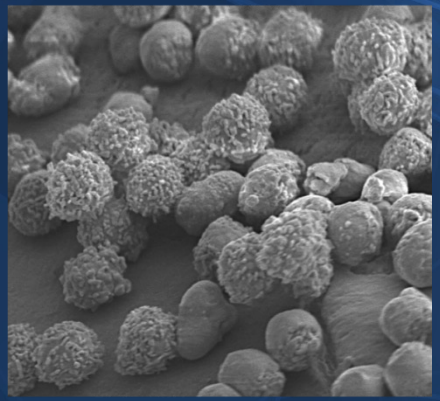


4th POSTGRADUATE

CLL Conference

Bologna
November 13-14
2023

Royal Hotel Carlton

President:
Pier Luigi Zinzani

Trafficking of CLL cells to and from the microenvironmental niche

Chris Pepper

brighton and sussex medical school

Disclosures of Chris Pepper

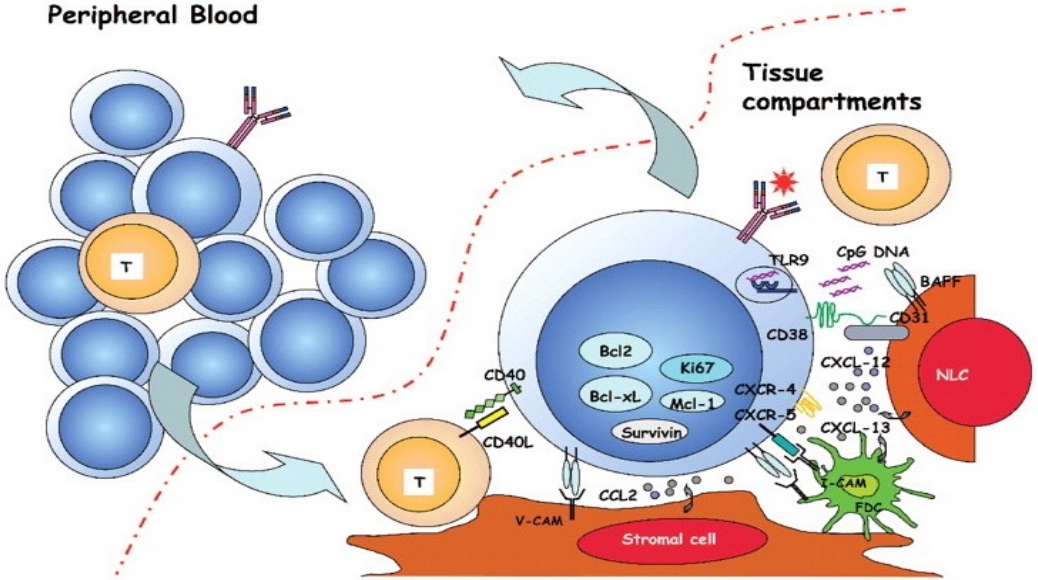
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

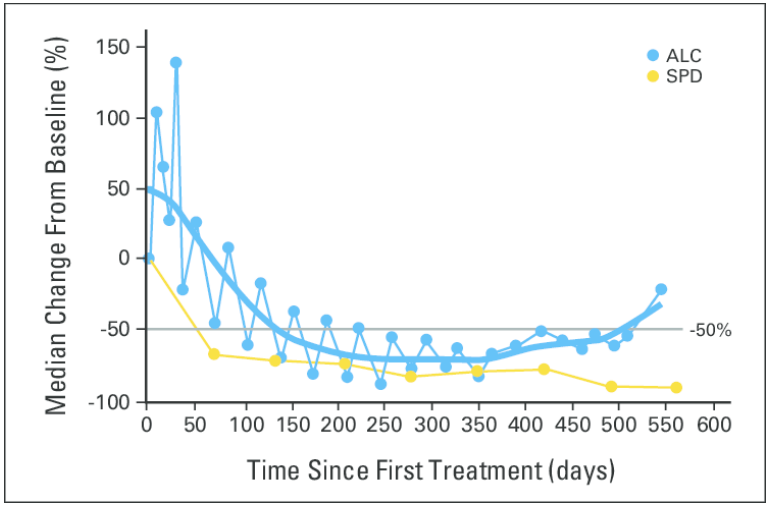
I have no relevant commercial disclosures

Outline

- Why is CLL cell trafficking important?
- What are the key molecular players involved in CLL cell trafficking?
- CLL signalling is not just about the BCR!
- Overcoming resistance by inhibiting migration and targeting CLL cells in the lymphoid niche

The unexpected class effect of BTK and PI3K targeted drugs tell us that trafficking to and from the lymphoid tissues really matters in CLL

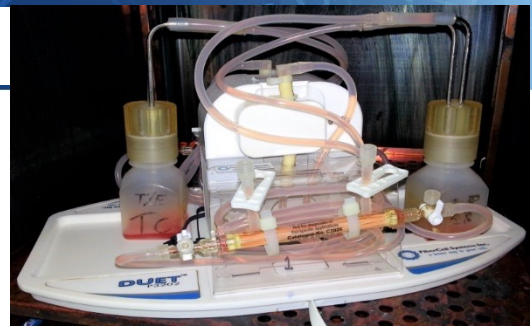


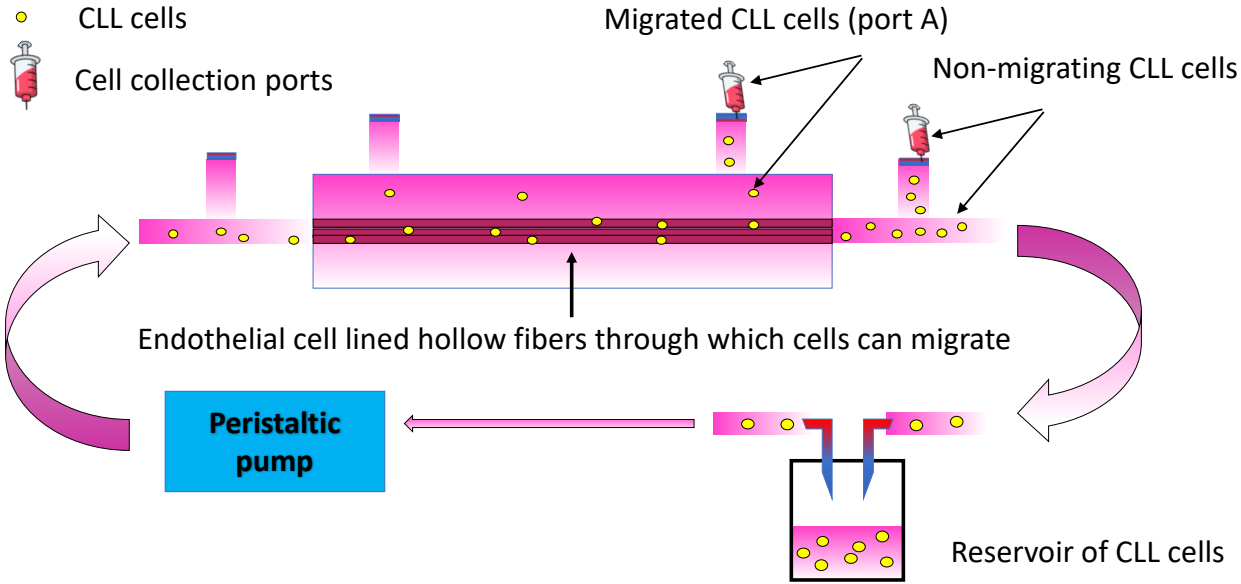


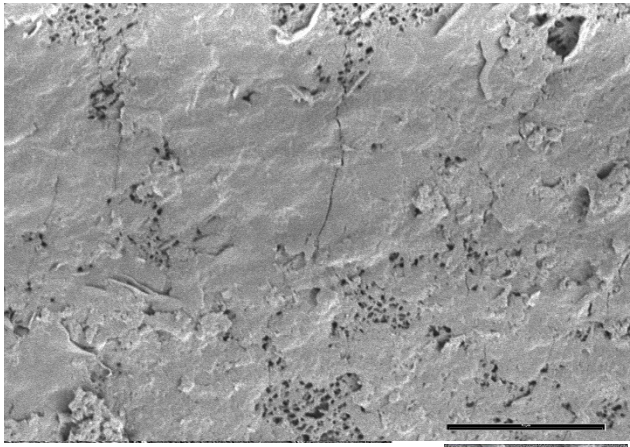
- Tissue redistribution - out of the lymph nodes into the peripheral blood
- These new peripheral blood CLL cells are enriched for $CD5^{bright}CXCR4^{dim}$
- **BUT** not all CLL cells leave the nodes

What are the key molecular drivers of CLL cell trafficking?

What are the key molecules involved in CLL cell trafficking?



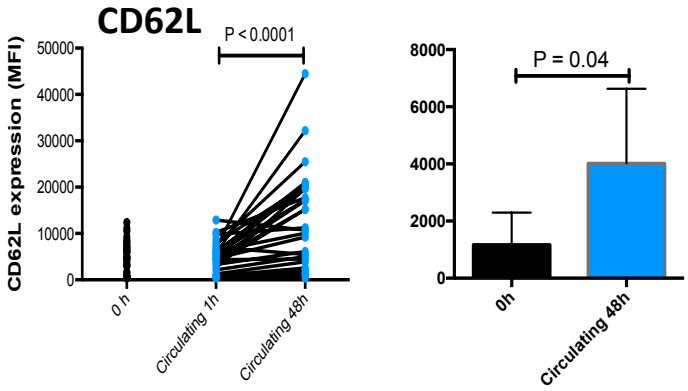


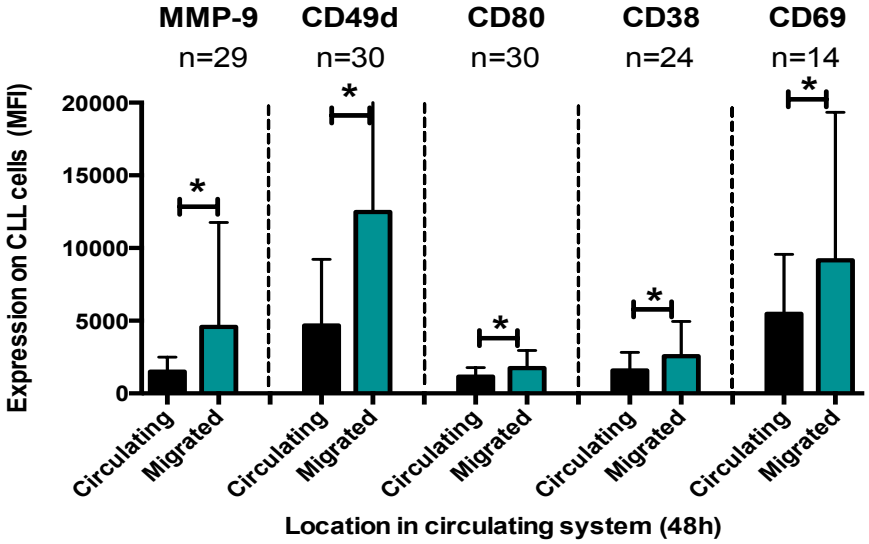


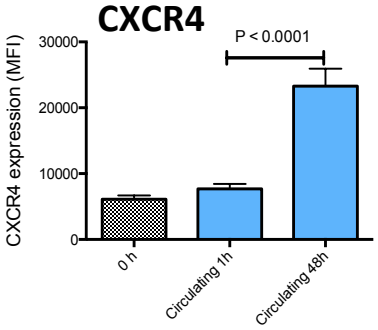
Walsby et al., Blood 2014

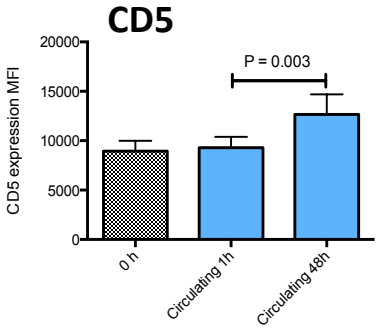
Distinct phenotype of CLL cells that migrate

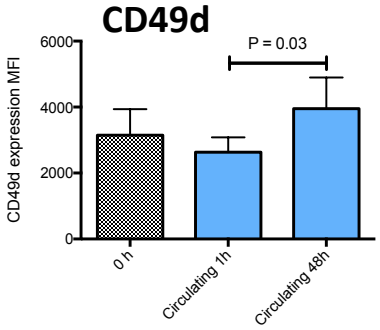
Phenotypic changes induced under shear

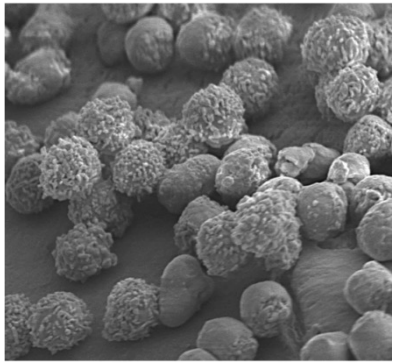






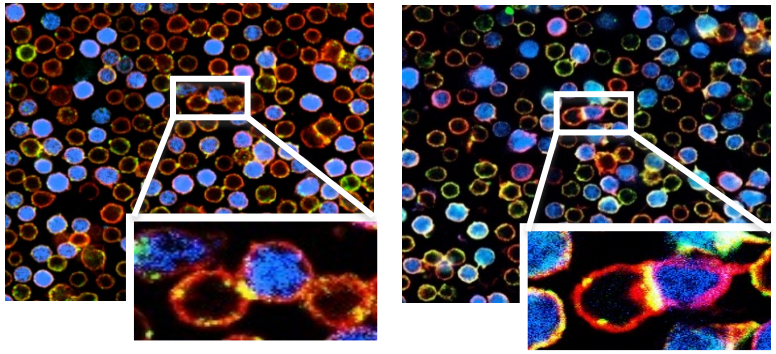


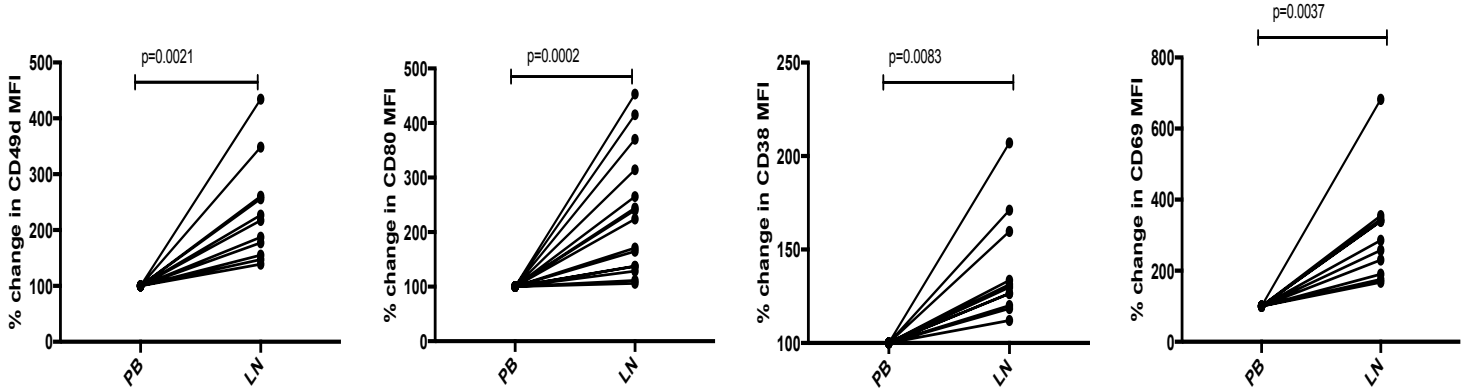




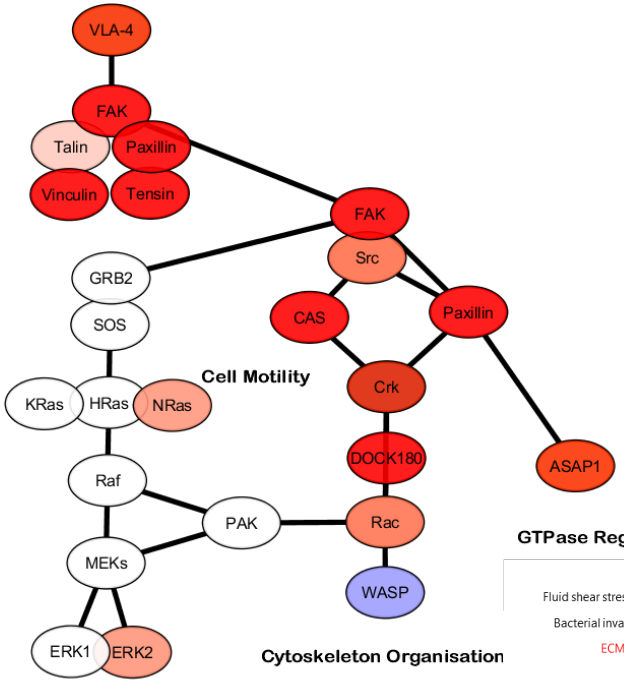
Pasikowska et al., Blood 2016

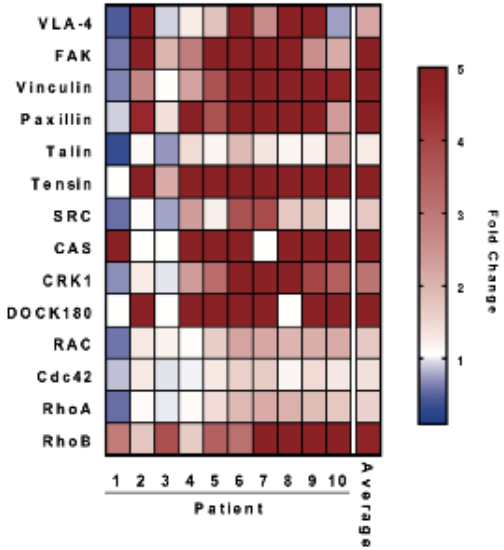
Lymph node-derived CLL cells have the same phenotype

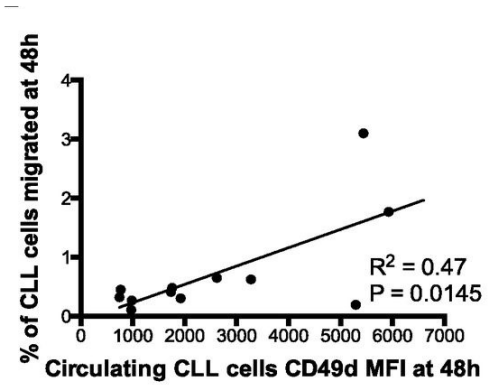


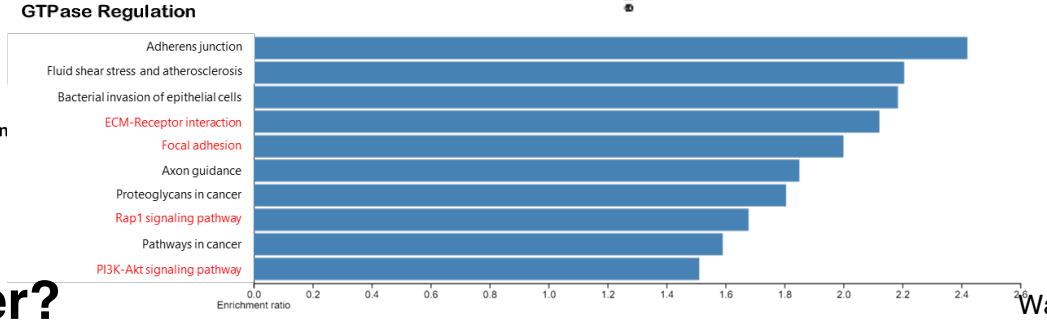


Migration leads to the transcriptional activation of CD49d (ITGA-4)





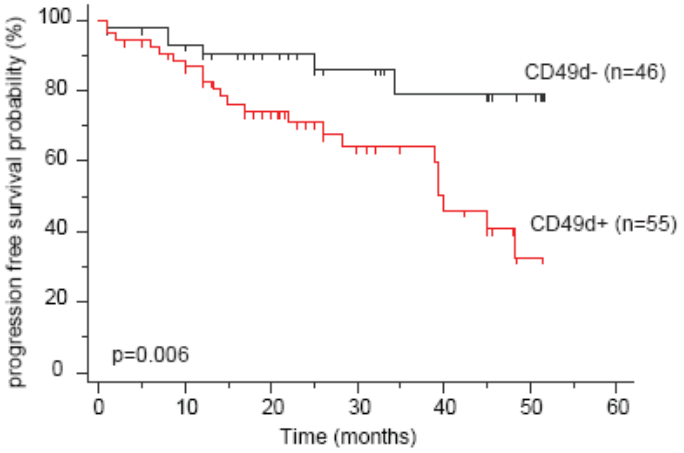


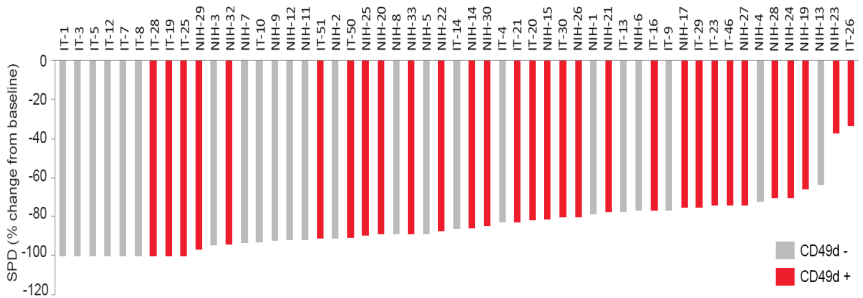


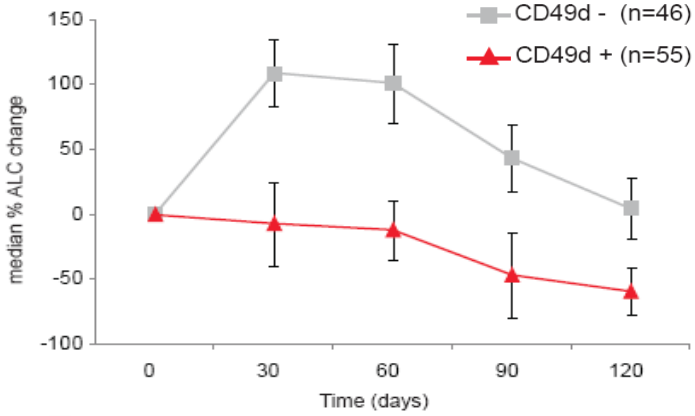
Why does this matter?

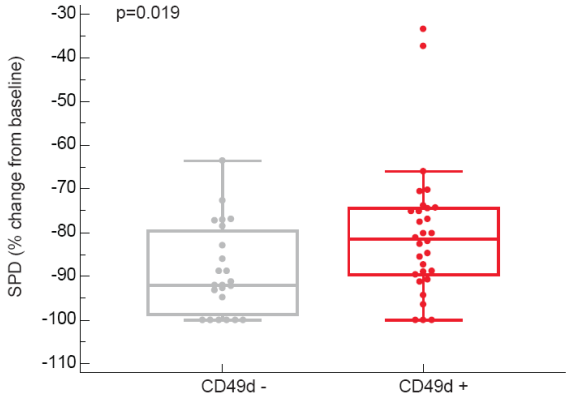
Walsby et al. Blood, 2014
Burley et al. (Cancers 2022)

CD49d expression predicts inferior response to Ibrutinib





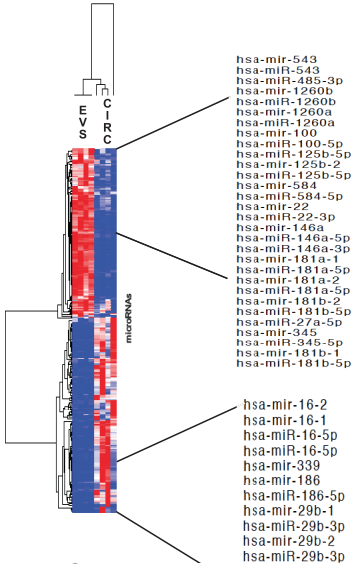




Poor response is associated with reduced tissue redistribution post ibrutinib

Tissino et al. J Exp Med, 2018

Migration also alters the miRNome of CLL cells

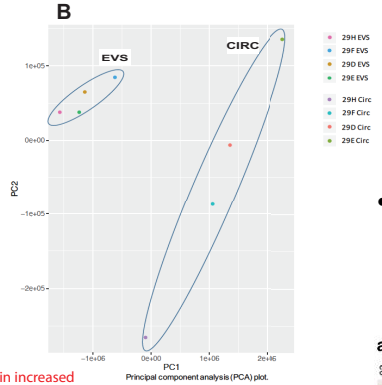


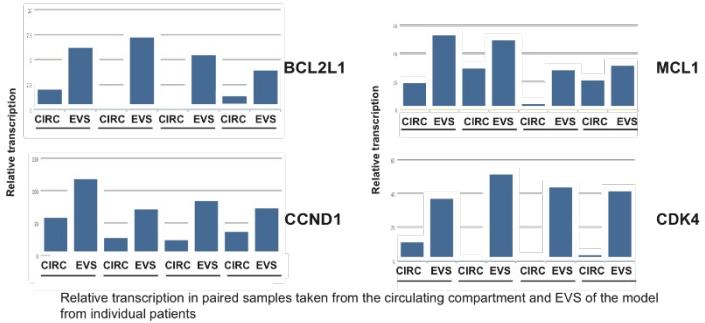
hsa-mir-543
hsa-miR-543
hsa-miR-485-3p
hsa-mir-1260b
hsa-miR-1260b
hsa-mir-1260a
hsa-miR-1260a
hsa-mir-100
hsa-miR-100-5p
hsa-miR-125b-5p
hsa-mir-125b-2
hsa-miR-125b-5p
hsa-mir-584
hsa-miR-584-5p
hsa-mir-22
hsa-miR-22-3p
hsa-mir-146a
hsa-miR-146a-5p
hsa-miR-146a-3p
hsa-mir-181a-1
hsa-miR-181a-5p
hsa-mir-181a-2
hsa-miR-181a-5p
hsa-mir-181b-2
hsa-miR-181b-5p
hsa-miR-27a-5p
hsa-mir-345
hsa-miR-345-5p
hsa-mir-181b-1
hsa-miR-181b-5p

Over expression promotes invasion, migration, angiogenesis and lymph node metasasis in other tumour models

hsa-mir-16-2
hsa-mir-16-1
hsa-miR-16-5p
hsa-miR-16-5p
hsa-mir-339
hsa-mir-186
hsa-miR-186-5p
hsa-mir-29b-1
hsa-miR-29b-3p
hsa-mir-29b-2
hsa-miR-29b-3p

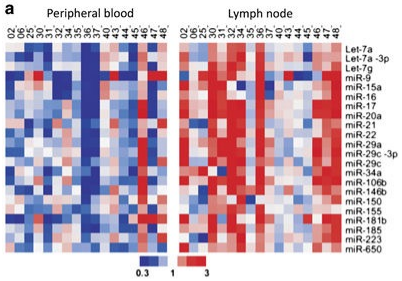
Repression results in increased expression of pro-survival BCL2 family genes and adhesion molecules



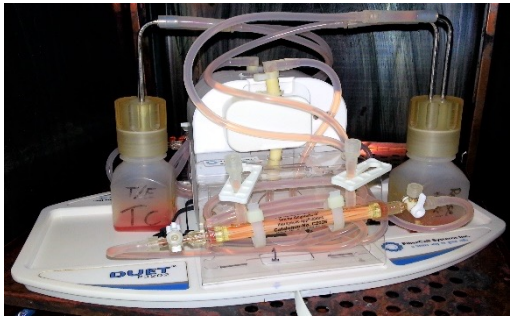


Relative transcription in paired samples taken from the circulating compartment and EVS of the model from individual patients

- miRNA signatures of *in vitro* migrated cells are similar to LN-derived CLL cells

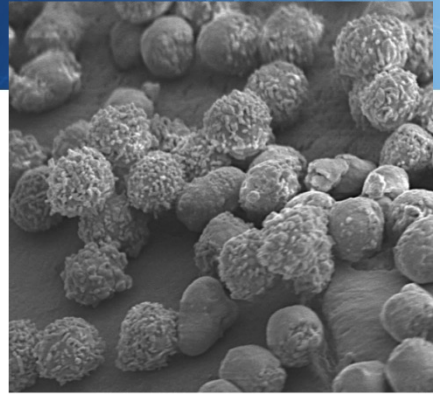


Leukemia, (2017)

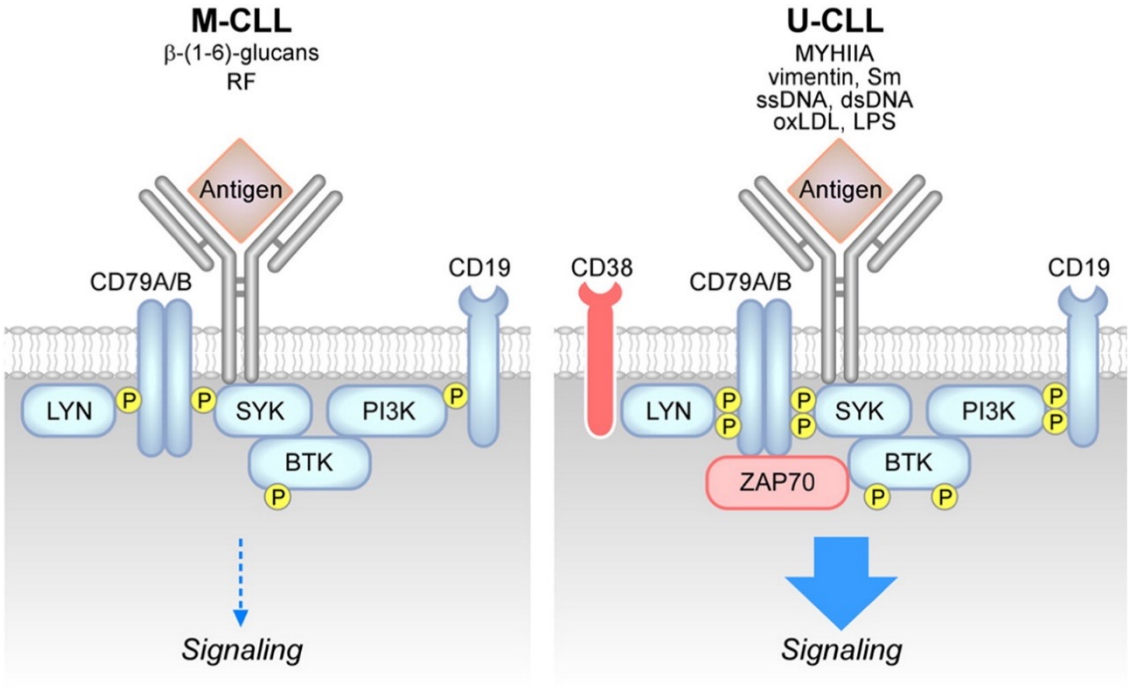


Use miRNomics /transcriptomics to identify possible new drug targets e.g.,

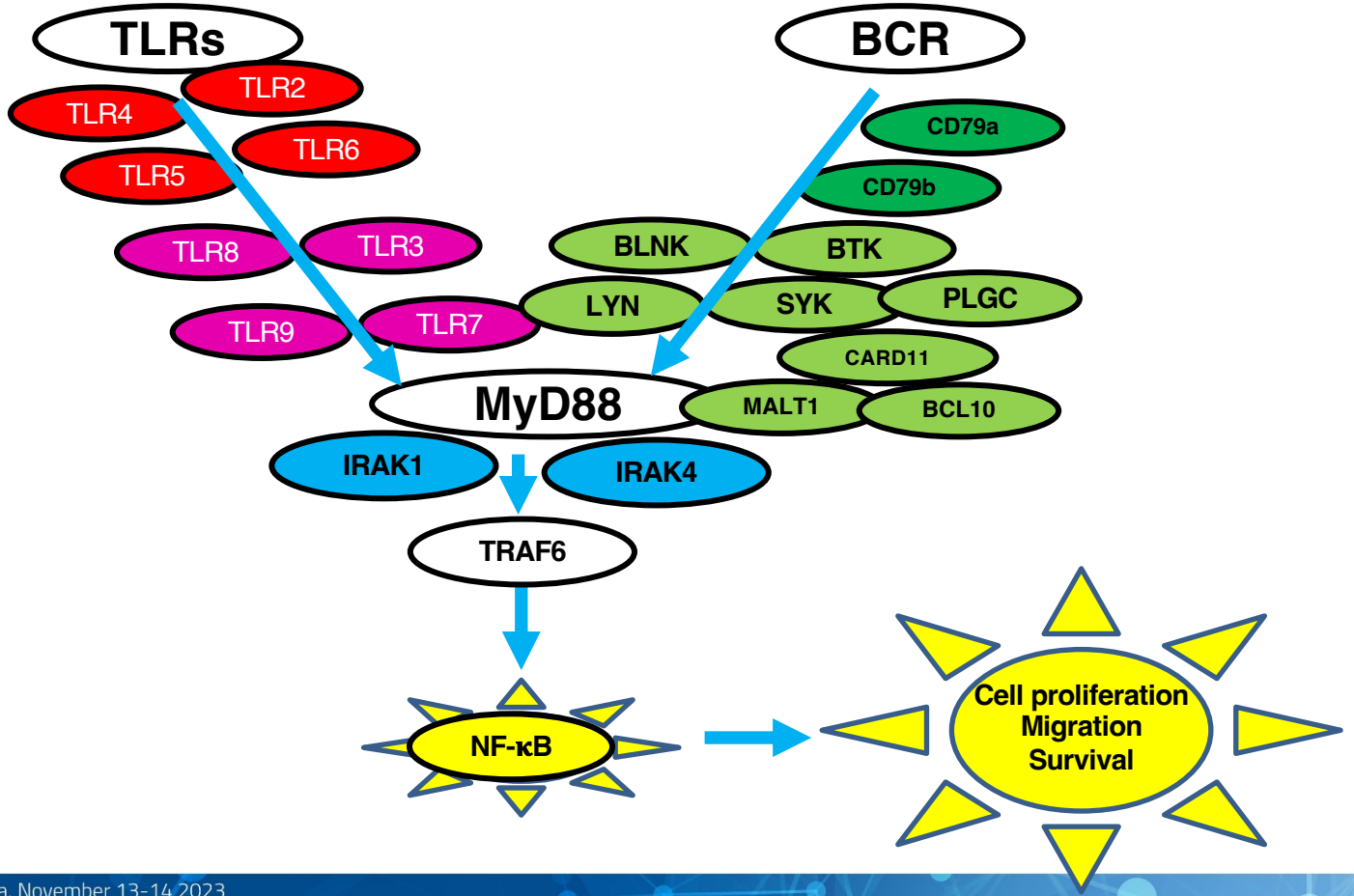
non-canonical NF-κB pathway is activated in migrated cells
(↑miR-322…↓TRAF3… ↑**NIK**)



CLL signalling is not just about the BCR!

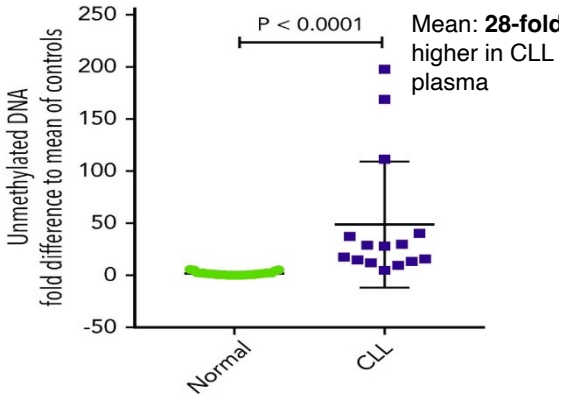


Toll-like receptor and B cell receptor signalling converge

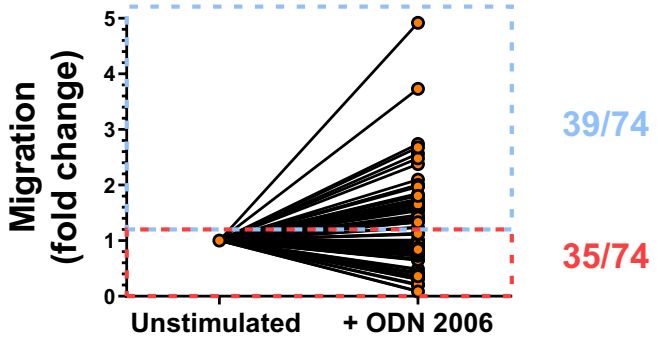


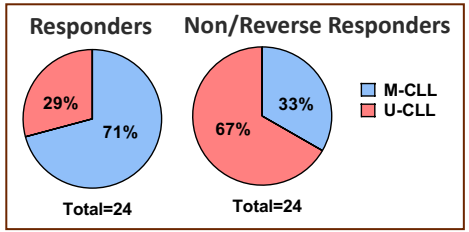
Evidence that TLR9 promotes CLL cell migration

CLL patient plasma contains the TLR9 ligand: unmethylated DNA



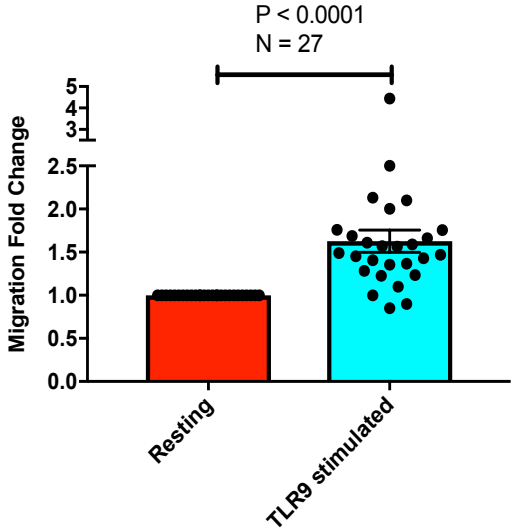
TLR9 activation induces a dichotomous migratory response in CLL patient samples





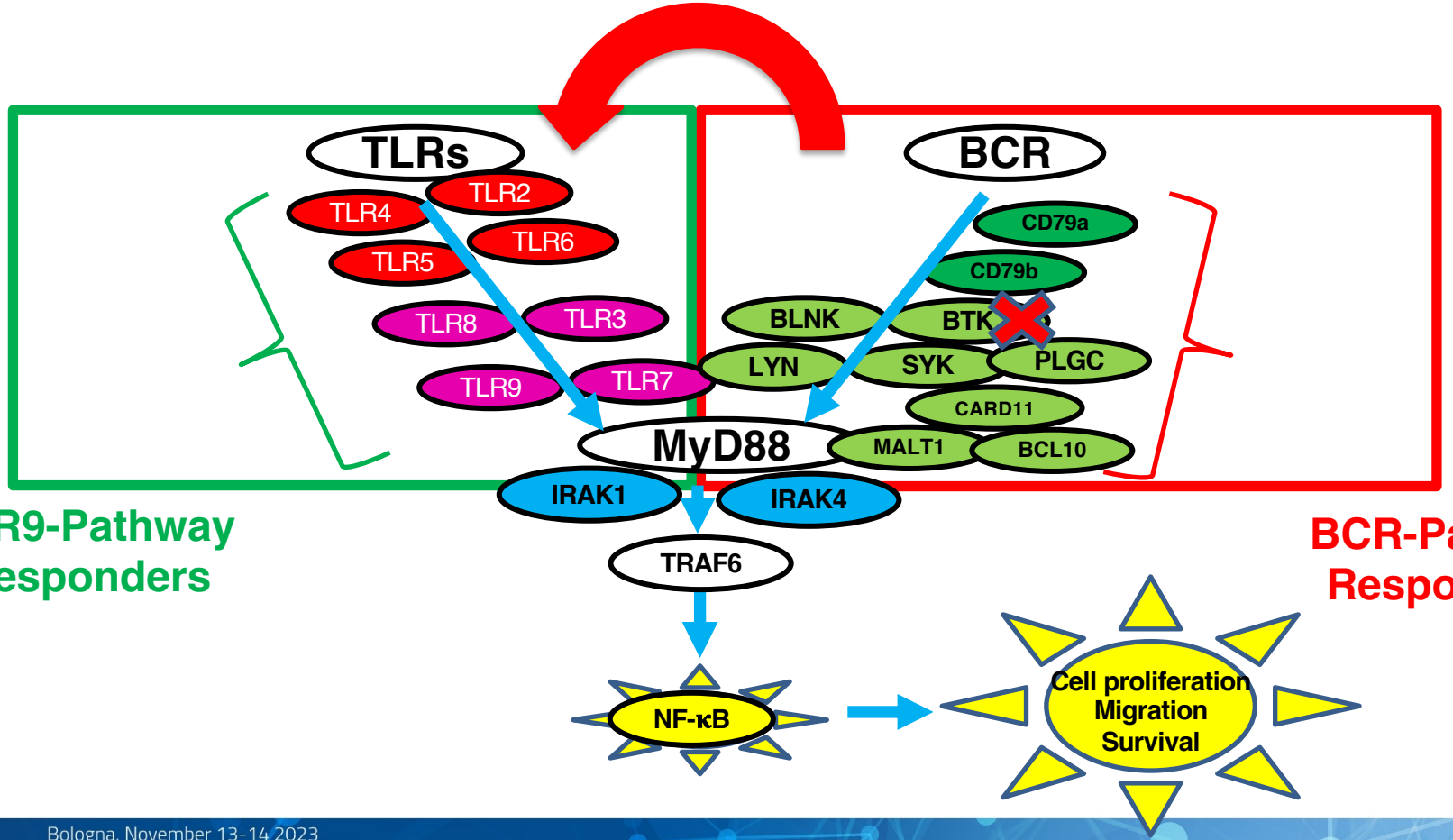
U-CLL cells exhibit high levels of **constitutive BCR signalling**

TLR9 stimulation of CLL cells increases migration

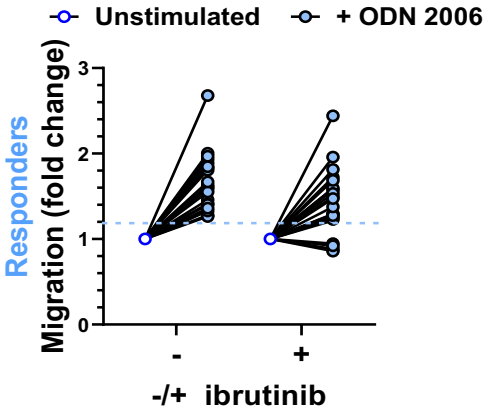


Kennedy et al., Blood, 2021

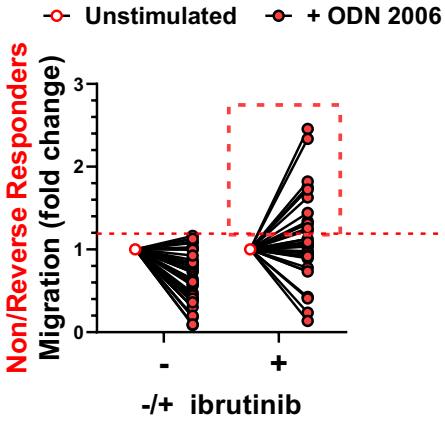
Can CLL cells switch signalling pathway as a drug resistance mechanism?



Different responses to TLR9 agonists +/- ibrutinib



Responders ✓



Sensitised ✓

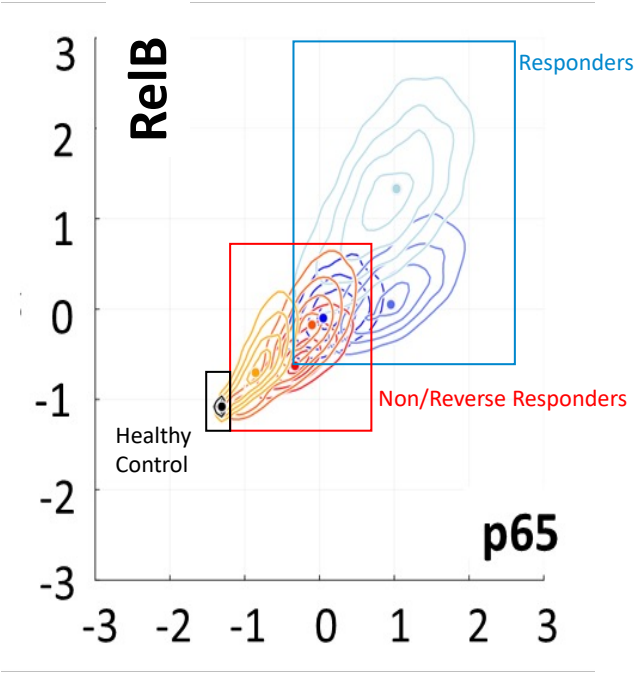
Non-sensitised ✗

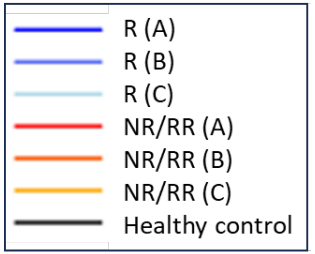
TLR9 signalling –
a potential mechanism of BTKi resistance

Responders and sensitised might benefit from dual targeting of BTKi and TLR9i

Unpublished data: Emma Kennedy

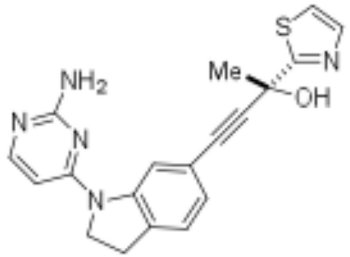
R and NR/RR samples have distinct 'NF-κB fingerprints'



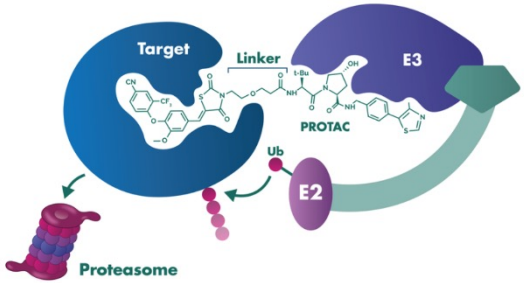


CONCLUSIONS

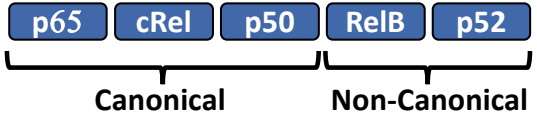
1) TLR9 activation may **promote CLL cell migration and BTKi resistance** in subgroups of CLL patients
2) We are evaluating samples from the FLAIR trial to establish whether our assay can predict response to ibrutinib
3) We are exploring a subunit-specific approach to NF-κB inhibition, to block BCR/TLR9 signalling in **Responder** and **'Sensitised'** patient subgroups



Small molecule inhibitors and PROTACs

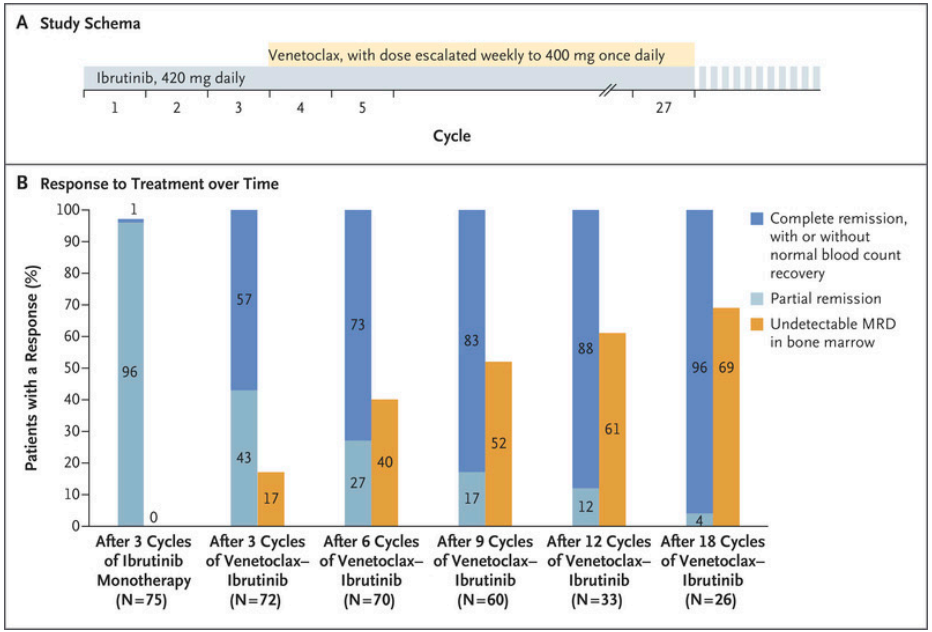


NF-κB subunits



Overcoming resistance by inhibiting migration and targeting CLL cells in the lymphoid niche

Ibrutinib and venetoclax are very effective



BUT…

REGULAR ARTICLE

blood advances

Microenvironmental agonists generate de novo phenotypic resistance to combined ibrutinib plus venetoclax in CLL and MCL

Kallesh D. Jayappa,[1,2] Craig A. Portell,[3,4] Vicki L. Gordon,[1,2] Brian J. Capaldo,[5] Stefan Bekiranov,[6] Mark J. Axelrod,[6] L. Kyle Brett,[7] Julia D. Wulfkuhle,[8] Rosa I. Gallagher,[8] Emanuel F. Petricoin,[8] Timothy P. Bender,[1,2] Michael E. Williams,[3,4] and Michael J. Weber[1,2,4]

[1]Department of Microbiology, Immunology, and Cancer Biology, University of Virginia, Charlottesville, VA; [2]Beirne B. Carter Center for Immunology Research, Charlottesville, VA; [3]Division of Hematology/Oncology, School of Medicine, [4]Cancer Center, and [5]Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA; [6]Gilead Sciences, Seattle, WA; [7]Medical Oncology, Utica Park Clinic, Tulsa, OK; and [8]Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA

blood

Regular Article

LYMPHOID NEOPLASIA

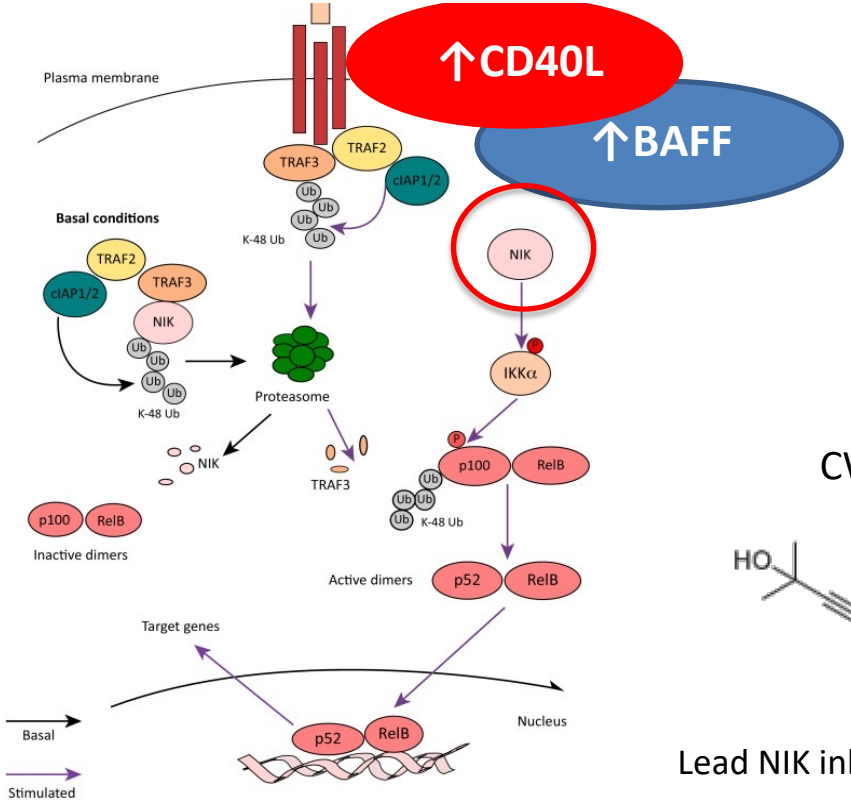
TLR9 expression in chronic lymphocytic leukemia identifies a promigratory subpopulation and novel therapeutic target

Emma Kennedy,[1,*] Eve Coulter,[2,3,*] Emma Halliwell,[4] Nuria Profitos-Peleja,[5] Elisabeth Walsby,[4] Barnaby Clark,[2] Elizabeth H. Phillips,[3,6] Thomas A. Burley,[1] Simon Mitchell,[1] Stephen Devereux,[3] Christopher D. Fegan,[3] Christopher I. Jones,[6] Rosalynd Johnston,[10] Tim Chevassut,[1,10] Ralph Schulz,[11] Martina Seiffert,[11] Angelo Agathanggelou,[12] Ceri Oldreive,[12] Nicholas Davies,[12] Tatjana Stankovic,[2] Triantafillos Liloglou,[13] Chris Pepper,[1,†] and Andrea G. S. Pepper[1,†]

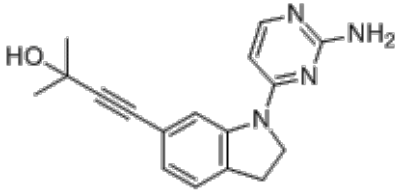
The focus of our current research is to understand how TLR9 signalling induces resistance to ibrutinib and venetoclax and develop TLR9 and non-canonical NF-κB inhibitors targeting specific NF-κB components

Activation of non-canonical NF-κB signalling via TLR9 activation

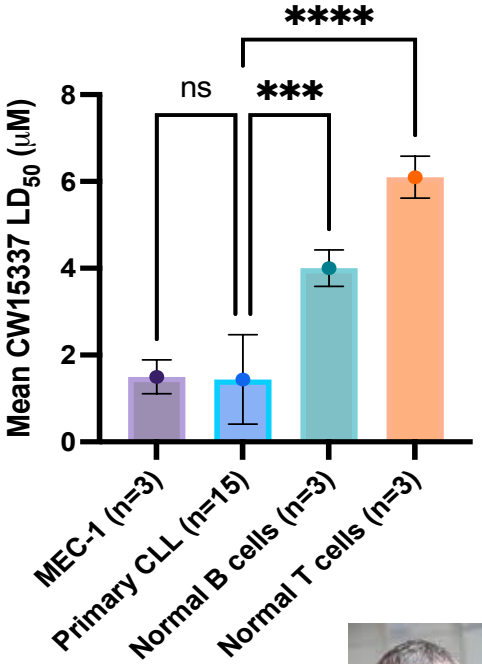
Non-canonical NF-κB activated by migration and is increased in the lymphoid tissues



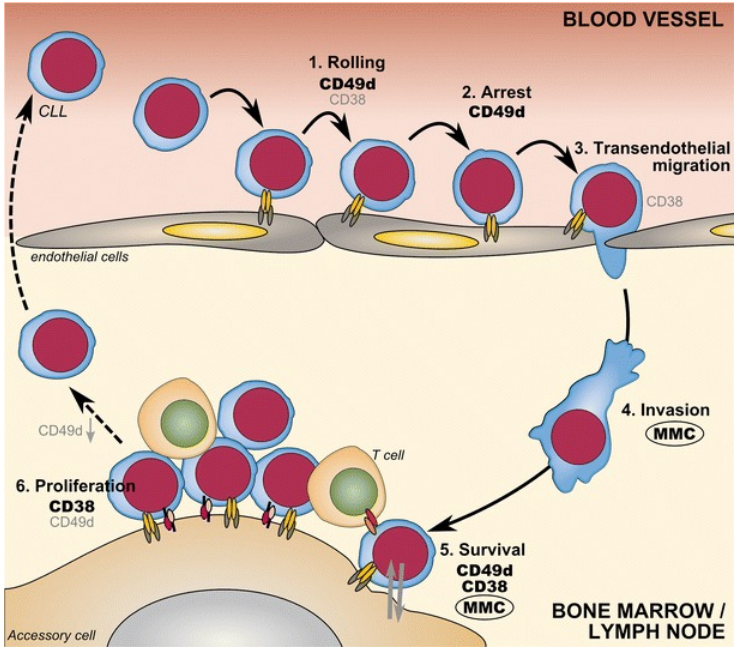
CW15337

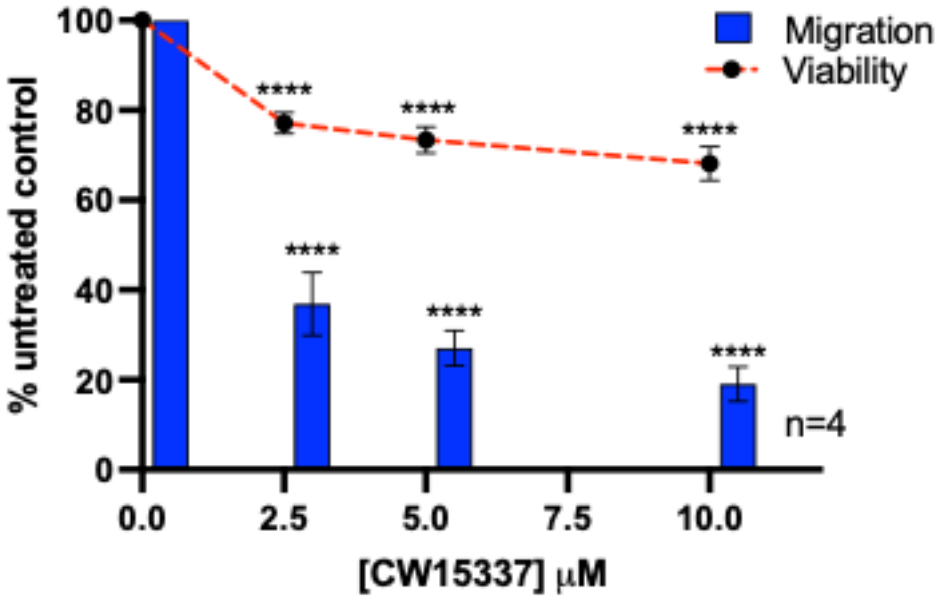


Lead NIK inhibitor (Ki = 2nM)

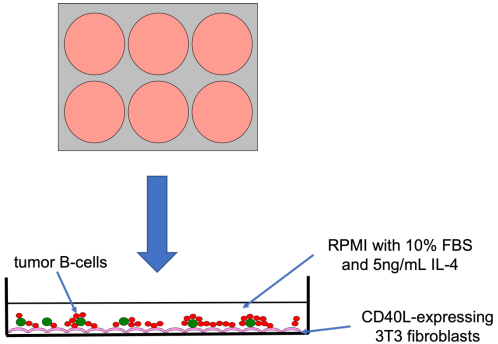


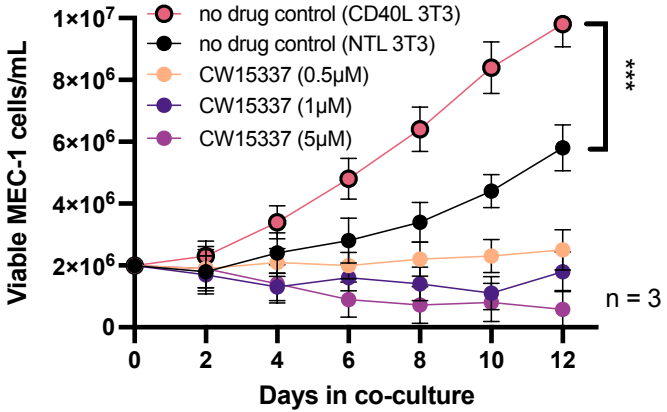
NIK inhibition blocks CLL cell migration



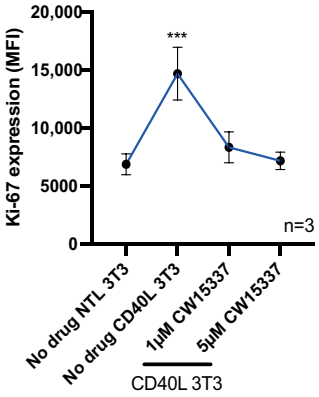


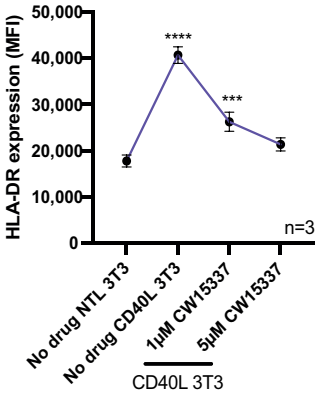
So, what about NIK inhibition in the lymphoid niche?

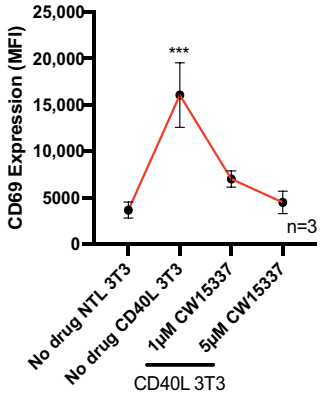


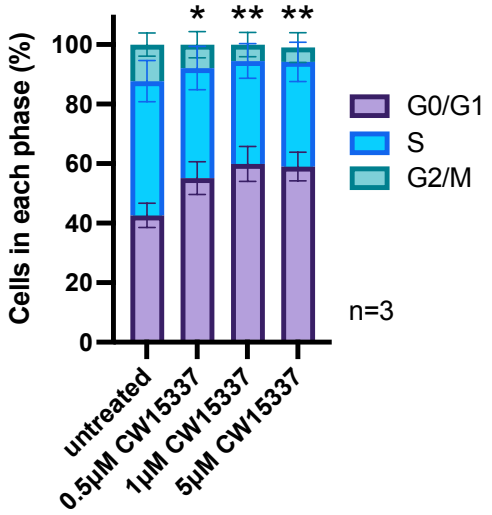


Co-culture on CD40L-expressing cells drives MEC-1 cell activation and proliferation, which is reversed by the addition of CW15337

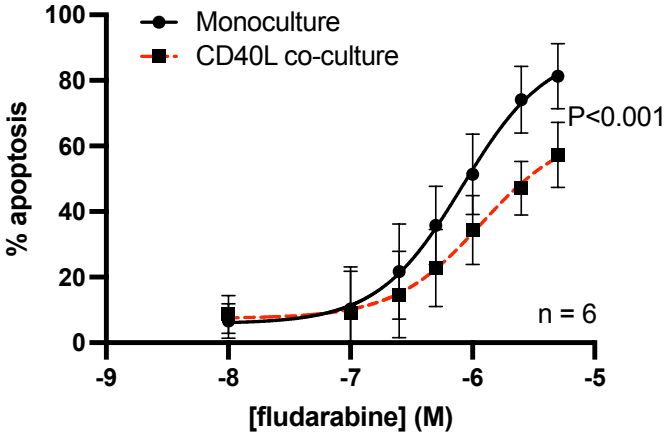


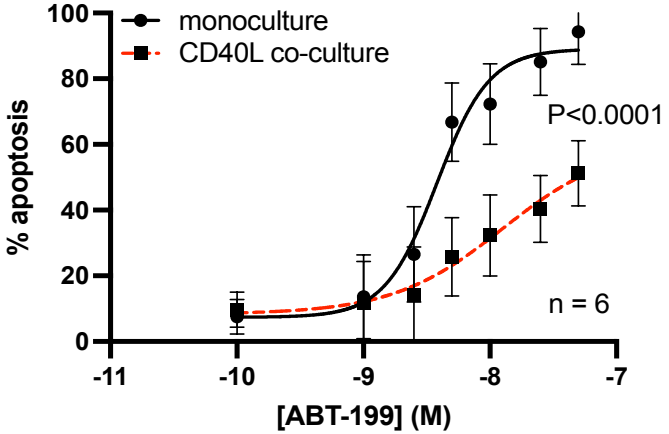


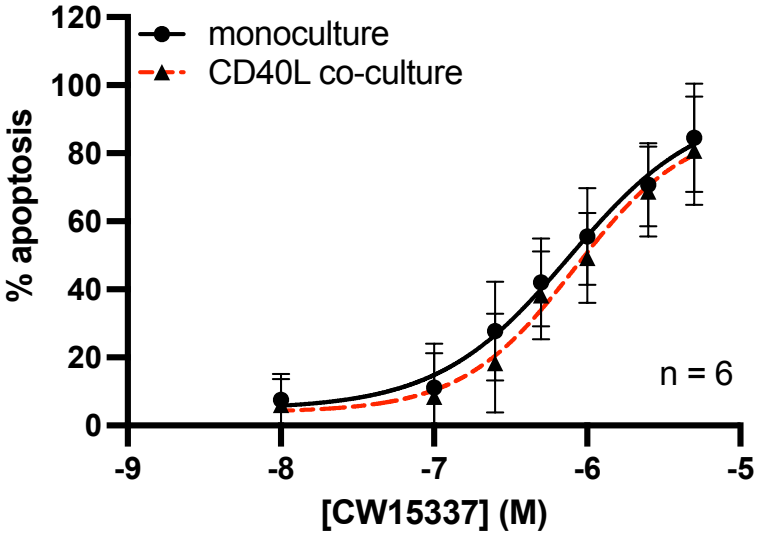




CD40L co-culture promotes resistance to Fludarabine and ABT-199



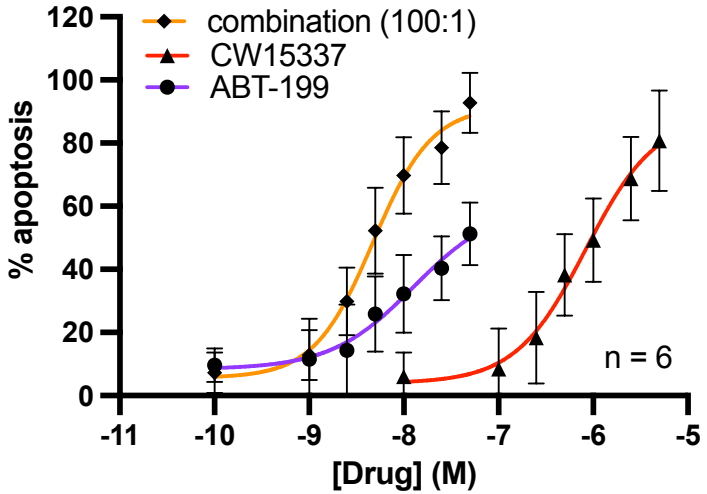


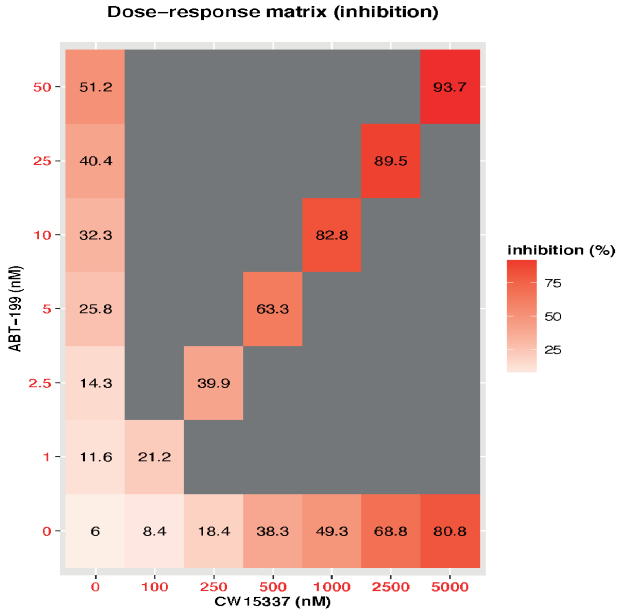


But this is not the case of CW15337

CW15337 resensitises CLL to venetoclax

CD40L co-culture

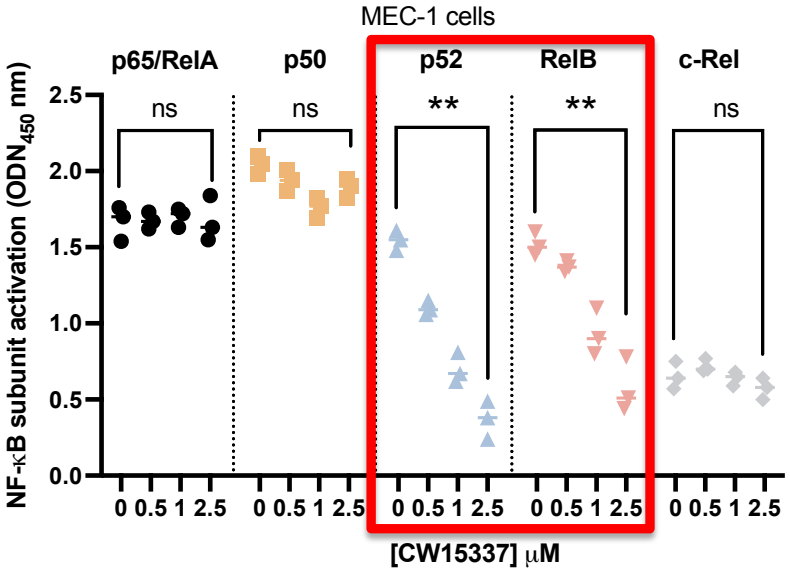


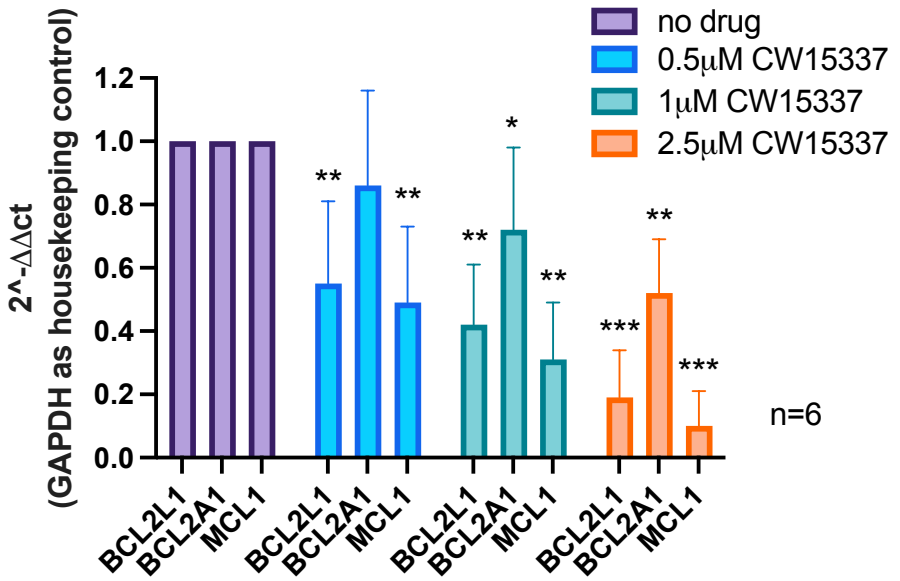


Drug combination	Synergy score	Most synergistic area score	Method
CW15337 - ABT-199	15.06	22.09	Bliss

NIK inhibition reverses CD40L-induced venetoclax resistance

CW15377 preferentially inhibits the non-canonical NF-κB





Final thoughts

- Treatment of CLL has come a long way in the last 20 years!
- Understanding tumour biology has accelerated the introduction of new and effective treatments
- Targeted agents are having a positive impact on CLL patients

  …But they are not curative and drug resistance is already starting to emerge
- Understanding how this resistance occurs is the key to overcoming it
- This is the focus of our research team at BSMS
  - Design and test new drugs that block migration and target tumour cells in the lymph nodes
  - TLR9 and non-canonical NF-κB subunits are two promising candidates

Blood cancer UK

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Brighton and Sussex University Hospitals NHS Trust

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