ANNE MÜLLER

CURRICULUM VITAE

Anne Müller, Ph.D.
Associate Professor of Experimental Medicine
Institute of Molecular Cancer Research, University of Zürich
Winterthurerstr. 190, 8057 Zürich
Phone 044 635 34 74, Fax 044 635 34 84, mueller@imcr.unizh.ch
www.imcr.uzh.ch/research/Muller.html

Personal Data

Marital status	married; three daughters (born 2003, 2007, 2013)
Date of birth	29.07.1971 (Mainz, Germany)
Nationality	German

Positions, Education and Training

Board of Directors	Institute of Molecular Cancer Research, University of Zürich	2017–present
Full Professor of Experimental Medicine	Institute of Molecular Cancer Research, University of Zürich	2021–present
Associate Professor	Institute of Molecular Cancer Research, University of Zürich	2012–2021
Assistant Professor	Institute of Molecular Cancer Research, University of Zürich	2006–2012
Post-doctoral fellow	Stanford University School of Medicine, Stanford, CA, USA Advisor: Prof. Dr. Stanley Falkow	2001–2006
Post-doctoral fellow	Max Planck Institute for Infection Biology Berlin, Germany Advisor: Prof. Dr. Thomas Rudel	2000–2001

Ph.D.	Max Planck Institute for Infection Biology Berlin, Germany Advisor: Prof. Dr. Thomas Rudel	1996–2000
M.S. Biology	Julius-Maximilians University Würzburg, Germany Advisor: Prof. Dr. Jörg Hacker	1990–1996

Fellowships, Awards and extramural funding (Funding since 2015 only)

Swiss Cancer League KFS-5228-02-2021 "Identifying and overcoming immune evasion strategies of diffuse large B-cell lymphoma" CHF 375 000	2021–2024
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
BSCGIO_157841/1
"Exploiting the immunomodulatory properties of H. pylori for the treatment of immunological disorders" CHF 1 992 150

Emmy Noether Post-doctoral research fellowship, 2002–2004
German Research Foundation (DFG)

Post-doctoral fellowship, Max Planck Society 2001–2002

Becton Dickinson dissertation award, 2001
German Society for Hygiene and Microbiology

Otto Hahn Medaille (young researchers' award) 2001
Max Planck Society

Institutional Responsibilities

Member and evaluation committee, 2006–present
Cancer Network Zurich and Cancer Biology PhD program

Member, Interfaculty MD/PhD committee 2014–present

Member, Scientific Advisory Board, 2016–2018
Hochschulmedizin Zürich

Member, Scientific Advisory Board, 2016–2018
Stiftung für wissenschaftliche Forschung der UZH

Member, research committee (Forschungskommission) 2016–present
of the Medical Faculty of UZH

Board of Directors, Cancer Research Center, UZH 2018–2020

OTHER Responsibilities

Scientific Advisory Board, ISREC foundation, Lausanne, Switzerland 2017–present

Elected member, SNF commission for post-doc mobility, Div. III, Bern, Switzerland 2018–2020

Elected member, SNF PRIMA commission, Div. III, Bern, Switzerland 2018–present

Elected member, SNF Ambizione commission, Div. III, Bern, Switzerland 2017–2019

Regular reviewing activities: Journal of Clinical Investigation, Nature, Gastroenterology, Blood, Journal of Immunology, PNAS, Pediatric Blood and Cancer, Infection and Immunity, Cellular Immunology, Clinical and Vaccine Immunology, Helicobacter, Journal of Bacteriology, PLOS Pathogens, Cell Host & Microbe, Allergy, Frontiers in Microbiology, and others

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.

Daniela Engler, **Anne Müller**. Compositions useful for inflammatory bowel diseases and coeliac disease. Application submitted in Jan. 2014. EP14153366.1; University of Zurich. Licensed to Geneva Biotech Center Inc., Geneva, Switzerland, 2016.

SELECTED PUBLICATIONS

Arnold, I.C., Dehzad, N., Reuter, S., Martin, H., Becher, B., Taube, C. and **Müller, A.** Neonatal infection with *Helicobacter pylori* prevents asthma through impaired dendritic cell maturation and induction of regulatory T-cells. *J. Clin Invest.* 121:3088–3093 (2011).

Toller. I.M., Neelsen, K., Steger, M., Hottiger, M.O., Stucki, M., Gerhard, M., Sartori, A.A., Lopes, M. and **Müller, A.** The carcinogenic bacterial pathogen *Helicobacter pylori* triggers DNA double strand breaks and a DNA damage response in infected host cells. PNAS 108, 14944-14949 (2011).

Arnold, I., Lee, J.Y., Amieva, M.R., Flavell, R.A., Sparwasser, T. and **Müller, A.** Tolerance rather than immunity protects from *Helicobacter*-induced gastric preneoplasia. *Gastroenterology* 140, 199–209 (2011).

Schmid, C.A., Robinson, M.D., Scheifinger, N.A., Müller, S., Cogliatti, S., Tzankov, A. and **Müller, A.** DUSP4 deficiency caused by promoter hypermethylation drives JNK signaling and tumor cell survival in diffuse large B cell lymphoma. *J Exp Med.* 212:775-92 (2015).

Koch, K.N., Hartung, M.L., Urban, S., Kyburz, A., Bahlmann, A.S., Lind, J., Backert, S., Taube, C. and **Müller, A.** *Helicobacter* urease-induced activation of the TLR2/NLRP3/IL-18 axis protects against asthma. *J. Clin Invest*. 125:3297-302 (2015).

Stelling A., Hashwah H., Bertram K., Manz MG., Tzankov A., **Müller A.** The tumor suppressive TGF-ß/SMAD1/S1PR2 signaling axis is recurrently inactivated in diffuse large B-cell lymphoma. *Blood.* 131:2235-2246 (2018).

Arnold, I.C., Artola-Borán, M., Tallón de Lara, P., Kyburz, A., Taube, C., Ottemann, K., van den Broek, M., Yousefi, S., Simon, H.U., and **Müller, A.** Eosinophils suppress Th1 responses and restrict bacterially induced gastrointestinal inflammation. *J Exp Med.* 215:2055-2072 (2018).

Kyburz, A., Fallegger, A., Zhang, X., Altobelli, A., Artola-Boran, M., Borbet, T., Urban, S., Paul, P., Münz, C., Floess, S., Huehn, J., Cover, T.L., Blaser, M.J., Taube, C., and **Müller A.** Transmaternal Helicobacter pylori exposure reduces allergic airway inflammation in offspring through regulatory T cells. *J Allergy Clin Immunol.* 143:1496-1512.e11 (2019).

Bauer M., Nascakova Z., Mihai A., Cheng P., Levesque M.P., Lampart S., Hurwitz R., Pfannkuch L., Dobrovolna J., Jacobs M., Bartfeld S., Dohlman A., Shen X., Gall T., Salama N. R., Töpfer A.,Weber A., Meyer T.F., Janscak P.* and **Müller A*.** The ALPK1/TIFA/NF-κB axis links a bacterial carcinogen to replication stress and DNA damage. *co-last authors *Nat. Communications* 11:5117 (2020).

Arnold I.C., Artola-Boran M., Gurtner A., Bertram K., Bauer M., Frangez Z., Becher B., Kopf M., Yousefi S., Simon H., Tzankov A. and **Müller A.** The GM-CSF/IRF5 signaling axis in eosinophils promotes anti-tumor immunity through activation of type I T-cell responses. *J Exp Med,* 217:20190706 (2020).

THE TWO FACES OF HELICOBACTER PYLORI

Anne Müller, Institute of Molecular Cancer Research, University of Zurich

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]

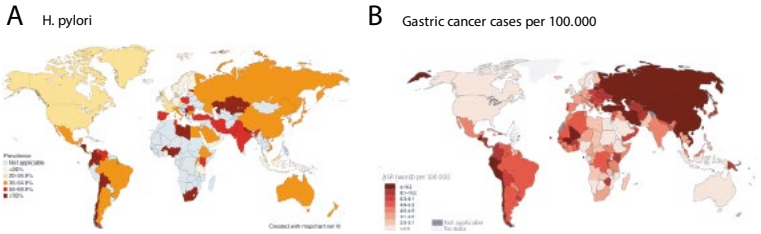

Figure 1. Global prevalence of H. pylori and of gastric cancer. *A, Prevalence of H. pylori, as estimated by Zamani et al.*[12] *B, Gastric cancer cases per 100 000 residents, as determined by Rawla et al.*[13]

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]

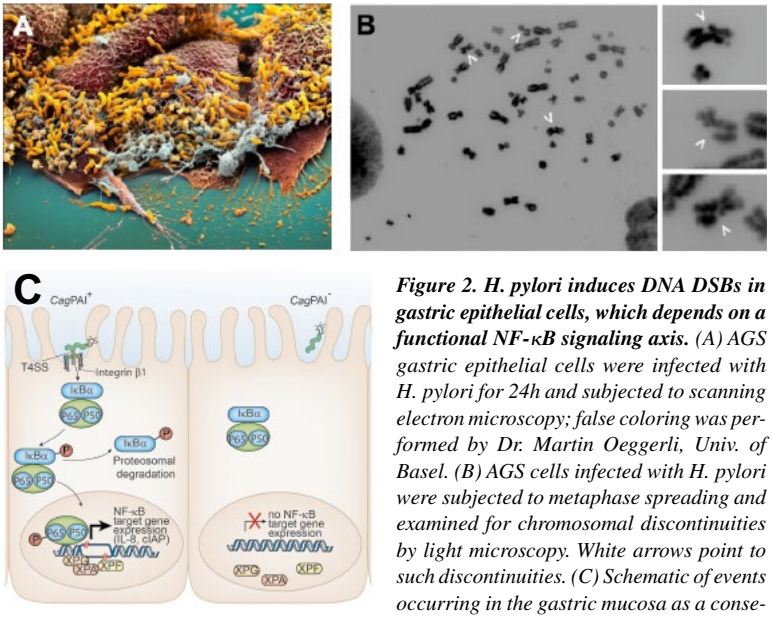

Figure 2. H. pylori induces DNA DSBs in gastric epithelial cells, which depends on a functional NF-κB signaling axis. *(A) AGS gastric epithelial cells were infected with H. pylori for 24h and subjected to scanning electron microscopy; false coloring was performed by Dr. Martin Oeggerli, Univ. of Basel. (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]

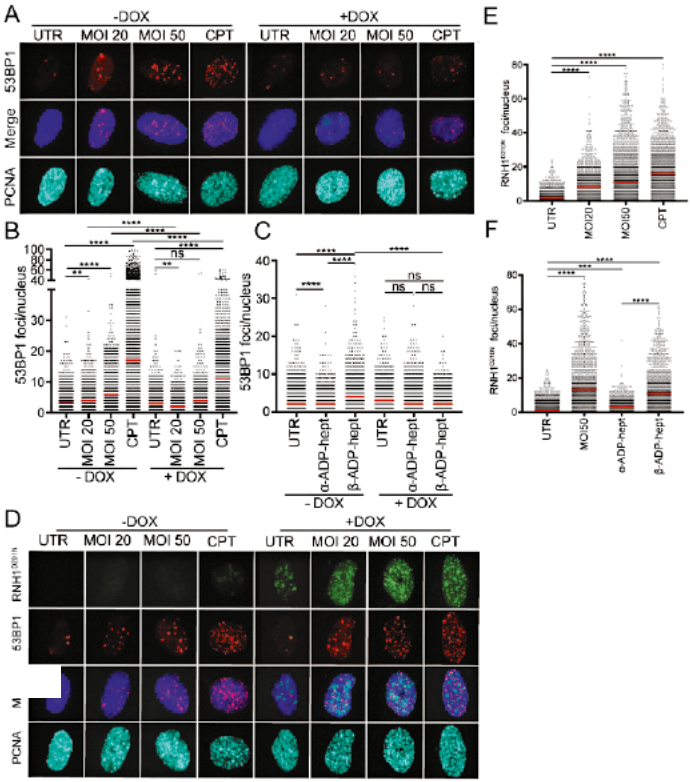

Figure 3. H. pylori-induced DNA damage and replication stress is prevented by over-expression of RNAse H1. *(A,B) U2OS cells were either infected for 6 hours with H. pylori P12 (MOI of 20 or 50), or treated with 100nM camptothecin (CPT), and were treated or not with doxycycline (-/+ DOX) to induce the 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.

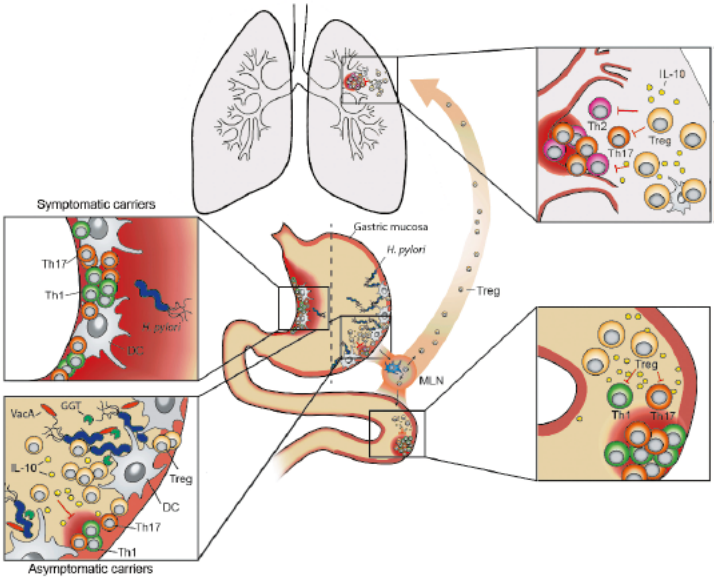

Figure 4. Dual role of the gastric pathobiont H. pylori. *H. pylori exclusively inhabits the gastric mucosa of humans. 10–20% of infected individuals will develop one of several gastric infection-associated diseases, such as chronic gastritis and gastric ulcers (shown in the upper left inset), that are 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]

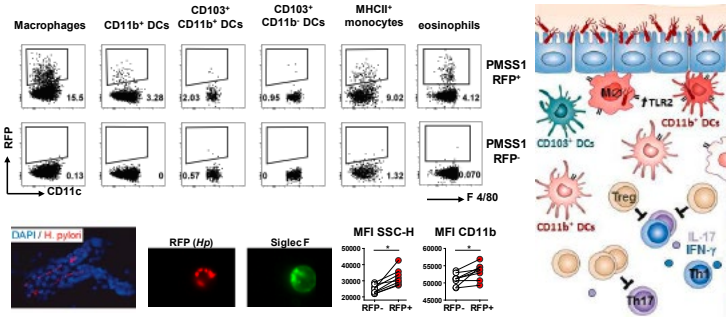

Figure 5. RFP⁺ H. pylori is sampled in the gastric lamina propria by monocytes, macrophages, DCs and eosinophils. *(A-E) Mice were infected with RFP⁺ or WT (RFP⁻) H. pylori for three months prior to the flow cytometric analysis of gastric lamina propria leukocyte populations. (A) Frequency of RFP⁺ cells 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]

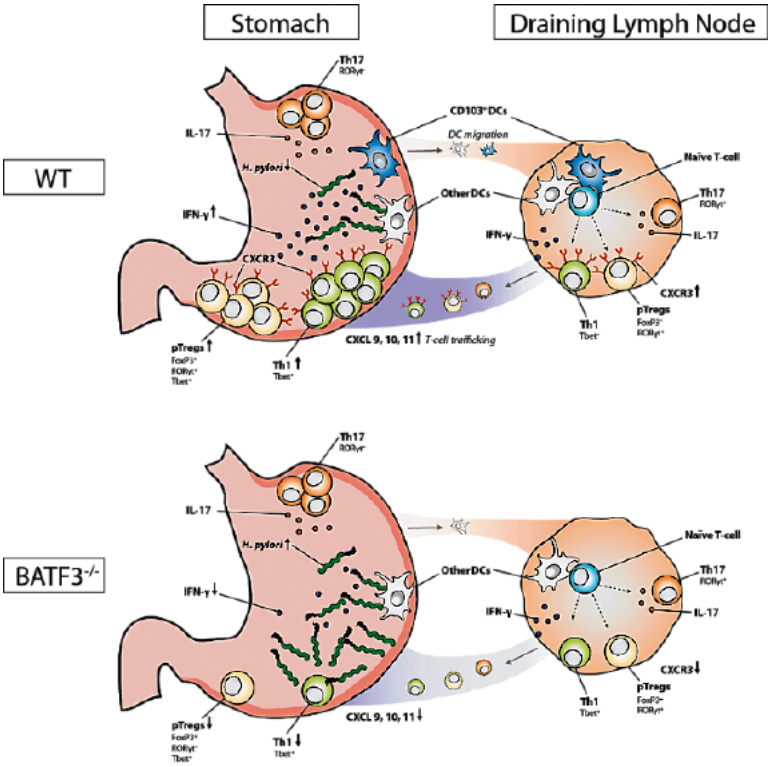

Figure 6. BATF3-dependent DCs drive immune control of H. pylori by producing chemokines and priming Th cells to express CXCR3. *In the gastric mucosa of wild type mice, BATF3-dependent DCs and several other myeloid lineages sample H. pylori and trigger a vigorous mixed Th1/Th17 response, which nevertheless 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).

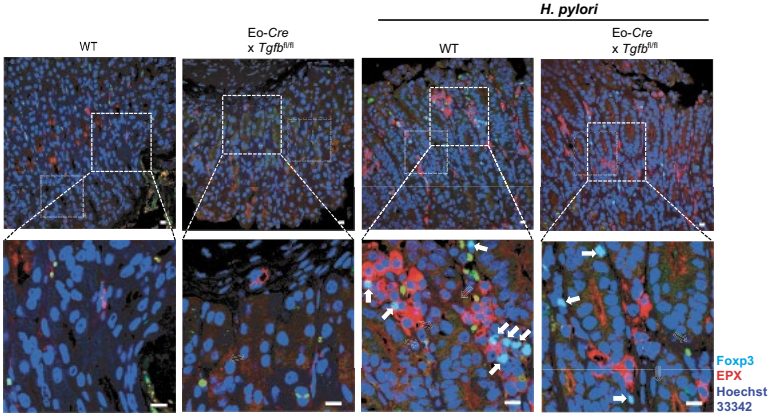

Figure 7. Tregs reside in close proximity to Tregs in the gastric mucosa of wild-type, but not Eo-Cre x Tgf$b^{fl/fl}$ mice. *Eo-Cre x Tgf$b^{fl/fl}$ mice and their wild-type littermates were infected with H. pylori strain PMSS1 for six weeks, or remained uninfected. EPX-positive eosinophils (red) and 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.

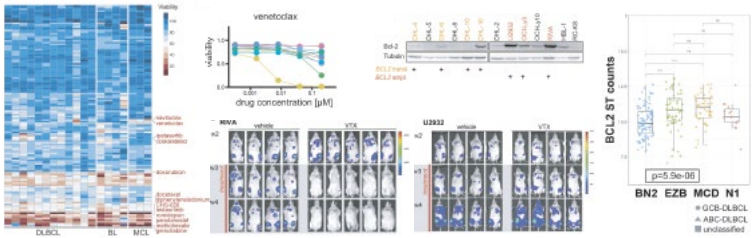

Figure 8. Venetoclax is effective in killing BCL-2^{hi} DLBCL cells in vitro and in vivo. *A, Heat map displaying the viability (calculated as the mean of the five concentrations assessed per drug) of 20 cell lines of the indicated entities, as assessed by CellTiter-Glo viability assay, after 48 hours 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

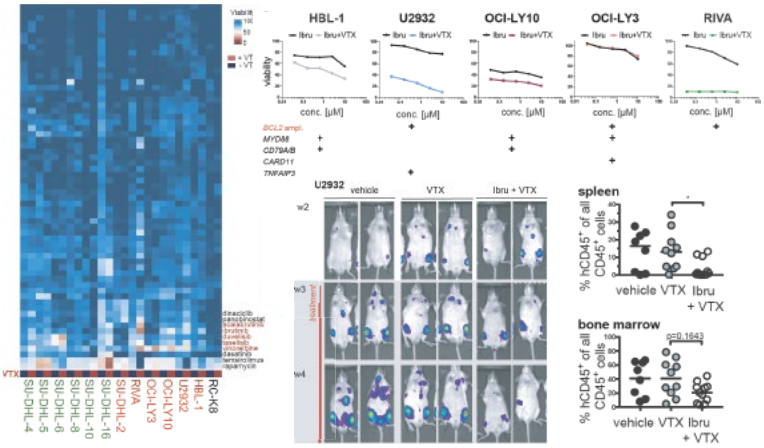

Figure 9. The BTK inhibitor ibrutinib synergizes with venetoclax in killing DLBCL cells in vitro and in vivo. *a, Heat map displaying the viability of 13 DLBCL cell lines after 48 hours of exposure to 64 manually selected compounds, with or without venetoclax. Select compounds that synergize with venetoclax 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

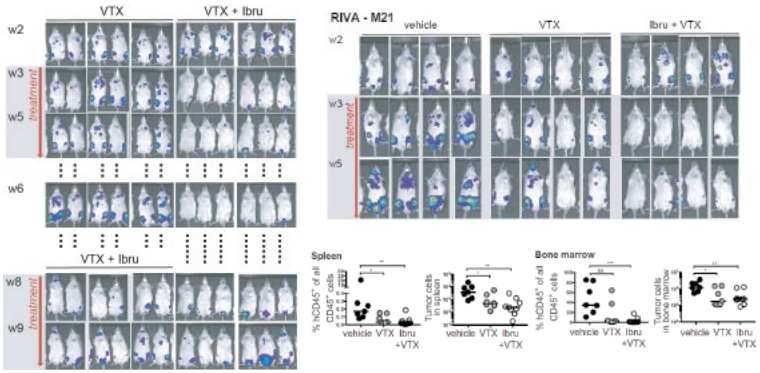

Figure 10. Acquired venetoclax resistance can be overcome by ibrutinib addition in vivo. *A, MISTRG mice were injected intravenously with 1 × 107 RIVA cells, and received twice-weekly doses of 40 mg/kg venetoclax, either alone or in combination with 10 mg/kg ibrutinib via oral gavage, initiated once 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.

Acknowledgments

I am grateful and honored to be selected by the Professor Dr. Max Cloëtta Foundation for this prestigious award. Research in my laboratory has been generously supported by various funding sources, in particular the Swiss National Science Foundation, the Swiss Cancer League, the Zurich Cantonal Cancer League and the University of Zurich (Clinical Research Programs Hematological Malignancies and Precision Oncology, and Comprehensive Cancer Center Zurich). I am blessed to be working with a fabulous technician, Mariela Artola-Boran, and have had the privilege to mentor several talented post-docs and master students. Most of all, I have had the pleasure to serve as advisor to numerous hard-working, motivated, smart and dedicated graduate students, without whom none of the above-listed achievements and successes would have been possible. You all have been a joy to work with, and an inspiration! Thank you! My heartfelt thanks to many collaborators, close by and far away, who have worked with me over the years, and to my colleagues and friends at IMCR and beyond, in particular: Isabelle Arnold, Maries van den Broek, Massimo Lopes, Alessandro Sartori. Thank you all for your support!

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