL2 status (yellow: BCL2 translocation; red: BCL2 amplification; black: wild type BCL2). D, MISTRG mice were injected intravenously with 1×10^7 cells of the two indicated cell lines; IVIS images were recorded once weekly. Mice received twice-weekly doses of 40 mg/kg venetoclax via oral gavage, initiated once lymphomas were clearly detectable in all mice of the cohort (after two weeks of growth, respectively; time on treatment indicated by grey shading). E, BCL2 gene expression of 206 DLBCL cases available through TCGA, stratified based on genetic subtype as assigned by Schmitz et al.[76] p-values were determined by Kruskal-Wallis test. Symbols indicate the subtype based on gene expression signature.*

We went on to conduct a combinatorial screen that included 64 manually selected compounds, with or without additional exposure to venetoclax. This screen revealed synergistic killing of numerous cell lines initially found to be resistant to venetoclax alone, in combinations with BTK inhibitors on the one hand, and PI3K inhibitors on the other (Figure 9A). The synergy of both combinations could be confirmed in individual viability assays (Figure 9B-D).

In a final set of experiments pertaining to the topic of drug synergy in DLBCL treatment, we were able to show that the addition of ibrutinib to the venetoclax treatment regimen not only overrides primary resistance, as seen with U2932 cells, but can also be used to overcome secondary resistance resulting from prolonged venetoclax exposure *in vivo*. Not only

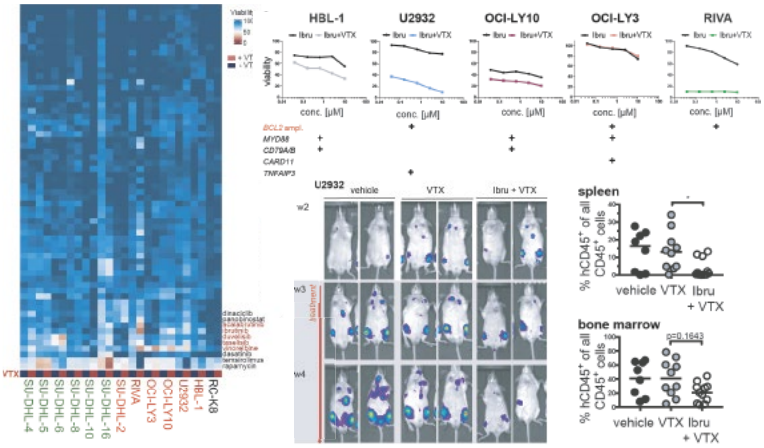

Figure 9. The BTK inhibitor ibrutinib synergizes with venetoclax in killing DLBCL cells in vitro and in vivo. *a, Heat map displaying the viability of 13 DLBCL cell lines after 48 hours of exposure to 64 manually selected compounds, with or without venetoclax. Select compounds that synergize with venetoclax to kill DLBCL cells are indicated. b, Viability curves of the indicated cell lines exposed to ibrutinib +/- venetoclax. c, MISTRG mice were injected intravenously with U2932 cells; IVIS images were recorded once weekly. Mice received twice-weekly doses of 40 mg/kg venetoclax, with or without 10 mg/kg ibrutinib via oral gavage.*

did the combination more effectively reduce the tumor burden when used as "first-line" treatment, but mice also relapsed later; the combination could further be used as "salvage" therapy in mice that had initially only received venetoclax (Figure 10A). A resistant clone, M21, that was harvested from a venetoclax-refractory donor, could be effectively controlled by the combination, but not venetoclax alone, in transplanted recipients (Figure 10B-D). Primary cells used in a PDX model also responded better to the combination than to venetoclax alone (data not shown).

Future work on our models of DLBCL will mostly revolve around mechanisms of immune escape, and treatment strategies that attempt to overcome immune escape mechanisms. We have recently found that some subtypes of DLBCL express large amounts of the immunoregulatory cytokine IL-10, which serves to cell-autonomously support proliferation

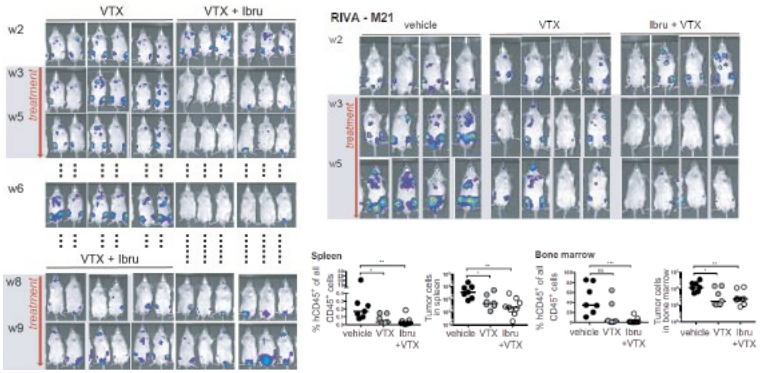

Figure 10. Acquired venetoclax resistance can be overcome by ibrutinib addition in vivo. *A, MISTRG mice were injected intravenously with 1 × 107 RIVA cells, and received twice-weekly doses of 40 mg/kg venetoclax, either alone or in combination with 10 mg/kg ibrutinib via oral gavage, initiated once lymphomas were clearly detectable in all mice of the cohort (after two weeks of growth; indicated by grey shading). An interval of two weeks without drug treatment lead to lymphoma recurrence, which was delayed in mice on combination treatment, and could to some extent be suppressed by salvage combination treatment. B-D, Venetoclax-resistant RIVA cells isolated from donor mouse M21 were re-transplanted into MISTRG recipients, which received twice-weekly doses of 40 mg/kg venetoclax, either alone or in combination with 10 mg/kg Ibrutinib via oral gavage, and were assessed with respect to their tumor burden at the study endpoint. E-G, Primary DLBCL cells were injected into MISTRG6 mice, which received twice-weekly doses of 40 mg/kg venetoclax, either alone or in combination with 10 mg/kg ibrutinib via oral gavage starting from week 4 post-transplantation. The tumor burden was assessed at 7 weeks post-transplantation.*

of the tumor B-cells on the one hand, and to recruit or locally expand regulatory T-cells on the other. The depletion of Tregs is sufficient to enable immune control of lymphomas by the immunocompetent host. A main research focus therefore will be to shed more light on the lymphoma B-cell/Treg axis, and to attempt to target it with suitable treatment strategies.

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Die Stiftung Professor Dr. Max Cloëtta

Die Stiftung Professor Dr. Max Cloëtta wurde am 27. September 1973 in Zürich von Dr. Antoine Cloëtta zu Ehren seines Vaters Prof. Dr. Max Cloëtta errichtet.

In Absatz 1 von Art. 3 der Stiftungsurkunde wird der Zweck der Stiftung wie folgt umschrieben:

"Die Stiftung bezweckt:
a) die Unterstützung und Förderung der medizinischen Forschung sowie der damit verbundenen naturwissenschaftlichen Hilfsdisziplinen in der Schweiz;
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zur Auszeichnung schweizerischer und ausländischer Persönlichkeiten, die sich in besonderer Weise um bestimmte Gebiete der medizinischen Forschung verdient gemacht haben."

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