BARCELONA • OCTOBER 7–9, 2026

7th NF-κB International Congress

CONGRESS BOOK

SCIENTIFIC PROGRAM
Invited speakers • Short talks • Poster abstracts

VENUE
Barcelona Biomedical Research Park (PRBB)

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WELCOME AND CONGRESS INFORMATION

Dear Friends and Colleagues,

Following the success of previous editions, we are delighted to welcome you to the **7th International NF-κB Congress**, taking place in Barcelona from **October 7 to 9, 2026**.

Over the years, this meeting has become an important forum for bringing together researchers from Europe and around the world who share a common interest in NF-κB biology. The Congress provides a unique opportunity to exchange new ideas, discuss recent advances, establish collaborations, and strengthen the international NF-κB research community.

This year's scientific program will feature dedicated sessions exploring the central roles of **NF-κB subunits and their key regulators** in a broad range of biological and pathological processes, with particular emphasis on **inflammation and immunity, development and cell differentiation, stemness, cancer, transcriptional regulation, and therapeutic targeting**. Through invited lectures, selected short talks, and poster presentations, we aim to provide a stimulating overview of both fundamental discoveries and emerging translational opportunities in the field.

We are especially pleased to bring together established leaders and younger investigators, creating an open and interactive environment in which new findings can be shared and discussed. We hope that the scientific sessions, poster discussions, and informal exchanges throughout the meeting will foster new connections and inspire future collaborations.

We warmly welcome you to Barcelona and hope that you will enjoy both the scientific program and the opportunity to meet colleagues from across the international NF-κB community.

Welcome to the 7th International NF-κB Congress!

The Organizing and Scientific Committees

Organizing Committee

Lluís Espinosa	Hospital del Mar Research Institute
Anna Bigas	Hospital del Mar Research Institute, Josep Carreras Institute

Scientific Committee

Véronique Baud	Université Paris Cité, Paris
Ruaidhri Carmody	University of Glasgow, UK
Nuno dos Santos	University of Porto, Portugal
Raquel Espín-Palazón	Iowa State University, USA
Alexander Hoffmann	University of California Los Angeles, USA
Daniel Krappmann	Molecular Targets and Therapeutics Center, Germany
Neil Perkins	Newcastle University, UK
Sonia Rocha	University of Liverpool, UK
Lienhard Schmitz	University Giessen, Germany
Laura Solé	Hospital del Mar Research Institute, Spain
Lluís Espinosa	Hospital del Mar Research Institute, Spain

Invited Speakers

Véronique Baud
Université Paris Cité, Paris

Anna Bigas
Josep Carreras Leukaemia Research Institute, Spain

Ruaidhri Carmody
University of Glasgow, UK

Emmanuel Dejardin
University of Liège, GIGA-Institute, Belgium

Raquel Espín-Palazón
Iowa State University, USA

Gourisankar Ghosh
University of California San Diego, USA

Alexander Hoffmann
University of California Los Angeles, USA

Michael Kracht
Justus-Liebig-University Giessen, Germany

Simon MacKay
Strathclyde University, UK

Rudi Beyaert
Ghent University, Belgium

Markus Bredel
University of Miami, USA

Matthew Cook
University of Cambridge, UK

Nuno dos Santos
University of Porto, Portugal

Guido Franzoso
Imperial College London, UK

Bodo Grimbacher
University of Freiburg, Germany

Tom Huxford
San Diego State University, California, USA

Daniel Krappmann
Molecular Targets and Therapeutics Center, Germany

Alexis Maillard
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Simon Mitchell
Brighton and Sussex Medical School, UK

Michael Naumann
Otto von Guericke University, Germany

Manolis Pasparakis
University of Cologne, Germany

Neil Perkins
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Sonia Rocha
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Lesley Stark
University of Edinburgh

Myong-Hee Sung
National Institute on Aging, NIH, USA

Henning Walczak
University of Cologne, Germany, University College London, UK

Vivien Wang
University of Macau

Jana Wolf
Max Delbrück Center, Berlin

Program Overview

Wednesday, October 7

SESSION I — TRANSCRIPTIONAL REGULATION BY NF-κB

Chairs: Gourisankar Ghosh and Alexander Hoffmann

SESSION II — NF-κB IN INFLAMMATION AND IMMUNITY

Chairs: Claus Scheidereit and Raquel Espín-Palazón

Key events: 09:20 OPENING KEYNOTE LECTURE • 13:00 Lunch time and poster viewing

Thursday, October 8

SESSION III — NF-κB IN DEVELOPMENT AND CELL DIFFERENTIATION

Chairs: Anna Bigas and Manolis Pasparakis

SESSION IV — NF-κB-REGULATORY ELEMENTS

Chairs: Tom Huxford and Vivien Wang

Key events: 12:55 Lunch time and poster viewing • 20:00 Congress dinner (not included)

Friday, October 9

SESSION V — NF-κB IN SOLID CANCERS, LEUKEMIAS & LYMPHOMAS

Chairs: Véronique Baud and Simon Mitchell

SESSION VI — THERAPEUTIC TARGETING AND FUTURE DIRECTIONS

Chairs: Lesley Stark and Myong-Hee Sung

Key events: 12:45 Lunch time and poster viewing • 16:25 Short Talk and Poster prizes • 17:00 Closure: Past, present and future of the NF-κB Congress • 17:30 Announcement of the 8th International NF-κB Congress

Detailed Program

Wednesday, October 7

08:45	On-site registration and welcome coffee	
09:20	**OPENING KEYNOTE LECTURE**	**Ranjan Sen**
	SESSION I — TRANSCRIPTIONAL REGULATION BY NF-κB	Chairs: Gourisankar Ghosh and Alexander Hoffmann
10:00	Structure-based biochemical analyses of NF-κB regulation by IκB and IKK	**Tom Huxford**
10:25	Functional analysis of NF-κB/RNA networks	**Lienhard Schmitz**
10:50	**Coffee break**	
11:15	The multifaceted role of NF-κB in hypoxia	**Sonia Rocha**
11:40	Bcl3 condensate formation in transcriptional regulation	**Vivien Wang**
12:05	Cancer-selective targeting of the NF-κB pathway: from target discovery to human trials	**Guido Franzoso**
12:30	Cell type-specific immune dysregulation in NFKB1-associated CVID	**Hanna Norbäck**
12:45	Regulation of ZBP1 signaling by CYLD and SPATA2	**Ulrich Maurer**
13:00	**Lunch time and poster viewing**	
	SESSION II — NF-κB IN INFLAMMATION AND IMMUNITY	Chairs: Claus Scheidereit and Raquel Espín-Palazón
14:30	Zooming in on NF-κB to understand inflammation and aging	**Myong-Hee Sung**
14:55	IKK/NF-κB and cell death signalling in inflammation: lessons from mouse models	**Manolis Pasparakis**
15:20	The signalling pathways that control the cellular decision to initiate inflammation	**Ruaidhri Carmody**
15:45	**Coffee break**	
16:10	NF-κB and cell death in the aetiology of inflammatory disease	**Henning Walczak**
16:35	NF-κB and B cell homeostasis	**Matthew Cook**
17:00	Regulation of CD8+T-cell responses to cancer by the NF-κB family of transcription factors	**Yenkel Grinberg-Bleyer**

Thursday, October 8

	SESSION III — NF-κB IN DEVELOPMENT AND CELL DIFFERENTIATION	Chairs: Anna Bigas and Manolis Pasparakis
10:00	In vivo NF-κB dynamics and their significance during development	**Raquel Espín-Palazón**
10:25	Novel inborn errors of the human alternative NF-κB pathway and their consequences	**Alexis Maillard**
10:50	Structural determinants of the non-canonical NF-κB pathway	**Emmanuel Dejardin**
11:15	**Coffee break**	
11:40	Imbalances in the NF-κB system disrupt lympho- and myelo-poiesis	**Alexander Hoffmann**
12:05	*NFKB1* mutations in humans: genotype and phenotype	**Bodo Grimbacher**
12:30	IκBα as a master regulator of pluripotency	**Anna Bigas**
12:55	**Lunch time and poster viewing**	
14:15	Notch/NF-κB crosstalk signaling controls SASP development in brain aging	**Bernd Baumann**
14:30	Combined deficiency of ABIN1 and A20 in mice results in T cell hyperactivation and transcriptional Th2 programming	**Jill Steels**
	SESSION IV — NF-κB-REGULATORY ELEMENTS	Chairs: Tom Huxford and Vivien Wang
14:45	Assembly and regulation of large p65 / RELA enhanceosomes	**Michael Kracht**
15:10	Ubiquitin ligases cooperate in balancing NF-κB activation in T cells	**Daniel Krappmann**
15:35	**Coffee break**	
16:00	Ubiquitin modulatory proteins in NF-κB signaling and disease	**Rudi Beyaert**
16:25	Subcellular proteome mapping provides mechanistic insight into the nucleolar-NF-κB inflammatory pathway	**Lesley Stark**
16:50	Selective pharmacological disruption of IκBα interaction with NF-κB and chromatin	**Ángela Montoto**
17:05	CYLD uncouples LUBAC-dependent NF-κB homeostasis from TNF-induced cell death	**Irina Antoshkina**
17:20	UBE2L3 safeguards intestinal epithelial homeostasis and limits inflammation at the gut barrier	**Femke DeMeyer**
20:00	**Congress dinner (not included)**	

Friday, October 9

	SESSION V — NF-κB IN SOLID CANCERS, LEUKEMIAS & LYMPHOMAS	Chairs: Véronique Baud and Simon Mitchell
09:30	RelB in metabolism and cancer	**Véronique Baud**
09:55	Mechanism of IKK2 activation and its implication in neurological diseases and cancer	**Gourisankar Ghosh**
10:20	Loss of IκBα reshapes chromatin in glioma	**Markus Bredel**
10:45	**Coffee break**	
11:10	The central role of NF-κB in mediating leukemic T cell interactions with the microenvironment	**Nuno dos Santos**
11:35	NDP-heptose regulated NF-κB: infection, inflammation and cancer	**Michael Naumann**
12:00	IKKα mediates crosstalk between the NF-κB and MAPK pathways through dual-specific phosphatases 1 and 5	**Dina Harti**
12:15	Inhibitory-κB kinase as a therapeutic target in colorectal cancer	**Kathryn Pennel**
12:30	NIK/MAP3K14 functions as a mitochondrial stress-immunity integrator and drives sex-polarized transcriptional outputs that govern glioblastoma pathogenesis	**Raquel Sitcheran**
12:45	**Lunch time and poster viewing**	
14:15	p52 inhibits anti-tumor immunity to promote glioblastoma growth	**Bakhtiar Yamini**
14:30	NF-κB signalling dynamics define survival dependencies in aggressive lymphomas	**Aimilia Vareli**
	SESSION VI — THERAPEUTIC TARGETING AND FUTURE DIRECTIONS	Chairs: Lesley Stark and Myong-Hee Sung
14:45	Computational approaches to NF-κB: from dynamic signaling to personalized disease models	**Jana Wolf**
15:10	**Coffee break**	
15:35	Precision targeting of NF-κB in cancer with systems biology	**Simon Mitchell**
16:00	The development of SU1644 – a potent and selective IKKα inhibitor with applications in prostate and colorectal cancer	**Simon MacKay**
16:25	**Short Talk and Poster Prizes**	
17:00	**CLOSURE: PAST, PRESENT AND FUTURE OF THE NF-κB CONGRESS**	**Neil Perkins**
17:30	**ANNOUNCEMENT OF THE 8TH INTERNATIONAL NF-κB CONGRESS**	

Invited Speaker Abstracts

Opening Keynote Lecture

Pr. Ranjan Sen, Biomedical Research Center, NIH, National Institute on Aging, USA

SESSION I • INVITED LECTURES (Chairs: Gourisankar Ghosh and Alexander Hoffmann)

Structure-based biochemical analyses of NF-κB regulation by IκB and IKK

Tom Huxford, San Diego State University, California, USA

Regulation of transcription factor NF-κB activity is primarily controlled through its direct noncovalent association with IκB inhibitor proteins. Over nearly three decades, X-ray crystallographic and structure-based biochemical analyses of engineered NF-κB:IκB complexes have provided considerable insight into how IκB proteins selectively bind distinct NF-κB dimers, influence NF-κB subcellular compartmentalization, and disrupt the propensity of NF-κB to bind target DNA. The recent release of AlphaFold3, an advanced generative AI model capable of predicting the structures and interactions of complex biomolecular assemblies, now provides an opportunity to extend structural analyses to biologically relevant, full-length NF-κB:IκB complexes. Comparison of these predictions with previously determined experimental structures reveals several differences that merit further investigation. More strikingly, regions of NF-κB and IκB that lack confidently predicted structure in the individual proteins generally show little evidence of acquiring defined structure or stable intermolecular contacts upon complex formation. Although generally less conserved in primary sequence than the stably folded domains, intrinsically disordered regions are retained throughout the NF-κB and IκB protein families and may contribute to regulation through mechanisms that do not require adoption of a stable three-dimensional structure. We propose that, in addition to harboring sites for post-translational modification and transient molecular interactions, these regions may tune the local physicochemical environment surrounding the folded NF-κB:IκB complexes and thereby influence their function within different compartments of the eukaryotic cell. Existing biochemical observations are consistent with aspects of this hypothesis, but new experimental approaches will be required to systematically evaluate how persistently disordered regions contribute to NF-κB regulation and inducible gene expression.

Functional analysis of NF-κB/RNA networks

Lienhard Schmitz, University Giessen, Germany

At the mesoscale level, the cell contains densely packed regions that form condensates or highly crowded environments, leading to slower diffusion and enhanced reaction rates. Macromolecular crowding also occurs in NF-κB signaling complexes, which are transiently formed in the cytosol or within the chromatin environment of the nucleus. The formation of these densely packed complexes requires a range of intermolecular interactions mediated by weak chemical forces, as well as interactions between post-translational modifications and interaction partners containing matching reader domains. As it is becoming increasingly clear that many cellular proteins are also capable of binding RNA, we screened the five DNA-binding NF-κB subunits for their ability to bind RNA using CLIP experiments. These experiments showed that p65 and p52 are capable of binding RNA. We are pursuing follow-up experiments with the strongly transactivating p65 subunit and identified p65-associated RNAs using CLIP-seq experiments. RNAs also function as important adhesive factors, as

mass spectrometry experiments demonstrated that limited digestion of the p65 immunoprecipitate with the endoribonuclease RNase 4 leads to a partial or complete loss of part of the p65 interactome. These experiments provide initial evidence for the importance of RNA as a structural component of p65 complexes. We are currently validating these findings through a complementary experimental approach by complexome profiling of cytosolic and nuclear NF-κB complexes isolated by high-resolution clear native PAGE. We hope to present the first data on these RNA-dependent NF-κB complexes at the 7th NF-κB International Congress.

The multifaceted role of NF-κB in hypoxia

Sonia Rocha, University of Liverpool, UK

Hypoxia, or reduced oxygen availability, is a condition frequently encountered during development and in various pathological states. Hypoxia induced a significant change in gene expression levels in different cellular backgrounds. Most hypoxia-induced genes are controlled by a master transcription factor named Hypoxia Inducible Factor (HIF). Similarly, analysis of RNA-sequencing datasets has revealed a conserved HIF signature as well as a broader hypoxia signature, which is conserved across a variety of tissues and cells. We and others have shown that hypoxia also induces NF-kappaB to control specific genes, including HIFs themselves. However, it remains unclear whether NF-kappaB activation results in a conserved gene expression signature under hypoxic conditions.

To address this question, we used a model cell system to deplete individual Rel members of the NF-kappaB family and examined their contributions to the hypoxia-induced gene expression profile. In addition, we have analysed publicly available RNA-sequencing datasets of different cellular backgrounds exposed to hypoxia, as well as our own datasets focusing on NF-kappaB target genes and hallmark signatures.

Our analysis revealed that hypoxia induces a distinct NF-kappaB hallmark signature across all analysed cell types. Importantly, our analysis revealed that NF-kappaB is necessary for more than 60% of all hypoxia-repressed genes, a response conserved across multiple cell systems. This revealed a previously unrecognised function for NF-kappaB in the control of the hypoxia response.

Cancer-selective targeting of the NF-κB pathway via GADD45β/MKK7 inhibition: From target discovery to Phase 2 trials

Guido Franzoso, Imperial College, London, UK

NF-κB transcription factors drive multiple myeloma (MM) aetiopathogenesis, malignant cell survival, and therapy resistance. However, developing clinically useful IKK/NF-κB inhibitors has proven impossible due to the dose-limiting on-target toxicities of systemic NF-κB blockade. To overcome this problem, we developed a targeted approach disrupting the essential, cancer-specific survival module GADD45β/MKK7 downstream of NF-κB, rather than NF-κB itself. This led to the first-in-class GADD45β/MKK7 inhibitor DTP3, which selectively kills MM cells via MKK7/JNK-driven apoptosis without toxicity to normal cells.

We report the results of a Phase-I dose-escalation study evaluating DTP3 monotherapy in patients with relapsed/refractory MM and diffuse large B-cell lymphoma (DLBCL). All patients had progressive disease following 4-11 prior lines of therapy. Seven MM patients (50.0%) had triple-refractory disease, and eight (57.1%) exhibited high-risk features.

DTP3 was well tolerated at doses up to 45 mg/kg, with no haematological toxicity in any study participant. The most common non-haematological toxicities were transient, grade-1/2 dysgeusia/perioral dysesthesia, dizziness, and QTc prolongation. Self-resolving grade-3 QTc prolongation occurred in two patients. Two patients treated at 67 mg/kg developed grade-3 hypotension/pre-syncope meeting dose-limiting toxicity criteria. The maximum tolerated dose was 45

mg/kg; 30 mg/kg was selected as recommended Phase-2 dose (RP2D). Notably, one of two MM patients treated at the RP2D exhibited a Very Good Partial Response (VGPR), with 98% reduction in tumour burden. Stable disease was observed in six patients treated at ≥6 mg/kg (66.7%).

Mechanistically consistent pharmacodynamic responses were observed in 50% of patients, with JNK activation and apoptosis in MM cells but not in normal cells, establishing cancer-selective target engagement. Pharmacokinetic analyses demonstrated dose-proportional systemic exposure and maximum plasma concentration, long plasma half-life, and no DTP3 accumulation following repeated administration.

These findings provide initial clinical validation of DTP3's safety and therapeutic efficacy and proof-of-concept for **a** cancer-selective NF-κB-targeting paradigm. A Phase-II dose-expansion study is ongoing.

Keywords: Oncology, Cancer Biology, Multiple Myeloma, Lymphoid Malignancies, Translational Research, Clinical Trials, NF-κB, Signal Transduction, Apoptosis, Drug Development, Precision Therapy.

SESSION II • INVITED LECTURES (Chairs: Claus Scheidereit and Raquel Espín-Palazón)

Zooming in on NF-κB to understand inflammation and aging

Myong-Hee Sung, National Institute of Aging, NIH, USA

The spatiotemporal complexity of NF-κB signaling, inevitable from its auto-regulatory network, is key to understanding the language of cellular and molecular systems mediated by NF-κB. Using endogenous NF-κB knock-in reporter mice, quantitative live-cell microscopy and spectroscopy, and mathematical modeling, we show how innate immune cells utilize the biological information encoded in distinct features of NF-κB RelA and c-Rel. Subunit-specific expression and signaling were important in pathogen sensing and responses to danger-associated molecular patterns and age-related damage products. Much remains to be understood about the complexity of the NF-κB system that has sustained through evolution.

IKK/NF-κB and cell death signaling in inflammation: lessons from mouse models

Manolis Pasparakis, University of Cologne, Germany

Inflammatory diseases are driven by complex interactions between immune activation, cell stress and regulated cell death. Although many inflammatory pathways have been intensively studied, therapeutic targeting of central signalling nodes has often been limited by toxicity, incomplete efficacy, or compensatory responses that undermine treatment benefit. A major challenge is therefore to understand how inflammatory signalling and cell-death pathways interact in vivo, and whether these interactions can be exploited to improve both the safety and efficacy of anti-inflammatory therapies. The IKK/NF-κB pathway regulates inflammatory and pro-survival signalling and has been implicated in the pathogenesis of many diseases. Using relevant genetic mouse models, we investigated how the cross-talk between IKK/NF-κB signalling and regulated cell death pathways affects inflammatory responses, tissue homeostasis and pathology. Our findings provide new insight into how IKK/NF-κB and cell death pathway interactions shape inflammatory pathology and may inform future strategies for the development of more effective anti-inflammatory treatments

The signaling pathways that control the cellular decision to initiate inflammation

Ruaidhri Carmody, University of Glasgow, UK

Toll-like receptors (TLRs) sense infection and injury and are critical inducers of inflammation. TLR-induced activation of the NF-κB and MAPK pathways drives secretion of inflammatory mediators such as cytokines and chemokines. Controlling this output is essential: excessive inflammatory responses cause tissue damage, while insufficient responses leave the host susceptible to infection. This presentation describes our recent work on how the activation properties of TLR-induced signalling pathways allow cells to discriminate genuine threats from background noise based on ligand concentration, preventing unwarranted inflammation. We show that these activation properties are TLR-specific, and that some TLRs are less effective at filtering signal from noise. We further demonstrate that a cell's decision to initiate inflammation can be changed by altering the activation properties of TLR-induced signals. Together, our data reveal mechanisms by which innate immune cells interpret extracellular signals that may be exploited for preventing inflammation.

ANKIB1-mediated lysine-11 (K11)-linked ubiquitination drives type-I/III interferon induction by cGAS–STING and Toll-like receptors 3 and 4

Henning Walczak, University of Cologne, Germany, University College London, UK

Linear ubiquitination by the linear ubiquitin chain assembly complex (LUBAC) is critical for NF-κB activation by several members of the tumour necrosis factor receptor (TNFR) superfamily but also downstream of several pattern recognition receptors (PRRs). The induction of type-I/III interferon (IFNs) by PRRs, including by toll-like receptor 3 (TLR3), TLR4 and cyclic GMP-AMP synthase (cGAS), together with the stimulator of IFN genes (STING), is critical for effective immunity. The induction of type-I/III IFN by these PRRs does not only require the activation of NF-κB but also of TANK-binding kinase 1 (TBK1). Activated TBK1 in turn activates the transcription factor IFN-response factor 3 (IRF3) to drive type-I/III IFN induction. However, the mechanism by which TBK1 is activated by these PRRs has remained elusive. We now found that lysine-11 (K11)-linked ubiquitination drives TBK1 activation by these PRRs. The E3 ligase ANKIB1 forms these K11-linked ubiquitin chains on various components of the TLR3- and cGAS–STING-induced signalosomes. This facilitates recruitment of Optineurin to these signalling complexes which, in turn, enables recruitment of TBK1, resulting in the activation of this kinase and, consequently, of IRF3. In mice, ANKIB1 deficiency dampens type I/III IFN induction via TLR3, reducing TLR3-dependent interferonopathy. Upon infection with herpes simplex virus 1 (HSV-1), cGAS–STING-induced type I/III IFN induction, known to be required for effective immunity to HSV-1, is also attenuated in ANKIB1-deficient mice, rendering the infection lethal. Hence, we uncover an unanticipated, critical role for ANKIB1-generated K11-linked ubiquitination in the immune response activated by TLR3, TLR4 and cGAS–STING.

NF-κB and B cell homeostasis

Matthew Cook, University of Cambridge, UK

Genetic manipulation of mice has been a cornerstone for discovery and elucidating the actions of NF-κB in both innate and specific immunity. Examples will be presented to show how recent progress in identification and characterisation of human inborn errors of immunity affecting NF-κB have complemented this knowledge base. First, these examples provide evidence of the consequences of NF-κB defects for individuals living in a complex environment. Second, the genetic architecture of pathogenic variants sometimes extends beyond null alleles. In particular, discovery of both gain- and loss-of-function alleles of the same gene have provided evidence of the narrow range of NF-κB activity necessary for maintaining immune homeostasis. Third, deep immune phenotyping of patients carrying pathogenic variants has identified novel aspects of NF-κB immune functions for T- and B-cell activation and differentiation. These insights may provide a bridge to the elucidation of more common immune diseases driven by non-monogenic dysregulation of NF-κB.

SESSION III • INVITED LECTURES (Chairs: Anna Bigas and Manolis Pasparakis)

In vivo NF-κB dynamics and their significance during development

Raquel Espín-Palazón, Iowa State University, USA

NF-κB is classically viewed as a central regulator of inflammation, immunity, and cancer. Much less is known about how NF-κB functions during normal development, or how the dynamics of NF-κB activity determine cellular behavior in vivo. Our work has challenged the view of NF-κB as simply an inflammatory switch and revealed an unexpected role for NF-κB in building the blood system.

Using the transparent zebrafish embryo, we developed destabilized fluorescent NF-κB reporters that allow NF-κB activity to be visualized in real time in living animals. Combining live imaging with transcriptomics, cell-cycle analysis, genetic and temporal manipulation of the canonical p65/p50 pathway, and lineage tracing, we investigated how the timing of NF-κB activation controls the emergence of hematopoietic stem and progenitor cells (HSPCs), a process that only occurs once in life during embryonic development. Conservation of these mechanisms was examined during human iPSC-derived hematopoietic differentiation.

Real-time visualization revealed a previously unresolved temporal organization of NF-κB signaling. NF-κB is activated in distinct waves, each associated with a different developmental outcome. An early p65-dependent wave is essential for establishing hemogenic endothelium (HE), the transient precursor that generates the lifelong blood system. Unexpectedly, NF-κB activation drives these endothelial precursors into a transient quiescent state, suppressing cell-cycle programs and promoting acquisition of hemogenic identity. This reveals cell-cycle quiescence as a previously unrecognized function of canonical NF-κB. A subsequent wave of NF-κB activity is reactivated as HSPCs emerge from the dorsal aorta, demonstrating that the same pathway is redeployed at a different developmental stage to control HSPC delamination. Similar p65-dependent regulation is observed during human hematopoietic differentiation.

These findings reveal that NF-κB has functions far beyond inflammation. By visualizing signaling dynamics in vivo, we uncover how the timing of NF-κB activation encodes distinct developmental decisions- from establishing a quiescent, hemogenic state to promoting HSPCs undergoing endothelial to mesenchymal transition. Thus, NF-κB functions not simply as an inflammatory switch, but as a dynamic developmental regulator that helps build the blood system.

Novel inborn errors of the human alternative NF-κB pathway and their consequences

Alexis Maillard, Imagine Institute, University of Paris, France

Human inborn errors of immunity provide unique insights into the functions of the alternative NF-κB pathway in host defense and immune tolerance. Genetic defects affecting NIK, IKKα, RelB, and NF-κB2 cause diverse combinations of antibody deficiency, impaired cellular immunity, and developmental abnormalities. Defects in upstream receptors and ligands or negative regulators further illustrate the contribution of this pathway to lymphoid organ organization, lymphocyte function, and immune homeostasis. Beyond effects on leukocytes, impaired signaling in medullary thymic epithelial cells disrupts central tolerance, leading to neutralizing autoantibodies against type I interferons and susceptibility to severe viral infections. Newly identified genetic defects extend this spectrum and help distinguish the respective contributions of individual pathway components. Together, these discoveries delineate the essential and redundant roles of alternative NF-κB signaling components across hematopoietic and stromal compartments in humans.

Structural determinants of the non-canonical NF-κB pathway

Emmanuel Dejardin, University of Liège, GIGA-Institute, Belgium

This study elucidates the structure-activity relationship between NIK and IKKα, the core kinases of the non-canonical NF-κB pathway. By leveraging AlphaFold3, we mapped a key interaction interface consisting of specific hydrogen bonds and hydrophobic contacts between the two kinases. We demonstrated that specific point mutations in NIK prevent IKKα recruitment in cellulo. Reciprocally, mutating specific residues within two newly identified stretches in IKKα completely abrogated NIK binding while preserving intrinsic NIK and IKKα kinase activity. To confirm the physiological relevance of this interface, we generated a novel IKKα knock-in mouse model. These mutant mice lacked secondary lymphoid organs, displayed disorganized spleens and thymi, and suffered from defects in the humoral response, perfectly mirroring the phenotypes of NIK-KO or IKKαAA-deficient mice. Together, these in cellulo and in vivo findings definitively validate our structural models of the NIK–IKKα interactome and also help to better define the pathogenicity of human NIK and IKKα variants.

Imbalances in the NF-κB System disrupt lympho-and myelo-poiesis

Alexander Hoffmann, University of California Los Angeles, USA

The effectiveness of the immune system declines with age and associated chronic inflammation, termed “Inflammaging”. Most marked is the reduction in B-cell populations, and their hematopoietic progenitors are myeloid biased. Furthermore, hematopoietic stem cells (HSCs) are functionally impaired to populate the bone marrow niche when transplanted, yet they become overabundant. To what extent these aging hallmarks are related, as cause or consequence, remains unclear. I will summarize our recent studies in which we developed experimental and mathematical models to dissect the age-associated dysregulation quantitatively and identified distinct mechanisms triggered by inflammation.

NFKB1 mutations in humans: genotype and phenotype

Bodo Grimbacher, University of Freiburg, Germany

Heterozygous germline variants in *NFKB1* can be classified into variants with ‘known protein defect’ and variants of ‘unknown protein effect’. However, beyond this, *NFKB1* variants may additionally be categorized into at least eight distinct variant classes. We conducted a literature review of 67 papers reporting on *NFKB1* variants and included four additional individuals, carrying previously unreported *NFKB1* variants. We identified 333 individuals with unique mutations in *NFKB1*. Our meta-analysis revealed that there is no sex predilection, the penetrance was 78%, and the mean age-of-diagnosis was 37 years following a diagnostic delay of 18.5 years. Individuals harboring variants with a known protein defect demonstrated a higher frequency of malignancies and autoimmune disorders. However, these individuals were less frequently classified as affected than individuals with variants with unknown protein defect. Carriers of whole *NFKB1* gene deletions had a significantly higher penetrance to being designated as “affected”, but beyond this, the clinical manifestations did not differ between variant classes.

IκBα as a master regulator of pluripotency

Anna Bigas, Hospital del Mar Research Institute and Josep Carreras Leukaemia Research Institute, Spain

IκB proteins are best known as cytoplasmic inhibitors of NF-κB transcription factors. However, specific IκB homologues also have additional, NF-κB-independent functions, including chromatin association, interaction with Polycomb components, and direct regulation of gene transcription. A clear example is phosphorylated and SUMOylated IκBα (pS-IκBα), which binds histones H2A and H4 in multiple stem and progenitor compartments, including skin, intestine, and blood. We have recently shown that IκBα

is also required for proper differentiation of mouse embryonic stem cells (ESCs), as its loss traps cells in a naïve pluripotent state. To dissect the chromatin-related role of IκBα in stemness from its canonical function as an NF-κB inhibitor, we used mouse and human pluripotent stem cells carrying separation-of-function mutants. Our results show that IκBα's role in regulating stem cell pluripotency is NF-κB-independent and instead relies primarily on its chromatin-associated function. The IκBα function in the regulation of chromatin structure will be discussed.

SESSION IV • INVITED LECTURES (Chairs: Tom Huxford and Vivien Wang)

Assembly and regulation of large p65/RELA enhanceosomes

Michael Kracht, Justus-Liebig-University Giessen, Germany

Despite its central role in immunity, inflammation, cancer and development, the canonical NF-κB pathway cannot yet be targeted at the level of subunit-specific chromatin regulation and gene transcription. We therefore aim to better understand how p65/RELA assembles with other nuclear proteins into the large multiprotein complexes referred to as enhanceosomes, and to define the underlying interactomes, their hierarchical organization, gene specificity, genomic distribution and potential druggability. To this end, we recently used proximity labelling with p65-miniTurbo fusion proteins to map the p65/RELA protein interactome in intact cells. This approach identified 366 p65/RELA interactors in untreated and interleukin-1α (IL-1α)-stimulated cells, among them 172 transcription factors (TFs, 47% of all interactors) and more than 50 epigenetic regulators (~20% of all interactors) belonging to several classes of chromatin-remodeling complexes, illustrating the extent of RELA cooperativity in a single cell type (1). To extend these observations beyond one cell type, we retrieved factors co-localizing with RELA on chromatin from a database of 23,577 ChIP-seq data sets covering 12 tissues and several hundred cell types. Intersecting the proteomic and ChIP-seq data sets yielded 128 proteins, which we regard as a p65/RELA consensus interactome, or canonical chromatin-associated RELA enhanceosome. This network is enriched in TFs and contains 15 SWI/SNF components, including a mixture of canonical and polybromo-associated BAF subunits. We then established a targeted, low-throughput proximity-labelling affinity-purification assay to validate individual enhanceosome components and to follow their assembly in subcellular fractions and at defined genomic loci, using immunoblotting and ChIP-qPCR. These data indicate that formation of RELA–BAF enhanceosomes depends on H3K27ac-associated enhancer activation and that such complexes are already present, and possibly pre-assembled, in the soluble nuclear compartment. Perturbation of SMARCA2/4 with catalytic inhibitors or PROTACs prevents the shuttling of selected BAF subunits to the chromatin fraction and strongly reduces transcription of prototypical IL-1α-NF-κB target genes at low nanomolar concentrations, leaving only residual gene expression. Together, these findings describe a large canonical p65/RELA enhanceosome, provide insight into its dynamics and hierarchical organization, and offer a framework for exploiting enhanceosome components to modulate p65/RELA-dependent gene expression.

(1) Leib, L. et al. (2025). The proximity-based protein interactome and regulatory logics of the transcription factor p65 NF-kappaB/RELA. EMBO Rep 26, 1144-1183, doi:10.1038/s44319-024-00339-8

Ubiquitin ligases cooperate in balancing NF-κB activation in T cells

Daniel Krappmann, Molecular Targets and Therapeutics Center (MTTC), Helmholtz Munich, Germany

The CARD11-BCL10-MALT1 (CBM) complex drives NF-κB signaling and MALT1 protease activation after T cell receptor (TCR) stimulation, forming a central signaling hub in adaptive immunity. Both linear ubiquitin chain assembly complex (LUBAC), consisting of HOIP, HOIL-1 and SHARPIN, and TRAF6 interact with the CBM complex. Here, it will be discussed how the coordinated activity of these E3 ligases contributes to controlling CBM activity. While TRAF6 is the main E3 ligase driving canonical NF-κB activation downstream of the CBM complex, it also restricts MALT1 protease activation in resting T cells, thereby keeping naive T cells in an inactive state. Furthermore, TRAF6 in conjunction with LUBAC coordinates the cleavage of substrates by the MALT1 protease. Thus, the accurate interplay of E3 ligases at the CBM complex in T cells is critical for immune activation, but it also counteracts chronic T cell activation to maintain immune homeostasis.

Ubiquitin modulatory proteins in NF-κB signaling and disease

Rudi Beyaert, Ghent University, Belgium

Ubiquitination controls multiple cellular processes through proteolytic and nonproteolytic mechanisms, including proteostasis, selective autophagy, protein trafficking, and cell signaling cascades such as those for NF-κB activation. Dysregulation of ubiquitin-dependent signaling leads to a variety of diseases such as cancer, neurodegenerative diseases, and immune disorders. The ubiquitin system encompasses E1, E2, and E3 enzymes that result in the transfer of ubiquitin to substrates, deubiquitinases that can remove ubiquitin from substrates, as well as proteins that bind to ubiquitinated proteins, determining their cellular fate. In this talk, using multiple conditional gene-targeted mouse models, I will highlight recent advances in our understanding of the biological roles of selected ubiquitin modulatory proteins in immune homeostasis and disease.

Subcellular proteome mapping provides mechanistic insight into the nucleolar-NF-κB inflammatory pathway

Lesley Stark, University of Edinburgh, UK

A key hallmark of senescence and ageing is an increase in the size of the nuclear organelle, the nucleolus. However, the mechanisms driving this enlargement, as well as its downstream consequences, remain poorly understood. We recently demonstrated that the RNA polymerase I complex component TIF-IA (RRN3) accumulates in senescent and aged cells, leading to both nucleolar enlargement and increased secretion of inflammatory factors via activation of NF-κB signalling. However, the mechanistic link between TIF-IA–mediated nucleolar alterations and NF-κB activation remains unclear. Elucidating this connection is important, as it may enable the development of therapeutic strategies to limit chronic inflammation in ageing tissues.

HCT116 colon cancer cells were transfected with GFP or GFP-TIF-IA to model TIF-IA accumulation observed in senescent and aging cells. Total proteomics were used to identify global changes to the proteome induced by TIF-IA accumulation. DIA-LOP, a label-free form of LOPIT, was used to identify significant changes in protein subcellular localisation. Proteins/pathways of interest were validated using standard procedures.

A total of 8305 proteins were quantified in the total proteomic dataset with 14 and 19 significantly (FC>2, P<0.05) up- and downregulated, respectively. Significantly downregulated proteins were enriched for GO terms “response to oxidative stress” and “chromatin remodelling” and included the NF-κB repressor, TNFAIP3 (A20). Global subcellular proteomics revealed that the majority of proteins (8174) remained in the same spatial space after TIF-IA expression in HCT116 cells. However, 1315 exhibited re-distribution across organelles. Mitochondrial reorganisation and endosomal trafficking of NF-κB regulators were particularly evident.

TIF-IA drives coordinated reorganisation of endosomal and biosynthetic pathways, converging on enhanced NF-κB-associated signalling.

SESSION V • INVITED LECTURES (Chairs: Véronique Baud and Simon Mitchell)

RelB activation and metabolic reprogramming in cancer cells

Véronique Baud, Université Paris Cité, France

A recent breakthrough in cancer research is realization that cancer cells exhibit bioenergetics alterations in order to meet their high-energy requirement, and deregulation of cellular energetics has been recognized as one of the hallmarks of cancer. High glucose consumption through aerobic glycolysis is well known as the “Warburg effect”, but it is now realized that deregulated mitochondrial oxidative phosphorylation, amino acid and lipid metabolism are also critical metabolic hallmarks. The alternative NF-κB pathway leads to the activation of RelB-containing dimers. However, how the alternative RelB NF-κB subunit impacts on cancer cell metabolism remains a central, poorly understood question. In this presentation, I will share some of our findings about RelB activation in cancer cells and its impact on reprogramming of cancer cell metabolism, particularly lipid metabolism.

Mechanisms of IKK2 activation and its implication on neurological diseases and cancer

Gourisankar Ghosh, University of California San Diego, USA

Inhibitor of NF-κB kinase (IKK) is a master regulator of cellular immunity and inflammation. In resting cells, IKK remains inactive but is poised for activation by a wide range of physiological and pathological stimuli. Despite decades of investigation, the molecular mechanism by which IKK transitions from its inactive to active state has remained elusive, in part because technical challenges have prevented the isolation of well-defined inactive and active complexes required for mechanistic studies.

The IKK complex consists of two catalytic subunits, IKKα/IKK1 and IKKβ/IKK2, and the regulatory subunit NEMO. Although the complex is often represented as a simple 1:1:2 assembly of IKK1, IKK2, and NEMO, these components undergo higher-order and distributed oligomerization. Moreover, obtaining a stable, inactive IKK complex from heterologous systems has been particularly challenging, limiting our ability to define the structural basis of activation.

I will describe a series of cryo-EM structures of IKK2 homo- and IKK1:IKK2 heterodimers that reveal previously unrecognized features of IKK architecture and provide a framework for understanding how IKK2 transitions from an inactive to an active state. I will then discuss how dysregulated IKK2 signaling orchestrates pathogenic programs in neurological diseases and cancer, and how selective, gentle modulation of IKK2 activity may provide a therapeutic strategy for suppressing chronic inflammation while preserving essential physiological immune functions.

Loss of IκBα reshapes chromatin in Glioma

Markus Bredel, University of Miami, USA

IDH mutations define most adult lower-grade gliomas and, through D-2-hydroxyglutarate, impose a hypermethylator phenotype (G-CIMP) associated with favorable but highly variable survival. Current

models cannot identify aggressive IDH-mutant tumors, a gap heightened by vorasidenib approval, which provides no guidance on patient selection or management when this favorable program fails. We identified a lesion overriding this program. Across 2,343 gliomas from 2,255 patients, haploinsufficient deletion of *NFKBIA*, encoding the NF-κB inhibitor IκBα, occurred in 8–13% of lower-grade gliomas, was disproportionately acquired at recurrence, and independently predicted shorter survival, exceeding the contribution of 1p/19q codeletion, TERT, or ATRX status in an externally validated nomogram. NFKBIA loss is mechanistically active: it reshapes DNA and histone (H3K27me3) methylation antipodal to IDH mutation, converts IDH-mutant tumors into an epigenetic phenocopy of IDH-wild-type disease, and produces a transcriptome resembling H3K27M-mutant pediatric midline glioma without an H3K27M mutation. This mechanism is separable from NF-κB signaling: chromatin-bound IκBα recruits PRC2 to differentiation loci and governs stem cell maturation and lineage specification. Using separation-of-function IκBα mutants, we find that IκBα loss in isogenic iPSC-derived neural progenitors sustains a NANOG/OCT3/4-high, pluripotency-like state and reduces H3K27me3 unless chromatin-competent IκBα is restored. We propose that *NFKBIA* haploinsufficiency reduces IκBα-directed PRC2 recruitment at lineage-fidelity loci, licensing a dedifferentiated, H3K27M-like state that subverts glial commitment and drives progression independently of, and potentially resistant to, IDH inhibition. Ongoing work in genome-edited iPSC-derived and patient-derived IDH-mutant models tests this mechanism, its effects on glial lineage commitment in vivo, and whether restoring chromatin-competent IκBα or targeting the PRC2 loss-of-function state limits progression alone or with vorasidenib. *NFKBIA* status may provide a predictive biomarker for IDH-inhibitor selection and therapeutic vulnerability. Beyond glioma, we identify an NF-κB-independent chromatin function for IκBα as a PRC2-directing determinant of lineage fidelity whose loss drives cancer through the epigenome rather than NF-κB signaling.

The central role of NF-κB in mediating leukemic T cell interactions with the microenvironment

Nuno dos Santos, University of Porto, Portugal

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy arising from the transformation of T-cell progenitors. About 40-50% of T-ALL patients express components of the T-cell receptor (TCR), and constitutive, tonic TCR-associated signaling has been linked to a pro-survival, canonical NF-κB program that supports leukemic growth. Here, we show that strong, antigen-driven TCR engagement in leukemic T cells activates canonical NF-κB signaling with an opposite outcome: rather than sustaining survival, this TCR-NF-κB axis drives apoptosis. This finding indicates that the leukemia-promoting and leukemia-suppressive role of NF-κB in T-ALL depend on the mode and intensity of upstream TCR stimulation and raises the possibility of therapeutically exploiting strong TCR engagement to selectively eliminate leukemic clones while sparing pathways required for normal T-cell function. In parallel, we investigated whether leukemic T cells influence their surrounding microenvironment through the same signaling axis. Our results show that TCR signaling activates TNF family ligands in an NF-κB-dependent manner. Leukemic T cells expressing these ligands, lymphotoxin in particular, activate NF-κB signaling in neighboring microenvironmental cells, revealing a bidirectional circuit in which TCR-driven leukemic activity reshapes the niche in which these cells reside. We speculate that this microenvironmental NF-κB activation may remodel stromal or immune support cells, potentially sustaining leukemic persistence or altering local immune surveillance.

NDP-heptose regulated NF-κB: infection, inflammation and cancer

Michael Naumann, Otto von Guericke University, Germany

Nucleotide-diphosphate (NDP)-heptose biosynthetic enzymes (HBEs) account for the synthesis of NDP-heptoses across kingdoms by a range of viruses, prokaryotes, and lower eukaryotes. NDP-heptoses translocate in cells of higher eukaryotic organisms and activate alpha-protein kinase 1

(ALPK1), which phosphorylates tumor necrosis factor (TNF) receptor-associated factor (TRAF)-interacting protein with a forkhead-associated domain (TIFA). Subsequent recruitment of other factors into so-called TIFAsomes culminates in the activation of innate immunity by activation of classical and alternative (nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) pathways. In the context of *H. pylori* infection, we investigated the complex NF-κB response as well as key regulators of the NF-κB system, the ubiquitin-processing deubiquitinylases (DUBs) and shed light on the mechanisms underlying rapid and sustained NF-κB activity. The inflammatory response triggered by *H. pylori* NDP-heptoses causes a variety of changes in the microbial environment of the stomach, leading to a slow and steady disruption of the gastric epithelial barrier. Interestingly, in addition to the innate immunity, NDP-heptoses influence a variety of other cell physiological processes including anti-tumor activity. Therefore, we have studied NF-κB regulators across a spectrum of gastric pathologies, including HP-gastritis, autoimmune gastritis (A-gastritis), reactive gastropathy (C-gastritis), Ex-HP-gastritis, adenomas, and adenocarcinomas, with nonpathological gastric mucosa serving as a baseline to gain deeper insights into their involvement in gastric pathologies. Our findings demonstrate differential expression of NF-κB and DUBs in HP-gastritis, adenomas, and adenocarcinomas.

SESSION VI • INVITED LECTURES (Chairs: Lesley Stark and Myong-Hee Sung)

Computational approaches to NF-κB: from dynamic signaling to personalized disease models

Jana Wolf, Max Delbrück Center, Germany

Cellular signaling networks generate dynamic responses that regulate how cells respond to external stimuli, perturbations or drugs. These responses can result in changes of cell fate, for example, transitions from apoptosis to survival or from quiescence to proliferation. Computational modeling combined with targeted experimentation have successfully elucidated the underlying mechanisms and functional consequences for individual pathways, e.g. the role of feedback or crosstalk. The models and insights are now being used to create large-scale mechanism-informed models that can integrate various data sets and drive the development of disease- and patient-specific models. Here I introduce an approach to model aggressive B cell lymphoma based on a detailed NF-κB network model capturing key cellular processes and the mapping of perturbation data of a cohort of around 300 lymphoma patients. The resulting personalized patient models capture patient heterogeneity and are used to analyze how the genetic alterations together shape cancer cell states. A systematic study of individual and combinatorial alterations identifies known and so-far-unknown cooperation effects and elucidates a strong context dependency of individual network alterations.

Precision targeting of NF-κB in cancer with systems biology

Simon Mitchell, Brighton and Sussex Medical School, UK

Poorly regulated NF-κB activity is oncogenic. In cancer NF-κB is activated by a complex combination of mutations and the tumour microenvironment (TME). Mechanistic computational biology models uniquely enable an integrated understanding of how both TME and mutations combine to drive cancer progression and treatment responses.

Here we show that mechanistic computational models, personalised through adjustment of parameters impacted by mutations in each virtual patient, can predict clinically significant differences in prognosis across multiple malignancies.

Through a combination of computational and experimental systems biology we find that non-canonical NF-κB signalling has the potential both to be modulated by, and modulate, the tumour microenvironment. Together this reveals a deleterious positive feedback loop between malignant B cells and T cells that may result in survival of malignant cells and dampening/exhaustion of T cells.

The development of SU1644- a potent and selective IKKalpha inhibitor with applications in prostate and colorectal cancer

Simon MacKay, Strathclyde University, UK

Selective inhibition of the non-canonical NF-κB pathway represents a promising therapeutic approach for inflammation-driven malignancies, including castration-resistant prostate cancer (CRPC) and colorectal cancer (CRC). The serine/threonine kinase IKKα is a key regulator of this signalling axis; however, the development of isoform-selective inhibitors has proven challenging due to the high sequence homology between IKKα and IKKβ and the conserved nature of kinase ATP-binding sites.

In this presentation, I will describe the work of our interdisciplinary team in the development of SU1644, a compound that exhibits sub-micromolar inhibition of IKKα-driven p100 phosphorylation and p52 nuclear translocation in cellular systems, with minimal impact on canonical NF-κB signalling. I will further demonstrate how modulation of this pathway translates into relevant functional and phenotypic outcomes in cellular and ex vivo models of CRPC and CRC.

Collectively, these findings establish SU1644 as a valuable benchmark compound for probing the therapeutic potential of pharmacologically targeting non-canonical NF-κB signalling in inflammation-driven cancers.

Closure: Past, present and future of the NF-κB Congress

Neil Perkins, Newcastle University, UK

Short Talk abstracts

ST01 • Wednesday 12:30

Cell type-specific immune dysregulation in NFKB1-associated CVID

Presenter: Hanna Norbäck (see also P04); University of Basel, Department of Biomedicine, Basel, Switzerland; EMBL Heidelberg, Heidelberg, Germany

Background: Common variable immunodeficiency (CVID) caused by NFKB1 haploinsufficiency is associated with impaired immune responses, autoimmunity and autoinflammation, yet the cell type specific regulatory mechanisms remain poorly defined. We aimed to define the molecular and cellular effects of NFKB1 haploinsufficiency across immune cell populations using integrated transcriptomic and epigenomic approaches.

Methods: PBMCs from clinically affected NFKB1-CVID patients and asymptomatic or mildly affected carriers were analyzed to distinguish mutation-driven effects from secondary disease-associated changes. The immune phenotype was assessed. Single-cell multiomic profiling integrating scRNA-seq and scATAC-seq was performed on PBMCs from NFKB1-mutated patients and healthy donors to characterize transcriptional and chromatin accessibility changes across immune cell populations. Additionally, sorted naïve CD4 T cells were analyzed by bulk RNA-seq under baseline and stimulated conditions, and gene regulatory networks inferred from CD4+ T cells were used to assess cell-type specific NF-κB activity. Functional consequences of NFKB1 insufficiency were further assessed using CD4+ T-cell differentiation assays and gene-edited CD4+ T cells.

Results: CD4 T cells consistently displayed Th1/cTfh1 skewing in both NFKB1-mutated patients and asymptomatic carriers. Naïve CD4 T cells showed altered regulation of NF-κB-associated target genes together with enrichment of inflammatory transcriptional programs and increased Th1 polarization in NFKB1-mutated and gene-edited cells. Single-cell multiomic analyses identified regulatory programs associated with reduced NF-κB activity in patient T cells and healthy Th1, supporting a model in which NFKB1 insufficiency biases CD4 T-cell states. These findings were observed across clinically affected individuals and asymptomatic carriers, suggesting mutation-driven immune alterations independent of overt disease manifestations.

Conclusions: Our findings support a CD4 T-cell-intrinsic role for NFKB1 haploinsufficiency in shaping immune dysregulation and inflammatory polarization in NFKB1-associated CVID. These results provide insight into molecular mechanisms underlying immunodeficiency and immune dysregulation associated with altered NF-κB signaling.

ST02 • Wednesday 12:45

Regulation of ZBP1 signaling by CYLD and SPATA2

Presenter: Ulrich Maurer (see also P02); Mater Research Institute - Institute of Molecular Medicine, University of Freiburg, Germany

Background: Pro-inflammatory signaling pathways activating NF-κB depend on the non-degradative, K63- and M1-linked ubiquitylation of various subunits of receptor signaling complexes. This is controlled by the activities of deubiquitinases such as CYLD, which is recruited to receptor complexes by the adaptor SPATA2.

Methods: Generation of conditional CYLD/SPATA2 knockout mice, flow cytometry, RTqPCR, CRISPR/Cas9-mediated gene knockout, Western blotting, ELISA

Results: Upon Cre-mediated deletion, CYLD/SPATA2 knockout mice exhibited strong induction of the Z-nucleic acid sensor ZBP1 in the lung, with an increase of macrophages and neutrophils in the bronchoalveolar lavage fluid, and we also observed high levels of ZBP1 in the liver and in BMDM from those animals. Likewise, CYLD/SPATA2 double-knockout MEFs expressed high levels of ZBP1, which was abrogated by loss of cGAS, by loss of the Interferon alpha/beta receptor or by inhibition of LUBAC.

CYLD/SPATA2 double-knockout MEFs were sensitized to ZBP1-mediated cell death, and ZBP1 drove inflammatory gene expression by CYLD/SPATA2 double-knockout MEFs, as they exhibited elevated, ZBP1-dependent expression of TNF and IL-6.

Conclusion: Together, our data reveal a ubiquitylation-dependent crosstalk of NF-κB and cGAS-STING signaling pathways.

ST03 • Wednesday 17:00

Regulation of CD8+T-cell responses to cancer by the NF-κB family of transcription factors

Presenter: Yenkel Grinberg-Bleyer (see also P32); Cancer Research Center of Lyon, France

CD8+ T cells are central effectors of the anti-tumor immune response and the main targets of current immunotherapies. Nevertheless, the transcriptional regulators orchestrating their activation, accumulation and cytotoxic function in the tumor microenvironment (TME), are largely unknown. In this project, we aimed at evaluating the roles of the different members of the NF-kappaB family of transcription factors, whose T-cell-intrinsic functions are so far blurry because of the lack of proper study models. We created a library of mouse models and engineered human T cells carrying CD8+ T-cell-restricted ablation of NF-kappaB subunits. We found that ablation of c-Rel, but not RelA or RelB, selectively led to increased tumor progression in transplanted models of melanoma. Mechanistically, c-Rel was required for T-cell accumulation in the TME by promoting their re-activation and cytotoxic function in situ. At the molecular level, c-Rel promoted Interleukin-2 expression, thus preventing T-cell exhaustion by limiting TOX expression. In line with this, c-Rel-deficiency completely abolished the response to checkpoint-blockade therapies in mice, and c-Rel activity correlated with enhanced response to anti-PD-1 in cohorts of melanoma patients. Conversely, overexpression of c-Rel in endogenous CD8+ T cells, was sufficient to reduce tumor growth in mice; such overexpression also optimized the accumulation and efficiency of engineered TCR-T and CAR T-cell therapy in mice. Together, this work establishes NF-kappaB c-Rel as a prime regulator of CD8+ T-cell function in cancer and paves the way toward NF-kappaB-modulated adoptive T-cell therapy for solid cancers.

ST04 • Thursday 14:15

Notch/NF-κB crosstalk signaling controls SASP development in brain aging

Presenter: Bernd Baumann (see also P13); Ulm University, Institute of Physiological Chemistry

Background: Brain aging is characterized by chronic low-grade inflammation and the development of a senescence-associated secretory phenotype (SASP) by glial cells. Although Notch and NF-κB signaling are key regulators of inflammaging and SASP formation, their functional and mechanistic interplay in the aging brain remains poorly understood.

Methods: Candidate SASP genes were analyzed in silico to identify regulatory DNA motifs potentially mediating Notch/NF-κB crosstalk signaling. Notch and NF-κB gene signatures were assessed by RNA sequencing and qRT-PCR in brain tissue and glial cells from transgenic mice with modulated NF-κB activity. Physical interactions between Notch and NF-κB pathway components were examined by co-immunoprecipitation and western blotting. Primary glial cultures were used to evaluate the impact of pharmacological Notch activation on NF-κB-driven SASP gene expression.

Results: Brain aging was associated with enhanced NF-κB activity and reduced Notch signaling. Astrocyte-specific NF-κB activation in young mice was sufficient to induce cell-autonomous and non-cell-autonomous transcriptomic and cellular alterations resembling premature brain aging and SASP development. This phenotype was characterized by robust induction of SASP-associated genes, enrichment of pro-inflammatory pathways, and increased immune cell infiltration into the brain parenchyma, together with suppression of the Notch gene signature. Mechanistically, the Notch intracellular domain (NICD) inhibited NF-κB signaling through multiple mechanisms, including competition for shared promoter elements, interference with RelA in both the cytoplasm and nucleus, and inhibition of activated IKK2, the central kinase of canonical NF-κB signaling. Consistently, pharmacological activation of Notch signaling reduced NF-κB-driven SASP gene expression in primary glial cells.

Conclusions: These findings identify Notch/NF-κB crosstalk as a central regulatory checkpoint of brain inflammaging and highlight this signaling axis as a potential therapeutic target for age-associated neuroinflammation and neurodegenerative diseases.

ST05 • Thursday 14:30

Combined deficiency of ABIN1 and A20 in mice results in T cell hyperactivation and transcriptional Th2 programming

Presenter: Jill Steels (see also P17); VIB-UGent Center for Inflammation Research, Department of Biomedical Molecular Biology, Unit of Molecular Signal Transduction in Inflammation, Ghent, Belgium

ABIN1 and its binding partner A20 have been described as negative regulators of different immune receptors and signaling pathways, including NF-κB signaling. The CARD–BCL10–MALT1 (CBM) signalosome connects T cell receptor (TCR) engagement to canonical NF-κB and MAPK cascades, indispensable for clonal expansion, effector differentiation, and T cell-mediated immunity. Despite its critical role and association with immunodeficiency, autoimmunity, and cancer, the molecular regulation of CBM signaling remains poorly defined.

Using MALT1 affinity purification and quantitative mass spectrometry, we delineated the MALT1 interactome in Jurkat T cells and identified ABIN1 (TNIP1) as a linear (M1-linked) ubiquitin-dependent CBM-interacting protein upon TCR stimulation. Although ABIN1 has previously been implicated as a negative regulator of NF-κB signaling by recruiting the deubiquitinase A20, ABIN1 deficiency in Jurkat T cells and primary $CD4^+$ _T cells did not significantly perturb TCR-induced IκBα degradation, NF-κB nuclear translocation, or NF-κB dependent gene expression. Strikingly, ABIN1 deficiency synergized with A20 loss to potentiate TCR signaling in vitro. Bulk RNA-seq of CD3-stimulated naïve $CD4^+$ _T cells revealed transcriptional enrichment for Th2 effector programs upon combined A20/ABIN1 deficiency, which was correlated with increased expression of GATA3+ and augmented (baseline) STAT6 phosphorylation.

In vivo, T cell specific ABIN1 deficiency was phenotypically silent, whereas T cell-specific A20 deficiency was associated with expansion of CD44hiCD62Llo effector-memory $CD4^+$ _T cells, reduction of naïve subsets, increased Treg numbers, and heightened activation-induced T cell death. Interestingly, these changes were much more pronounced upon combined ABIN1/A20 deficiency. Moreover, combined A20 and ABIN1 deficiency resulted in increased amounts of $ST2^+$ _$CD4^+$ _T cells in the spleen.

Collectively, our data demonstrate a non-redundant, cooperative axis between ABIN1 and A20 in restraining CBM-driven T cell effector activation and Th2 programming. This knowledge will help to better understand the role of A20 and ABIN1 as susceptibility genes for several immune diseases in humans.

ST06 • Thursday 16:50

Selective pharmacological disruption of IκBα interaction with NF-κB and chromatin

Presenter: Ángela Montoto (see also P03); Hospital del Mar Research Institute, Spain

Background: IκBα is classically defined as the canonical inhibitor of the NF-κB pathway. However, growing evidence indicates that chromatin-bound IκBα also acts as a non-canonical epigenetic regulator, scaffolding the Polycomb Repressive Complex 2 (PRC2) to maintain the repression of stemness and developmental programs. In the intestine, IκBα loss triggers a fetal-like regenerative state characterized by YAP1 activation. While IκBα loss correlates with aggressive tumor phenotypes, whether this is driven by NF-κB hyperactivation, loss of chromatin-associated activity, or both remains unclear. Separating these two activities is essential to clarify their specific contributions to colorectal cancer (CRC) progression.

Methods: In collaboration with Chemotargets, we developed small molecules specifically targeting the IκBα-NF-κB interaction interface. Compounds were screened via immunoprecipitation (IP) assays in HT29 CRC cells to evaluate the IκBα-p65 interaction. Intracellular localization was assessed by

immunofluorescence (IF). To analyze downstream transcriptomic changes, RNA-sequencing (RNA-seq) was performed on human CRC patient-derived organoids treated with the selected candidates.

Results: IP assays successfully identified compounds that significantly reduced the interaction between IκBα and p65. IF analyses showed increased nuclear localization of both proteins upon treatment, consistent with the disruption of their physical interaction, enhanced p65 nuclear translocation, and preservation of IκBα chromatin-associated functions. Concurrently, RNA-seq analyses in human CRC organoids revealed NF-κB signaling as the top upregulated pathway following treatment, functionally confirming the inhibition of the canonical interaction. Strikingly, treated organoids also displayed reduced stemness-associated transcriptional programs.

Conclusions: Selective pharmacological disruption of the IκBα-NF-κB interaction modulates tumor plasticity while preserving the non-canonical, chromatin-associated functions of IκBα. This supports the feasibility of therapeutically targeting specific IκBα interactions. Future work will focus on developing second-generation compounds and designing molecules targeting the chromatin-binding region to further dissect its role in intestinal identity and fetal-like reprogramming.

ST07 • Thursday 17:05

CYLD uncouples LUBAC-dependent NF-κB homeostasis from TNF-induced cell death

Presenter: Irina Antoshkina (see also P12); Helmholtz Munich / SAT, Germany

Background: Non-degradative ubiquitination regulates immune and inflammatory signaling by controlling NF-κB activation and cell death pathways. At the tumor necrosis factor receptor 1 (TNFR1) complex, the Met1- and Lys63-specific deubiquitinase CYLD is recruited via SPATA2 to HOIP, the catalytic subunit of the linear ubiquitin chain assembly complex (LUBAC) signaling. Upon TNF stimulation, CYLD counteracts NF-κB activation and enhances apoptotic/necroptotic cell death pathways, but how CYLD executes these functions or fulfills additional tasks remains largely unknown.

Methods: We investigated CYLD functions under basal and stimulated conditions by generating individual and combined KO cells for CYLD, SPATA2, and LUBAC components, complemented by genetic reconstitutions using mutant constructs. NF-κB activation and translocation, protein interaction and ubiquitination assays, as well as cell death responses are used to interrogate the roles of CYLD.

Results: We identify two non-canonical functions of CYLD that extend beyond its established role in stimulus-induced signaling: First, CYLD suppresses basal NF-κB activity in resting cells by counteracting LUBAC-driven activity independent of canonical SPATA2-binding to the HOIP PUB domain. Thereby, CYLD retains LUBAC in an inactive state and limits TAK1-dependent IKK–NF-κB signaling in the absence of stimulation. Second, CYLD promotes TNF-induced apoptosis through a mechanism that does not require LUBAC. Although LUBAC deficiency sensitizes cells to TNF-induced death, CYLD facilitates apoptotic signaling upon TNFR1 engagement by trimming RIPK1 ubiquitination even in the absence of LUBAC.

Conclusions: CYLD enforces NF-κB homeostasis not only during receptor engagement but also under basal conditions, preventing aberrant LUBAC activation. Collectively, CYLD functions as a molecular rheostat in inflammatory signaling, dampening basal LUBAC-driven NF-κB activation while promoting apoptosis when inflammatory signaling is perturbed. These dual LUBAC-dependent and -independent roles expand the current understanding of CYLD in immune homeostasis and cell fate regulation.

ST08 • Thursday 17:20

UBE2L3 safeguards intestinal epithelial homeostasis and limits inflammation at the gut barrier

Presenter: Femke DeMeyer (see also P30); VIB-IRC, University of Ghent, Belgium

Although inflammation is a hallmark of inflammatory bowel diseases (IBD), increasing evidence suggests that defects at the intestinal epithelial barrier actively contribute to disease onset and progression. How IBD-associated genetic variants affect epithelial stress responses and inflammatory signaling remains incompletely understood. Polymorphisms in UBE2L3, encoding a ubiquitin-conjugating enzyme involved

in ubiquitin signaling, have been linked to increased IBD risk, yet its epithelial-specific function and contribution to NF-κB regulation remain poorly characterized.

To investigate the role of UBE2L3 in intestinal epithelial cells (IECs), we generated IEC-specific Ube2l3 knockout mice and performed phenotypic analyses under homeostatic conditions and in a dextran sulphate sodium (DSS)-induced colitis model. Complementary ex vivo/in vitro studies were conducted using UBE2L3-deficient mouse colon organoids and HT29 cell lines. NF-κB pathway activation was assessed using luciferase reporter assays, qPCR and WB, while ubiquitin-dependent signaling mechanisms were investigated using linear ubiquitin chain pull-down assays. Additional analyses included assessment of epithelial stress responses, cell death, and barrier integrity. Loss of IEC-specific UBE2L3 disrupted IEC homeostasis and significantly increased susceptibility to DSS-induced colitis in mice. This was accompanied by enhanced epithelial stress and pronounced barrier dysfunction. Mechanistically, UBE2L3 deficiency resulted in dysregulated inflammatory signaling, including aberrant NF-κB pathway activation. Furthermore, linear ubiquitin pull-down experiments suggested altered regulation of LUBAC-dependent signaling, pointing to a defect in NF-κB signal modulation at the level of ubiquitin chain assembly. These findings were obtained in UBE2L3-deficient HT29 cells, supporting a cell-intrinsic role for UBE2L3 in controlling epithelial signaling responses.

Collectively, our data identify UBE2L3 as a critical regulator of intestinal epithelial homeostasis that regulates NF-κB activation through ubiquitin-dependent mechanisms. Loss of UBE2L3 amplifies inflammatory signaling, leading to barrier dysfunction and increased susceptibility to colitis. These findings identify UBE2L3 as an essential epithelial regulator linking genetic risk to barrier dysfunction and intestinal inflammation in IBD.

ST09 • Friday 12:00

IKKα mediates crosstalk between the NF-κB and MAPK pathways through dual-specific phosphatases 1 and 5

Presenter: Dina Harti (see also P16); Hospital del Mar Research Institute, Barcelona, Spain

Constitutive activation of the MAPK pathway is a major driver of colorectal cancer (CRC) progression and therapeutic resistance. Although genetic alterations in KRAS, BRAF, or EGFR sustain MAPK signaling in many tumors, the mechanisms maintaining ERK activity in specific metastatic contexts remain incompletely understood. We used colorectal cancer cell lines, patient-derived organoids, and in vivo metastasis models with genetic and pharmacological inhibition of IKKα to investigate MAPK regulation.

NF-κB-dependent control of DUSP1 and DUSP5 was assessed through transcriptional and functional assays. Therapeutic vulnerability was evaluated using the MEK inhibitor selumetinib, and clinical relevance was explored through analysis of human CRC datasets. Here, we identify a previously unrecognized role for IKKα in restraining MAPK signaling through NF-κB dependent regulation of the ERK phosphatases DUSP1 and DUSP5. Loss or pharmacological inhibition of IKKα led to downregulation of DUSP1/5, resulting in sustained ERK activation and enhanced proliferative capacity of CRC cells. Restoration of DUSP1 or DUSP5 expression reverted ERK hyperactivation and reduced tumor cell proliferation in vitro. Importantly, pharmacological inhibition of the MAPK pathway using selumetinib suppressed metastatic outgrowth of IKKα-deficient tumors in vivo without affecting initial metastatic seeding, identifying MAPK signaling as a key driver of metastatic expansion in this context. Analysis of human CRC datasets further supported the clinical relevance of the IKKα-DUSP axis.

Collectively, our findings uncover an IKKα–NF-κB–DUSP signaling axis that restrains MAPK activity and highlight a therapeutically actionable vulnerability in colorectal cancer metastasis.

ST10 • Friday 12:15

Inhibitory-κB kinase as a therapeutic target in colorectal cancer

Presenter: Kathryn Pennel (see also P06); Department of Experimental Therapeutics, School of Cancer Sciences, University of Glasgow, Glasgow, UK

Background: The canonical NF-κB pathway, driven by inhibitory-κB kinase beta (IKKβ), has been implicated in colorectal cancer (CRC) development and progression. However, inhibition of IKKβ has been unsuccessful in clinical trials. Research has shifted to focus on non-canonical NF-κB signaling, regulated by inhibitory-κB kinase alpha (IKKα). High tumour epithelial expression of IKKα has been recently established as a marker of poor prognosis in CRC. This project aims to establish the therapeutic potential of a novel first-in-class selective IKKα inhibitor utilising a range of in vitro/ex vivo CRC models.

Methods: CRC cells (HT29, HCT116, SW837) and patient-derived organoids (PDOs) from colon cancer (n=5) and rectal cancer (n=2) were profiled for response to selective inhibition of IKKα using a novel compound, SU1644. Responses were measured via cell viability and apoptosis assay. Immunofluorescent (IF) staining was used to establish the effect of SU1644 on Ki67 expression in PDOs. SU1644 was also tested in coculture models of tumour cells with cancer-associated fibroblasts (CAFs) and monocyte-derived macrophages. Patient-derived explants (PDEs) were used to assess response in a short-term model with complete tissue architecture.

Results: Inhibition of IKKα using SU1644 significantly reduced the viability of HT29 ($p<0.001$), HCT116 ($p<0.001$) and SW837 cells ($p<0.001$) in a dose-dependent manner. SU1644 showed additive effects when used in combination with chemotherapeutic agent 5-fluorouracil (5FU) and radiotherapy ($p=0.039$) in HT29 cells. SU1644 reduced cell viability and altered apoptosis in PDOs. There was a reduction in Ki67 expression (proliferation) in PDOs treated with 3μM SU1644 compared to vehicle control. Culture with primary CAFs induced therapeutic resistance in HCT116 treated with SU1644 and 5FU. In a subset of PDEs, SU1644 reduced expression of proliferation index.

Conclusions: IKKα represents a promising therapeutic target for colorectal cancer. In recapitulative disease models, first-in-class selective IKKα inhibitor SU1644 showed a potent anti-tumour activity.

ST11 • Friday 12:30

NIK/MAP3K14 functions as a mitochondrial stress-immunity integrator and drives sex-polarized transcriptional outputs that govern glioblastoma pathogenesis

Presenter: Raquel Sitcheran (see also P29); Texas A&M University Vashisht College of Medicine (USA)

Background: Glioblastoma (GBM) is uniformly lethal and exhibits a marked sex bias, with males showing higher incidence and shorter survival that remain mechanistically unexplained. The GBM tumor microenvironment (TME), dominated by microglia and macrophages, drives immune suppression and therapy resistance. NF-κB–Inducing Kinase (NIK/MAP3K14), the obligate activator of non-canonical NF-κB, is overexpressed in GBM, selectively enriched in microglia, and expressed at higher levels in male vs female GBM patients. We recently showed that NIK also localizes to mitochondria and supports GBM metabolic fitness independently of NF-κB signaling. Here we define how mitochondrial NIK rewires brain-wide transcriptional programs in a sex-polarized manner that shapes GBM progression.

Methods: Orthotopic GL261 and SB28 tumors were grown in male and female NIKKO and microglia-specific NIKCx3Cr1-cKO mice. Sex-stratified bulk RNA-seq of NIKKO vs NIKWT brains, combined with proteomics analysis of NIK-deficient GBM cells, defined NIK-dependent immune and mitochondrial pathways. NIK-mitochondrial interactions and putative transmembrane (TM) motifs were interrogated by IP/MS, PLA, imaging, and Seahorse metabolic assays.

Results: High NIK marks clinically aggressive GBM subgroups with poor survival. Systemic and microglial NIK loss selectively reduces tumor burden and prolongs survival in male, but not female, hosts. Strikingly, ~80% of shared DEGs invert between sexes: NIK loss in males amplifies antigen presentation and interferon effector programs, whereas in females it exacerbates mitochondrial dysfunction and promotes ECM permissiveness. Mechanistically, NIK physically associates with TIM50, TIM8a, and DRP1, and conserved GxxxG and WN TM motifs in NIK are required for maximal OXPHOS independently of RelB/non-canonical NF-κB. Proteomics reveal that NIK loss coordinately impairs oxidative phosphorylation and MHC-I antigen presentation pathways.

Conclusions: These data reframe NIK as a mitochondrial stress integrator whose NF-κB-independent respiratory function operates within a broader sex-biased brain transcriptional landscape to drive

microglia-specific, male-selective GBM pathogenesis — positioning NIK as a sex-stratified immunometabolic target.

ST12 • Friday 14:15

p52 inhibits anti-tumor immunity to promote glioblastoma growth

Presenter: Bakhtiar Yamini (see also P23); University of Chicago (USA)

Background: Nuclear p52 is generally maintained at a low level in non-cancer cells. We find that over half of glioblastomas (GBM) have elevated nuclear p52 at baseline that is linked to poor patient survival.

Methods: CRISPR/Cas9 was used to delete Nfkb2/p52 from GBM cells. In vitro and intracranial mouse GBM studies were performed. RNA sequencing identified pathways regulated by p52. Flow cytometry was used to study immune cell distribution in mouse GBM and to evaluate immune cell responses in vitro. Tumor interstitial fluid (TIF) was harvested from mice and metabolomic and functional studies performed.

Results: Although loss of p52/Nfkb2 (p52-KO) attenuates cell cycle progression, expression profiling indicates that in GBM cells p52 primarily up-regulates processes associated with the extracellular compartment. Using an immunocompetent mouse GBM model, we find that mice with p52-KO intracranial tumors have significantly improved survival compared to those with p52-WT. This difference in survival is completely lost if tumors are grown in nude mice or in immunocompetent mice in the presence of CD4/CD8 T-cell depletion. Analysis of the immune cell landscape of mouse brain tumors indicates that p52-KO tumors have fewer macrophages and decreased T-regulatory cells compared to p52-WT tumors. Given that p52 modulates extracellular processes, we looked at the extracellular fluid (TIF) from intracranial GBM and identified p52-dependent metabolites that attenuate T-cell-dependent cytotoxicity.

Conclusions: While p52 has generally been associated with modulating cell-intrinsic processes, this work indicates that in GBM cells p52 primarily up-regulates factors that modulate the surrounding tumor microenvironment. More specifically, high p52 in GBM tumors induces macrophage recruitment and alters the extracellular metabolite composition leading to inhibition of anti-tumor immunity facilitating tumor growth.

ST13 • Friday 14:30

NF-κB signalling dynamics define survival dependencies in aggressive lymphomas

Presenter: Aimilia Vareli (see also P22); Brighton and Sussex Medical School (United Kingdom)

Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive blood cancer treated with a “one-size-fits-all” immunochemotherapy regimen that fails in up to 40% of patients. A major barrier to targeted therapy is molecular heterogeneity within NF-κB signalling and downstream BCL2-family targets, as well as tumour microenvironment (TME) inputs such as CD40L that alter therapeutic responses. However, how NF-κB signalling dynamics encode apoptotic dependency remains unclear. We developed mechanistic Ordinary Differential Equation (ODE) models linking NF-κB signalling dynamics with BCL2-family regulation. Our analyses show that redistribution of NF-κB inhibitory complexes controls p100 availability for NIK-mediated processing to p52, thereby constraining non-canonical NF-κB output and anti-apoptotic signalling.

Methods: ODE models were simulated under monoculture and CD40L conditions. Model outputs were reduced into a predictive “Mediators of Apoptosis Combinatorial Score” (MAC-Score) to resolve apoptotic dependency by integrating relative anti-apoptotic protein abundance. Experimental validation was performed using flow cytometry, immunofluorescence, and Western blotting to quantify NF-κB subunit activation (RelA, RelB and cRel), BCL2-family protein expression, and cell death.

Results: Simulations revealed that NF-κB signalling dynamics encode apoptotic dependency states across DLBCL models, with basal and CD40L-induced signalling driving distinct anti-apoptotic programmes. These states were constrained by basal NF-κB activity and reflected dynamic buffering between canonical and non-canonical signalling that shapes anti-apoptotic protein balance. Experimental

measurements confirmed that NF-κB-driven shifts in anti-apoptotic protein balance underpin resistance under TME-mimicking co-culture conditions. MAC-Scores identified pan-tumour strategies, including NIK inhibition, and tumour-specific vulnerabilities such as BTK or MCL1 targeting. Model-predicted co-targeting enabled effective cell killing at substantially reduced doses (up to four orders of magnitude lower), supporting low-dose combination strategies.

Conclusions: These findings demonstrate that NF-κB signalling dynamics determine apoptotic dependency in DLBCL. Combinatorial protein scores derived from signalling behaviour enable prediction and experimental validation of therapeutic vulnerabilities while retaining mechanistic resolution, enabling model-driven personalised therapy in aggressive blood cancers.

Poster Abstracts

P01 • IκBα involvement in Neural-to-GBM Malignant Transformation

Authors & Affiliations: Daniel Alvarez-Villanueva, University of Miami and Markus Bredel, University of Miami

Background: NF-kappa-B inhibitor alpha (IκBα) is a marker of poor prognosis in glioblastoma (GBM), with hemizygous deletion of *NFKBIA* gene correlating with reduced patient survival. Beyond its canonical role in nuclear factor kappa B (NF-κB) inhibition, IκBα regulates stem cell maintenance and cancer cell stemness; however, its function in neural lineage transformation remains unclear.

Results and Methods: Here, we used IκBα separation-of-function (SOF) mutants to dissect its chromatin-associated activity independently of NF-κB signaling. Inducible iPSC-derived neural progenitor cells (NPCs) expressing IκBα SOFs enabled interrogation of its role during malignant transformation. IκBα chromatin immunoprecipitation (ChIP) identified RE-1 silencing transcription factor (REST) as a potential chromatin partner of IκBα. Given its established role in neural differentiation, REST emerges as a key regulator in this context. Consistently, bulk RNA sequencing of wild-type and IκBα knockout iPSCs showed that genes identified as IκBα chromatin targets are markedly downregulated upon IκBα loss. In parallel, we are integrating Hi-C data to further characterize the role of IκBα in three-dimensional chromatin organization.

Analysis of public datasets revealed a correlation between REST and IκBα expression in GBM, with low REST levels associated with poor prognosis. Taking advantage of patient-derived gliomaspheres, we are assessing the functional relationship between REST and IκBα. Ongoing studies are evaluating their impact on GBM cell growth and tumor-initiating capacity.

Conclusions and future perspectives: We are currently developing iGBM-iPSC models to generate mesenchymal, proneural, and classical subtypes to define subtype-specific roles of IκBα using iSOF systems. Together, these approaches aim to define the contribution of IκBα to GBM initiation and progression and identify potential therapeutic vulnerabilities.

P05 • IKKα-High Tumours Exhibit EMT-Enriched NF-κB Signalling Associated with Poor Survival

Authors & Affiliations: Molly McKenzie [1], Kathryn Pennel [1], Guang-Yu Lian [1], Leonor Patricia Schubert Santana [1], Phimmada Hatthakarnkul [1], Jean A Quinn [1], Assya Legrini [1], Yoana Doncheva [1], Claire Kennedy Dietrich [1], Colin Wood [1], Gerard Lynch [1], Noori Maka [2], Nigel Jamieson [1], Joanne Edwards [1]. [1]Wolfson Wohl Cancer Research Centre, School of Cancer Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK, G61 1BD, [2]Department of Pathology, Queen Elizabeth University Hospital, G51 4TF

Background: NF-κB driven inflammation has been strongly associated with progression of colorectal cancer (CRC). Recent work suggests IKKβ, which drives canonical NF-κB signalling, is a suboptimal target for therapeutic intervention due to its essential role in maintaining normal tissue homeostasis. Consequently, there have been efforts to inhibit IKKα, a non-canonical NF-κB kinase, as an alternative

avenue to reduce inflammation. There is a need to delineate the mechanism-of-action of IKKα signalling in CRC. This study investigates the prognostic role of IKKα in CRC and explores the spatial landscape of IKKα and IKKβ expression in patients.

Methods: Immunohistochemistry (IHC) for IKKα and IKKβ was performed on a cohort of 787 CRC patients. TempO-Seq® bulk transcriptomics (BioClavis) was used to examine differences between IKKα high and low expressing groups. Mini-bulk spatial transcriptomics (GeoMx®, Bruker) was performed to delineate transcriptomic profile of distinct tissue compartments. Transcriptomic results were validated using sequential immunofluorescence (seqIF™, 20-plex) staining by Lunaphore COMET™, employing the SPYRE-IO-core, and bespoke NF-κB panels.

Results: In tumour tissue, IKKα-high IKKβ-low expressing patients had the poorest cancer specific survival (CSS) (p=0.017), compared to IKKα-low IKKβ-high group (p=0.001). In bulk-transcriptomics, IKKα-high patients demonstrated enriched stemness markers (eg: L1TD1) and a loss of adhesion genes (eg: DSG2). In pan-cytokeratin+ segments, an enrichment of *hallmark cholesterol homeostatic* and *epithelial to mesenchymal (EMT)* gene sets (p<0.05), were observed in IKKα-high patients. In 20-plex seqIF™ data, EMT markers were expressed in inflamed tumours.

Conclusions: In tumour tissue, IKKα-high IKKβ-low expression is associated with the poorest CSS compared to other IKK status. Patients with high IKKα protein expression demonstrated enriched EMT gene expression across bulk and spatial compartments, which was validated at protein level. These data highlight a functional insight into the mechanism within IKKα-high tumours, highlighting an avenue for patient stratification in CRC treatment.

P07 • Digital Twins for Mechanistic Investigation of Stress and NF-κB Signalling in Triple-Negative Breast Cancer

Authors & Affiliations: Isabelle Jupp [1], Aimilia Vareli[1], Sarah Becker [2], Fabio Simoes [1], Shubhankar Sinha [2], Emma Kennedy [1,3], Ayaka Ichikawa [3], Mariko Okada [3], Melanie Flint [2], Simon Mitchell [1]. [1]Brighton and Sussex Medical School, Department of Clinical and Experimental Medicine, Brighton and Sussex Medical School, University of Brighton and University of Sussex, Brighton, BN1 9PX, UK. [2]University of Brighton, School of Pharmacy and Biomolecular Sciences, University of Brighton, Centre for Stress and Age-related Diseases, Moulsecoomb, Brighton BN24GJ, United Kingdom. [3]Institute for Protein Research, Osaka University, Suita, Osaka 565-0871, Japan.

Background: Triple-negative breast cancer (TNBC) is an aggressive cancer characterised by a lack of efficacious targeted therapies. Psychological stress and glucocorticoid signalling can promote drug resistance. The transcription factor NF-κB regulates cellular proliferation, metastasis and immune evasion. Links between NF-κB and GR are predominantly antagonistic across multiple cellular contexts, but elevated NF-κB and GR activity both correlate with poor clinical outcomes in TNBC. Links between NF-κB, the cell cycle, apoptosis and treatment responses have been elucidated in other malignancies through computational systems biology models but have not been tailored to TNBC or GR signalling. It is not known whether NF-κB RelB or cRel are modulated by GR activity in TNBC.

Methods: High-throughput immunofluorescence, flow cytometry, and western blotting were employed to assess GR and NF-κB subunit abundance and localisation under GC-activated conditions. Live-cell FUCCI imaging was employed to quantify cell cycle phases. The gene encoding GR (NR3C1) was knocked down with siRNA. Existing TNFα models were incorporated into the multiscale ODE-based modelling framework and adjusted with TNBC-specific gene expression.

Results: Experimental results revealed that TNBC cell lines have the highest GR abundance, compared with ER+ and HER2+. While NF-κB RelA was found to have significantly higher cytoplasmic sequestration and lower whole-cell abundance in TNBC. We found that RelB and cRel subunit abundance and localisation were heterogeneous across breast cancer subtypes, with TNFα inducing nuclear localisation

of RelA, cRel and RelB in TNBC. While GR activation decreased the abundance of NF-κB in ER+ and HER2+ cell lines, we found selective increases in cRel in TNBC

Conclusions: A novel model of TNBC captured the multiple cell fate outcomes seen in BC responding to TNFα, including necroptosis and proliferation. By integrating experimental data with computational models, we investigate NF-κB and GR as digital twins of TNBC to enable the identification of new therapeutic strategies.

P08 • *NFKBIA* deletion rewires differentiation programs in triple negative breast cancer

Authors & Affiliations: Sindhu Nair, Markus Bredel. Affiliations – Department of Radiation Oncology, Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida, USA

Background: Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer (BC) associated with poor median overall survival (OS) and lacking well-defined molecular targets, posing a significant clinical challenge. The deletion of the endogenous NF-κB repressor gene *NFKBIA*, is significantly associated with the TNBC subtype in patients. Additionally, the constitutive activation of NF-κB has been associated with the aggressive basal-like subtype which is hypothesized to derive from mammary stem cells exhibiting high levels of stemness markers. We identified that *NFKBIA*-encoded IκBα, which inhibits NF-κB, exerts a novel chromatin-regulatory function that drives the differentiation of human induced pluripotent cells. We hypothesize that IκBα depletion rewires differentiation programs in TNBC cells promoting stem-like traits and driving a more aggressive phenotype.

Methods: We retrieved genomic data and clinical attributes of > 5000 human BC to assess *NFKBIA* status and correlated the results with patient survival, metastases and subtypes. To discern the role of IκBα in TNBC, we generated IκBα wild type and knockout models in immortalized and patient derived TNBC cell lines. We are evaluating stemness markers using immunoblotting, immunofluorescence and qPCR and will employ separation-of-function IκBα mutants and utilize ChIP-seq and scRNA-seq to specify the role of IκBα in NF-κB signaling versus chromatin binding and differentiation in TNBC.

Results: *NFKBIA* was deleted in 10.8% of breast cancers and significantly associated with TNBCs (32.8%) and the basal-like subtype (36.7%). Deletion of *NFKBIA* was strongly associated with poor overall, recurrence-free, and metastasis-free survival and tumor stage. We are currently validating the role of IκBα depletion in promoting stemness in TNBC models in vitro, with results to be presented at the meeting.

Conclusions: Deletion of *NFKBIA* in TNBC is prognostically relevant and linked to the aggressive basal-like subtype, which exhibits stem-like traits. Our results aim to clarify the mechanism by which IκBα depletion drives this phenotype.

P09 • Disrupted immune homeostasis caused by a novel human MALT1 mutation

Authors & Affiliations: Constanze Sixt [1], Thomas O'Neill 1, Sofia Coelho [1], Katja Lammens [2], Safa Baris [3], Daniel Krappmann [1]; [1]RU Signaling and Translation, Helmholtz Munich, [2]Gene Center, LMU Munich; [3]Faculty of Medicine, Marmara University, Istanbul.

Background: MALT1 is a key regulator in maintaining immune homeostasis, exhibiting a dual function upon activation in T cells. MALT1 acts as a protease and as a scaffolding protein mediating NF-κB activation via the TRAF6-IKK-IκBα axis. To date, a number of MALT1 patient mutations have been described that are mostly associated with combined immunodeficiency, often caused by MALT1 deficiency due to truncating or destabilizing mutations. Here, we report on a unique homozygous MALT1 mutation, which is associated with symptoms of immune deficiency (recurrent infections) and autoimmunity (dermatitis and ulcerative colitis) in the adolescent patient.

Methods: We studied the influence of the MALT1 patient mutation under basal and stimulated conditions by analyzing patient PBMCs, reconstitution of MALT1 KO Jurkat cells, and evaluating an in vitro MALT1 activity assay. NF-κB activation, protein stability, MALT1 substrate cleavage and inhibition have been assessed to characterize the patient mutation.

Results: We show that the missense mutation neither affects the MALT1 stability nor scaffolding function leading to NF-κB activation. However, by reconstitution of MALT1 KO Jurkat T cells, we demonstrate that the mutation reduces MALT1 protease function and substrate cleavage. In addition, the mutation yields a natural variant of MALT1, which impairs inhibition of MALT1 protease by allosteric inhibitors, which are currently evaluated in clinical trials.

Conclusion: Studying this human MALT1 variant reveals that a slight structural alteration outside the catalytic binding pocket strongly affects MALT1 protease activation and substrate cleavage. Further, our data reveal that uncoupling of MALT1 protease and scaffolding functions in humans has a strong impact on immune activation and maintenance of immune homeostasis.

P10 • Notch/NF-κB crosstalk signaling controls SASP development in brain aging

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Background: Brain aging is characterized by chronic low-grade inflammation and the development of a senescence-associated secretory phenotype (SASP) by glial cells. Although Notch and NF-κB signaling are key regulators of inflammaging and SASP formation, their functional and mechanistic interplay in the aging brain remains poorly understood.

Methods: Candidate SASP genes were analyzed in silico to identify regulatory DNA motifs potentially mediating Notch/NF-κB crosstalk signaling. Notch and NF-κB gene signatures were assessed by RNA sequencing and qRT-PCR in brain tissue and glial cells from transgenic mice with modulated NF-κB activity. Physical interactions between Notch and NF-κB pathway components were examined by co-immunoprecipitation and western blotting. Primary glial cultures were used to evaluate the impact of pharmacological Notch activation on NF-κB-driven SASP gene expression.

Results: Brain aging was associated with enhanced NF-κB activity and reduced Notch signaling. Astrocyte-specific NF-κB activation in young mice was sufficient to induce cell-autonomous and non-cell-autonomous transcriptomic and cellular alterations resembling premature brain aging and SASP development. This phenotype was characterized by robust induction of SASP-associated genes, enrichment of pro-inflammatory pathways, and increased immune cell infiltration into the brain parenchyma, together with suppression of the Notch gene signature. Mechanistically, the Notch intracellular domain (NICD) inhibited NF-κB signaling through multiple mechanisms, including competition for shared promoter elements, interference with RelA in both the cytoplasm and nucleus, and inhibition of activated IKK2, the central kinase of canonical NF-κB signaling. Consistently, pharmacological activation of Notch signaling reduced NF-κB-driven SASP gene expression in primary glial cells.

Conclusion: These findings identify Notch/NF-κB crosstalk as a central regulatory checkpoint of brain inflammaging and highlight this signaling axis as a potential therapeutic target for age-associated neuroinflammation and neurodegenerative diseases.

P11 • Genetic Variation in NF-κB motifs of the HIV-1C LTR Promotes Stringent Latent Reservoirs

Authors & Affiliations: Hrimkar Buch, Rahul Khaleri, Manisenthil Shanmugam, Swarnima Mishra, Udaykumar Ranga. Jawaharlal Nehru Centre for Advanced Scientific Research, India

The persistence of latent reservoirs remains the principal barrier to curing HIV-1 infection. While the molecular basis of latency has been extensively studied, the contribution of the NF-κB signaling pathway at the viral promoter remains underexplored in a subtype-specific context. The long terminal repeat of

HIV-1C contains a unique heterotypic cluster of three NF-κB motifs, including the subtype-specific C-κB site, defined by A→G and T→G transitions and increased GC richness.

We propose that the C-κB motif functions as a high-affinity binding site for p50–p50 homodimers under basal conditions, promoting transcriptional repression. Critically, this repressive architecture necessitates intact adjacent canonical H-κB sites for productive transcriptional activation. We hypothesize that genetic variation within these H-κB sites disrupts cooperative NF-κB binding, thereby reducing reactivation potential and elevating the activation threshold. Analysis of patient-derived HIV-1C sequences revealed that all subjects harbor variation in one or more NF-κB motifs, with ~40% intrahost diversity, supporting a model in which motif heterogeneity reinforces latency by destabilizing activation-competent configurations.

To directly address the impact of NF-κB motif variation on latency, we employ both sub-genomic reporter systems and full-length viral constructs. At the chromatin level, we provide evidence of differential NF-κB occupancy and binding dynamics using CUT&RUN and EMSA, linking sequence variation to altered transcription factor engagement.

Additionally, targeting the classical NF-κB pathway for latency reversal has been limited by broad inflammatory effects. In this context, we are further exploring alternative NF-κB pathway activation in HIV-1C and are optimizing an immunotherapeutic strategy utilizing OX40/CD40 agonists to selectively engage this axis.

Collectively, our findings identify a subtype-specific NF-κB regulatory mechanism in which variation in canonical H-κB sites, in the context of a repressive C-κB motif, elevates the threshold for reactivation and contributes to a more stringent latent reservoir potentially aiding the barrier to cure.

P14 • Elevated Cytoplasmic IKKα in Meibomian Glands Drives NF-κB–Mediated Meibocyte Dysfunction and Age-Related Dry Eye Disease

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Background: Dry eye disease (DED) is a common multifactorial ocular surface disorder affecting over 20% of adults over 40, with higher prevalence in the elderly. It is often driven by meibomian gland dysfunction (MGD), as the meibomian glands (MGs) produce meibum, a lipid-rich secretion essential for tear film stability. Chronic inflammation is a hallmark of DED/MGD, and NF-κB overactivation across the ocular surface likely contributes to disease progression, though its activation mechanisms remain unclear. Notably, conjunctival biopsies from DED/MGD patients show elevated IKKα levels, but its functional role in pathogenesis remains unexplored. The lack of suitable models—human studies rarely include MGs, and current mouse models rely on short-term induction—limits understanding of DED/MGD. Here, we use C-IKKα mice, which display increased cytoplasmic IKKα in MG basal cells, providing a clinically relevant model that recapitulates key features of human DED/MGD, including sustained NF-κB activation, glandular inflammation, and progressive age-related changes.

Methods: C-IKKα mouse DED/MGD model, with histopathology, RNA-seq, flow cytometry, functional assays, and genomic comparisons to assess similarity to human disease.

Results: To assess the impact of increased IKKα and NF-κB activation on DED/MGD, we analyzed C- and N-IKKα mice. Nuclear IKKα does not affect MG homeostasis, whereas cytoplasmic IKKα triggers sustained NF-κB activation, chronic inflammation, impaired meibocyte differentiation, reduced lipid production, glandular dysfunction, and spontaneous DED/MGD. The model mirrors patient signatures, including a four-gene signature (IFITM1, HLA-A/H2-K1, CTSS, CCL4) that distinguishes DED/MGD patients from controls; highlights Cyclin D1 and PPARγ dysregulation as initiating events, and reveals

genetic alterations in hormone and lipid metabolism, acinar proliferation/differentiation and adhesion genes that may drive disease onset and progression.

Conclusions: Elevated cytoplasmic IKKα in MG basal cells drives DED/MGD development, recapitulating key features of the human disease and providing a valuable platform to investigate disease mechanisms, identify biomarkers, and evaluate new DED/MGD therapies.

P15 • Structural determinants of the non-canonical NF-κB pathway

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We have studied the structure-activity relationship between the two main kinases (NIK and IKKalpha) of the non-canonical NF-κB pathway. Using Alphafold3 and Alphafold multimer, we identified a set of amino acids within NIK that form hydrogen bonds and hydrophobic interactions with IKKalpha. Mutation into Alanine of specific amino acid within NIK prevents the recruitment of IKKalpha in cellulo. Conversely, we identified two stretches of amino acids in IKKalpha and mutation of particular amino acids abrogates the binding to NIK without inhibiting IKKalpha kinase activity.

We next challenged our in silico/in vitro finding by generating an IKKalpha knock-in mouse with specific point mutation. Our mice do not develop secondary lymphoid organs, show disorganised spleen and thymus and inflammatory disorders, all phenotypes reminiscent to NIK KO or IKKalpha deficient mice. In conclusion, our in cellulo and in vivo results validate our structural predictions for the interplay between NIK and IKKalpha.

P18 • A Systems-Biology Approach Investigating the Impact of NF-κB-Signalling and Crosstalk on the Expression of Factors Influencing the Lymph Node Microenvironment

Authors & Affiliations: Abigail Mary Edwards[1], Fabio Simoes[1], Sean Lim2, Aimilia Vareli[1], Simon Mitchell[1]. [1]Brighton and Sussex Medical School, [2]University of Southampton

Diffuse Large B-cell Lymphoma (DLBCL) is an aggressive haematological malignancy, with approximately 6000 cases per year in the UK. DLBCL is a strikingly heterogeneous disease; despite this, most patients receive a one-size-fits-all immunochemotherapy regimen, conferring a relapse/refractory (R/R) rate of 30-40%. Chimeric Antigen Receptor T-cell (CAR-T) therapies have improved outcomes for R/R-DLBCL; however, despite their improved specificity, they only extend progression-free survival (PFS) by approximately 1 year. The tumour microenvironment (TME) represents a key point of CAR-T failure.

This project focuses on the heterogeneity in a molecular network that is central to DLBCL's response to therapies: the nuclear factor kappa-B (NF-κB) pathway, and how this network influences the TME. The project addresses this challenge by combining computational and experimental approaches. An established computational model that simulates NF-κB signalling and induction of NF-κB target genes is used to investigate how heterogeneity in two NF-κB pathways (canonical/non-canonical) promotes the development of heterogeneous TMEs. Many TME-modulating cytokines are NF-κB target genes, and pathway-specific control of cytokines can be investigated through activation of the NF-κB pathways in-vitro and in-silico.

This systems-biology approach is informed by novel experimental and published data to inform parameters and has revealed a potentially transformative new link between the non-canonical NF-κB subunit RelB and the T-cell co-stimulatory molecule CD70. This interaction may be central to the failure of CAR-T approaches in DLBCL, therefore highlighting non-canonical NF-κB as a potential therapeutic target in synergy with CAR-T approaches.

P19 • Selection and Characterization of Inhibitory DNA Aptamers Targeting Fatty Acid-Binding Protein 5

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Background: Fatty acid-binding protein 5 (FABP5) is an intracellular lipid chaperone that transports long-chain fatty acids between cellular compartments, thereby regulating gene expression and signal transduction. FABP5 plays a multifaceted role in immune regulation [1,2]. It enhances the inflammatory response in dendritic cells, limits macrophage polarization, and promotes cytokine production through reactive oxygen species and protein kinase C-mediated activation of the NF-κB signalling pathway. Overexpression of FABP5 has been linked to autoimmune diseases and multiple cancer types. Hence, FABP5 inhibitors are promising for the control of inflammation [3]. This study aimed to develop high-affinity inhibitory DNA aptamers specifically targeting FABP5.

Methods: FABP5 protein was expressed in E. coli cells and purified by affinity chromatography. DNA aptamers against recombinant FABP5 protein were selected using magnetic-based SELEX (Systematic Evolution of Ligands by Exponential Enrichment) method. Binding affinity of the selected aptamer was characterized using isothermal titration calorimetry (ITC). Fluorescence measurements using 11-(Dansylamino)undecanoic acid (DAUDA) were performed to reveal the inhibitory potential of the aptamer.

Results: SELEX method yielded a DNA aptamer with high affinity for FABP5. The aptamer demonstrated potential to interfere with FABP5-ligand interactions, suggesting inhibitory potential over NF-κB-associated downstream inflammatory signalling.

Conclusion: FABP5-specific inhibitory DNA aptamer developed in this study represents a promising molecular tool for modulating the NF-κB-driven inflammatory pathways. Strong binding affinity and target specificity highlight the potential for future therapeutic applications in inflammatory diseases, autoimmune disorders, and FABP5-overexpressing cancers.

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P20 • p52 haplo-sufficiency (?)

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Background: Immuno-pathogenic mechanisms in severe NFKB2-related diseases commonly originate from excess IκB-like activity mediated by un-processible mutant p100. In contrast, heterozygous mutations in NFKB1 mostly cause haplo-insufficiency of p105/p50. Based on almost identical domain structures of the paralogous NF-κB1 and NF-κB2 proteins, the apparent rareness of pathogenic NFKB2 variants with haplo-insufficiency of p100 and p52 (i.e. without mutant proteins being present) appears paradox. We hypothesized that, if genetic conditions with heterozygous loss-of p100/p52 expression are associated with rather mild clinical symptoms or even remain asymptomatic, detrimental variants should be prevalent in public databases.

Methods: We screened a representative series of NFKB2 missense variants, identified in our IEI patient cohort or listed in public databases such as gnomAD, for protein-damaging effects. For this purpose, we used well-established in vitro diagnostic tests for deleterious NF-κB protein defects, relying on ectopic over-expression of EGFP-fused p100 and p52 in HEK293T cells.

Results: We found a huge spectrum of severe p52 damages, which likely lead to protein loss in vivo. The typical signs – reminiscent of harmful p50 variants - included overall limited expression levels, devastating protein decay, sub-nuclear accumulation, loss-of-DNA binding activity and protein-interaction defects which were all caused by single amino acid changes residing within the N-terminal Rel-homology domain.

In contrast, the corresponding p100 precursors appeared to be largely unaffected by these missense variants.

Conclusions: Heterozygous loss of p52 might be associated with haplo-sufficiency (non-pathogenic) rather than with insufficiency (pathogenic) and might therefore be underdiagnosed. Sequencing data of patients carrying a NFKB2 missense variant should be carefully reconsidered if a genetic disease is assumed but causality remains elusive, particularly in cases where a variant has been filtered out or is assumed to be benign, solely because it is listed in population databases.

P21 • Therapy-induced fetal reprogramming and autophagy in colorectal cancer: a potential role for NF-κB signaling

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Colorectal cancer cells can adopt fetal-like transcriptional programs in response to therapeutic stress, but the mechanisms underlying this plasticity and its contribution to treatment resistance remain incompletely understood. We have identified suppression of Polycomb Repressive Complex 2 (PRC2) activity as a central driver of this adaptive response.

Treatment-naïve fetal-like colorectal tumors display reduced expression of PRC2 components and attenuation of MYC-dependent transcription. Moreover, chemotherapy further suppresses PRC2 in colorectal cancer patient-derived organoids, while pharmacological or genetic PRC2 inactivation is sufficient to induce a fetal/EMT-like transcriptional state. This transition is accompanied by reduced proliferative activity and increased resistance to chemotherapy, suggesting that PRC2 loss promotes the acquisition of a drug-tolerant cellular state.

We found that fetal conversion is also associated with increased autophagic activity. Chemotherapy and PRC2 inhibition induce LC3B accumulation, and fetal-like or therapy-induced states show increased dependency on lysosomal/autophagic pathways, supporting a functional connection between cellular reprogramming, autophagy and therapeutic resistance.

Interestingly, transcriptional analyses consistently identify TNFA SIGNALING VIA NFKB signaling among the pathways associated with fetal conversion. We are currently investigating whether chemotherapy- and PRC2-induced autophagy is accompanied by activation of the NF-κB pathway by analyzing the cellular levels and localization of p65 and IκBα.

Together, our results identify PRC2 suppression as a mechanism linking therapeutic stress to fetal reprogramming, autophagy and drug resistance in colorectal cancer, and suggest a potential involvement of NF-κB signaling in the establishment or maintenance of this adaptive state.

P24 • Targeting p52 Dimerization: Novel Potential Anticancer Inhibitors

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Cancer remains a substantial impact on the global healthcare system and remains the second leading cause of mortality, according to the World Health Organization. Due to the profound impact of this disease, both in terms of the cost in human lives and the pressure it imposes on healthcare resources, there is an urgent need for increased research efforts to mitigate the consequences of cancer care.

The non-canonical NF-κB pathway involves processing p100 into p52, and overexpression of the non-canonical NF-κB subunit p52 has been reported in epithelial cancers. Therefore, targeting p52 represents a promising therapeutic approach in cancer treatment.

The purpose of this study was to rationally design and synthesize novel potential p52 inhibitors, focusing on blocking the formation of p52:52, p52:RelA, and p52:RelB dimers through structure-based drug design. A virtual screening of 48 billion compounds from Enamine's REAL Space was performed. The resulting hits were evaluated against the residues of the dimerization domain of chain B (p52 monomer) of the p52:p52 homodimer (PDB: 1A3Q) through AutoDock Vina. Based on the docking score analysis, promising scaffolds, namely xanthone and estradiol derivatives, were selected for synthesis. Ongoing studies include biological evaluation in cancer cell lines and NMR spectroscopy to elucidate the mechanism of action of the most promising compounds.

This work highlights p52 dimerization as a relevant anticancer target. The identified small molecules provide a promising starting point for the development of a first class of therapeutic inhibitors.

P25 • Crosstalk Between NF-κB Signalling and the Tumor Microenvironment in Prostate Cancer

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Background: Prostate cancer (PC) is the second most commonly diagnosed cancer and the fifth leading cause of cancer-related death among men globally. The inflammatory response has been implicated in PC progression. NF-κB activation and many other signals associated with inflammation are known to contribute to prostate cancer malignancy. Inflammatory signals have also been associated with the development of castration resistance and resistance against other androgen depletion strategies, which remains a major therapeutic challenge.

Methods: We employed immunohistochemistry (IHC) to identify the prognostic value of NF-κB markers (such as IKKα and IKKβ) and multiplex immunofluorescence (mIF) to understand the interaction between NF-κB tumour signalling and the surrounding immune cells.

Results: Patients with High IKKα experienced poorer survival compared to those with low IKKα expression within their tumours. It is also involved with adverse clinical pathological factors in PC cohort. Using Lunaphore COMETTM platform, 20-plex staining (SPYRETM Immuno-oncology core panel with NF-κB markers; IKKα and IKKβ, phospho-p65, pSTAT3, pSTAT1, CLK2) demonstrated an association between NF-κB signalling in tumours and tumour microenvironment (TME) in PC cohort.

Conclusion: The effect of IKKα driven NF-κB signalling confirms our hypothesis that inflammatory signalling is involved in disease progression in PC cohort. The relationship with TME warrants further study to understand how tumour and immune cells interactions lead to the poor prognosis in PC patients.

P26 • Biphasic NF-κB activity in the Helicobacter pylori infected gastric mucosa

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Background: The gastric pathogen Helicobacter pylori is a Group 1 carcinogen and a known risk factor for several gastric diseases, including gastric cancer. Nearly half of the global population is colonized by H. pylori. NF-κB activation is a well-established response to H. pylori infection. ADP-heptose, an intermediate in lipopolysaccharide biosynthesis, induces rapid and transient NF-κB activation via ALPK1 and TIFA, however the contribution of other factors remains unclear.

Methods: H. pylori strain P1 and isogenic mutants were used to identify the bacterial determinants of NF-κB activation in infected AGS cells. CRISPR/Cas9-mediated knockout of ALPK1 and TIFA in AGS cells

was used to depict their role in NF-κB signaling. Other factors were validated by siRNA-mediated knockdown with a scrambled siRNA control. NF-κB activation was assessed by immunoblotting for phosphorylation of NF-κB signaling intermediates (IKKα/β, IκBα).

Results: H. pylori infection induced a biphasic NF-κB response in gastric epithelial cells. ADP-heptose-deficient bacteria failed to induce the first phase of NF-κB signaling while CagA mutant bacteria failed to induce sustained NF-κB activation. The early, rapid phase (1–3 h) required ALPK1 and TIFA, whereas the delayed and sustained phase (4–12 h) remained largely unaffected by ALPK1 or TIFA deletion. TRAF6 knockdown abrogated both early and delayed NF-κB activation, whereas MyD88 and IRAK1 knockdown led to partial attenuation of sustained NF-κB signaling.

Conclusion: Taken together, we defined distinct bacterial and host mechanisms underlying the biphasic NF-κB response to H. pylori infection, providing a refined framework for understanding how bacterial effectors shape the temporal dynamics of NF-κB signaling in gastric epithelial cells.

P27 • A computational multi-cell model of canonical NF-κB signaling

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The NF-κB signaling system responds to a large variety of stimuli in a cell-type-, context- and stimulus specific manner resulting in the transcription of a diverse set of targets. These transcription targets include the pathway's own negative and positive regulators, e.g., members of the IκB family. A special case is TNFα, which is not only transcribed in a NF-κB dependent manner but also results in NF-κB activation via the TNFR receptor cascade acting as positive feedback. During the early inflammatory response macrophages respond to bacterial metabolites with TNFα secretion alerting neighboring cells to the invasion. This NF-κB -mediated process is inherently multi-cellular necessitating a multi-scale, multi-cell perspective.

We utilize a computational model of canonical NF-κB signaling activated by TNFα and LPS to examine the impact of spatial properties on cell-cell communication. Starting from an established model for the intracellular dynamics of canonical NF-κB signaling, we expand into a model coupling cells via TNFα and analyze pathway dynamics during the early inflammatory response. Furthermore, to investigate the interaction between different cell types and stimuli, we adapt the model to represent two NF-κB-expressing cell types in the intestinal crypt: sentinel macrophages and epithelial cells. In this setting, LPS-activated macrophages secrete TNFα to elicit an immune response in the epithelium exemplifying the importance of a cell type-specific NF-κB response.

We conducted computational analysis to identify which model parameters, and their corresponding biochemical processes, have the strongest impact on signal transmission speed and loss of signal. In addition, we quantify measures on single-cell level, like the strength of NF-κB activity. Intercellular processes have a large influence on the system, emphasizing the importance of studying cells in context.

P28 • IKK/NF-κB signaling in astrocytes critically affects TBI outcome

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Background: Traumatic brain injury (TBI) is a complex disease condition in which multiple pathophysiological mechanisms can shape disease progression and long-term outcome. Beyond the initial mechanical insult, secondary neuroinflammatory responses driven by glial cells and infiltrating peripheral immune cells are central determinants of clinical recovery. The IKK/NF-κB signaling pathway has been broadly implicated in CNS injury, however the cell-type-specific mechanisms balancing beneficial versus

detrimental secondary processes remain poorly defined. Given that long-term consequences of TBI severely compromise quality of life and are strongly linked to neurodegenerative and neuropsychiatric disorders, characterization of IKK/NF-κB-mediated processes is instrumental for the development of effective therapeutic strategies.

Methods: We employed NF-κB reporter gene mice and conditional mouse models allowing the astrocyte-specific modulation of IKK/NF-κB signaling. RNA sequencing and flow cytometry were performed on primary brain cells, complemented by histological, western and MRI analyses to monitor the post-traumatic wound-healing process.

Results: Here, we show that TBI-mediated IKK/NF-κB activation in astrocytes impairs their homeostatic functions, amplifies the post-traumatic neuroimmune response and disturbs the multicellular CNS scar development in a male mouse model of TBI. Our results further demonstrate a specific deficit in the formation of the glial limitans border and establish that paracrine signaling pathways induced by NF-κB-activated astrocytes can prevent a beneficial restoration of the CNS integrity after TBI. Interestingly, TBI promotes an aging-like phenotype which can be repressed by NF-κB inhibition in astrocytes. Moreover, leukocyte composition in peripheral blood was already altered before injury and remained dysregulated thereafter, underscoring a profound disturbance of brain-periphery immune communication.

Conclusion: These findings establish astrocytic NF-κB signaling as a critical driver of maladaptive post-traumatic pathology and a compelling therapeutic target.

P31 • CARD14 hyperactivation in intestinal epithelial cells promotes colorectal cancer

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CARD14 is an NF-κB signalling protein that has primarily been studied in the context of skin inflammation, with gain-of-function mutations leading to psoriasis. However, we recently showed that intestinal epithelial cell-specific expression of a psoriasis-associated CARD14(E138A) gain-of-function mutant in mice also drives subclinical intestinal inflammation. Moreover, CARD14 hyperactivation sensitized mice to the development of colon polyps in response to the DNA-damaging drug azoxymethane. We previously showed that CARD14 signalling can be regulated by expression of A20, an inhibitor of NF-κB signaling and cell death that is often downregulated in human colorectal cancer (CRC). We therefore also studied the effect of CARD14(E138A) expression in an A20-deficient background. CARD14(E138A)/A20 KO mice were found to suffer from severe inflammation that is characterized by body weight loss, strong neutrophil infiltration in the lamina propria, and intestinal cell death. Strikingly, colon tumors appeared as early as 8 weeks after birth. To study direct IEC-intrinsic mechanisms in the absence of potential immune and microbial influences, we also employed 3D intestinal organoid cultures derived from our genetically engineered mouse models. This revealed high spontaneous cell death in organoids derived from CARD14(E138A)/A20 KO mice. Ongoing studies focus on further determining how CARD14(E138A) activation in A20-deficient IECs triggers cell death and influences the immune and microbial microenvironment in the intestine, eventually promoting CRC tumor formation. In conclusion, our findings reveal an unexpected novel role of CARD14 hyperactivation in driving CRC in mice, which is further supported by human RNAseq datasets showing increased CARD14 expression and mutations in colon adenocarcinoma that correlate with poor prognosis.

P33 • Context dependent role of the NF-κB regulator Bcl3 in Colorectal Primary Tumorigenesis and Liver Metastasis

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NF-κB signalling participates in a wide range of cellular processes such as inflammation and immune regulation and has been shown to have both tumour promoting and suppressive roles in a context

dependent manner. The NF-κB regulator Bcl3 is overexpressed in colorectal cancer (CRC), the second most common cause of cancer related deaths worldwide, and high Bcl3 expression has been reported to correlate with worse patient survival. Using genetically engineered mouse models (GEMMs) of CRC with whole body or epithelial-specific Bcl3 loss, we observed context dependency in the role of Bcl3 during primary tumorigenesis and liver metastasis. Whole body Bcl3 knockout promoted colonic tumour formation in Apc-loss driven CRC mouse models. Meanwhile, epithelial-specific Bcl3 loss extended survival and did not promote colonic tumorigenesis, suggesting a contrasting role of Bcl3 in a cell type dependent manner during primary tumorigenesis. Context dependency also extends to the level of metastasis. In a Kras mutant, Notch driven metastatic CRC GEMM, whole body Bcl3 knockout also promoted colonic tumour formation. Yet, whole body Bcl3 knockout prevented liver metastasis, and this was accompanied by a reduction in neutrophil abundance in the blood. Allografts of tumour cells derived from these mice showed Bcl3-deficient tumour cells had reduced ability to establish liver metastasis. This demonstrates that epithelial tumour-intrinsic Bcl3 promotes liver metastasis and positions Bcl3 as an exciting therapeutic target for CRC liver metastasis.

P34 • Multi-layered regulation of nucleic acid-induced innate immune signaling by protein ADP-ribosylation in lung cancer

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Background: Lung cancer cells adapt to chronic innate immune stress by rewiring signaling pathways that balance survival and immune evasion. A hallmark of many tumors is the accumulation of mislocalized self-nucleic acids (“viral mimicry”), which activate pattern-recognition receptors and trigger TBK1-NF-κB/IRF3 and type I interferon (IFN) signaling.

Results: Here, we investigated the role of ADP-ribosyltransferases in regulating nucleic acid-driven signaling networks in lung cancer. PARP7-mediated ADP-ribosylation stabilized the AP-1 transcription factor FOSL1/FRA1 and suppressed aberrant nucleic acid-induced signaling. Pharmacological inhibition of PARP7 or mutation of the FRA1 ADP-ribosylation site promoted FRA1 degradation, leading to enhanced NF-κB /IRF3-dependent cytokine expression and increased type I IFN production. This immune activation was accompanied by cell cycle disruption, DNA damage signaling, and caspase-8-mediated apoptosis. Subsequently, the IFN-responsive network itself induced expression of additional ADP-ribosyltransferases, including PARP14 and PARP10. We identified SQSTM1/p62 and the E3 ubiquitin ligase RNF114 as PARP14 substrates modified at cysteine residues, forming distinct ADP-ribosylated cytoplasmic foci. Given the established role of RNF114 in antiviral responses and NF-κB/IRF3 signaling, we further examined its regulation in this context. Knockdown of p62 or PARP14, as well as PARP14 inhibition, increased RNF114 protein levels and reduced expression of NF-κB/IRF3 target genes, such as CCL5.

Conclusions: Together, our findings identify ADP-ribosylation of proteins as a central regulator of nucleic acid-induced innate immune signaling in lung cancer at multiple levels.

P35 • IκBNS controls Treg cell function during Leishmania major infection

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Atypical IκB proteins, like IκBNS, are nuclear proteins that co-regulate NF-κB activity. IκBNS is vital for Treg cell development by inducing Foxp3 transcription and regulates Th1 and Th17 cell differentiation. Leishmania major, an intracellular parasite, causes cutaneous infections. In rodents and humans, these lesions heal over time, leaving some active parasites. Th1 cells are crucial for the immune response, while Treg cells help to maintain a small parasite pool and immunological memory. Hence, L. major infection is a suitable model to dissect the importance of IκBNS in Th1 versus Treg cells.

We infected T cell-specific IκBNS knockout mice with L. major, resulting in reduced ear thickening, parasite burden, and infected cell frequencies compared to control mice. Consistently, we detected elevated IFNγ and decreased IL-10 expression. Treg cell frequencies were lower in lymph nodes, but surprisingly, more Tregs were found at the infection site, suggesting impaired function rather than migration or number.

To investigate the role of IκBNS in Treg function, we generated Treg-specific knockout mice and performed RNA sequencing on sorted naïve and effector Treg populations. IκBNS deficiency affected cell-cycle-associated genes in naïve Treg cells, while effector Treg cells showed widespread dysregulation of immune-relevant pathways. Functional enrichment analyses revealed impaired NF-κB signaling, T cell activation, and calcium signaling in IκBNS-deficient effector Treg cells. Key genes and pathways associated with Treg suppressive function, including Ctla4 and Klrg1, and intracellular calcium flux were significantly downregulated.

We conclude that IκBNS controls distinct transcriptional changes in naïve versus effector Treg cells and regulates Treg suppressive function.

P36 • NF-κB RelA and RelB promote melanoma progression through both shared and selective mechanisms

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NF-κB has been involved in most hallmarks of cancer, including recently in several mechanisms of escape from the immune system. Nevertheless, the specific contributions of distinct NF-κB subunits in these processes are still unclear, preventing the development of safe and specific inhibitors for cancer therapy. In this project, we investigated the roles of RelA and RelB as prototypical subunits of the canonical and alternative NF-κB pathway, respectively, in mouse models of melanoma. Thus, we created knock-out B16F10 and BRAFV600E.Pten-/- (BP) cell lines using CRISPR/Cas9. Surprisingly, ablation of either RelA or RelB did not alter the proliferation or survival of melanoma cells in vitro. In contrast, the progression of RelA- and RelB-deficient B16F10 and BP melanomas was dramatically impaired upon transfer to immunocompetent syngeneic mice. Spectral flow cytometry analyses revealed major alterations in the immune microenvironment at end point, with in particular increased infiltration of cytotoxic CD8+ T cells at the expense of CD4+ effector and regulatory T cells. Strikingly, the altered progression of NFκB-deficient melanomas was entirely rescued upon transfer to mice lacking B and T cells, or upon depletion of CD8+ T cells. This demonstrated that escape from the T-cell response is a major function of NF-κB subunits in this disease. Mechanistically, RNA-sequencing experiments revealed both overlapping and specific regulation of gene expression by RelA and RelB, with in particular involvements in antigen presentation and cellular plasticity. This project provides a comprehensive analysis of the contribution of NF-κB subunits in melanoma.

P37 • Targeting Non-canonical NF-κB Signalling in Richter Transformation: Rationale for NIK Inhibition as a Microenvironment-Directed Therapeutic Strategy

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Background: Richter transformation (RT) is an aggressive evolution of chronic lymphocytic leukaemia (CLL) with poor outcomes and limited treatment options. Unlike circulating CLL, RT is primarily a lymph node–based disease, shaped by strong microenvironmental cues. In CLL, lymph node signalling, particularly CD40–CD40L interactions, drives dependence on non-canonical NF-κB signalling via NF-κB-inducing kinase (NIK). We investigated whether RT shares this dependency and whether it represents a therapeutic vulnerability.

Methods: NF-κB pathway activity was assessed in primary RT patient-derived xenografts (RT-PDX) and the U-RT1 cell line, alongside primary CLL cells under basal and CD40-stimulated conditions. Canonical and non-canonical NF-κB activation were evaluated by Western blotting, nuclear ELISA, and transcriptional profiling. Cytotoxicity and combination studies used selective NIK inhibitors (B022, Amgen16) ± venetoclax. In vivo efficacy was tested in a subcutaneous RT-PDX model.

Results: RT cells exhibited elevated basal non-canonical NF-κB activity compared with CLL, with further activation following CD40 stimulation. NIK inhibition selectively suppressed non-canonical signalling, including under microenvironmental stimulation, with minimal effects on canonical NF-κB. CD40 stimulation induced venetoclax resistance, associated with increased BCLXL and MCL1 expression. NIK inhibition reversed this resistance and demonstrated strong synergy with venetoclax across RT models, including intrinsically resistant contexts. In vivo, NIK inhibition, particularly in combination, reduced tumour growth and improved survival.

Conclusions: Non-canonical NF-κB signalling is a central, targetable dependency in RT. NIK inhibition overcomes microenvironment-driven resistance and synergises with BCL2 inhibition, providing a strong translational rationale for NIK-targeted strategies in RT and venetoclax-resistant CLL.

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