PO-01 Transcriptional dimensions provide a framework for describing tumor heterogeneity in CLL. Julie E. Feusier, Rosalie G. Waller, Michael J. Madsen, Brian Avery, Nicola J. Camp. University of Utah, Salt Lake City, UT.

Our goal is to define multiple informative transcriptome variables that capture sources of heterogeneity in chronic lymphocytic leukemia (CLL) cells, for flexible modeling in epidemiology and clinical studies. CD19+/CD5+ B-cells were sorted from whole blood of 227 CLL patients and RNA was sequenced on the HiSeq4000 or NovaSeq platforms as part of the ORIEN Avatar Initiative. Transcript-based read counts were generated from FASTQ files using Salmon. High-quality genes were selected and read counts internally normalized and corrected for batch effects using ComBat. Preprocessing resulted in a final set of 8,716 quality-controlled, autosomal, protein-coding genes. PCA was performed and, using a scree test, we selected 14 dimensions that represented 55.9% of the total variance across the CLL patients' transcriptomes. Fourteen quantitative, orthogonal CLL dimension variables were calculated for all 227 patients. By design, these CLL dimensions capture transcriptome variance and provide novel multi-gene expression biomarkers. We assessed whether these CLL transcriptome dimensions captured known clinically relevant molecular differences. First, we investigated associations with IGVH mutational status (determined using MiXCR). CLL dimension variables 1, 5, 6, and 8 predicted IGHV mutational status ($p=4.6x10-16$). Next, we investigated associations with ZAP70 and CD38 biomarkers, calculated by their expression in the RNA sequencing data using a separate pipeline and correcting for batch effects by ComBat (neither gene was in the 8,716 genes retained for PCA). CLL dimension variables 3, 5, 6, 7, and 8 significantly predicted Zap70 expression ($p=1.6x10-38$). CLL dimension variables 2, 3, 5, and 6 significantly predicted CD38 expression ($p=3.1x10-31$). Transcriptome dimension variables provide a flexible intrinsic framework to describe heterogeneity across CLL patients. We have shown that our transcriptome dimensions capture IGHV mutational status and ZAP70 and CD38 expression, all biomarkers for prognosis. Future work will include exploring the ability of the 14 dimensions to capture other known important molecular markers for CLL, including somatic deletion of 17p deletion, somatic mutational patterns, microsatellite instability, and previously described expression-based subgroups. Transcriptome dimensions are designed for utility as predictor variables, alongside other covariates, in parametric modeling, and have the potential to improve both epidemiology and clinical studies.

PO-04 Noncoding mutations in mantle cell lymphoma disrupt regulation of HNRNPH1 by alternative splicing. Krysta M. Coyle, Quratulain Qureshi, Prasath Pararajalingam, Nicole Thomas, Timothy E. Audas, Ryan D. Morin. Simon Fraser University, Burnaby, BC, Canada.

Non-Hodgkin lymphomas (NHL) are a collection of cancers with each malignancy having distinct clinical management and prognosis. Mantle cell lymphoma (MCL) is particularly genetically heterogeneous and is considered incurable. Through a combination of exome, genome, and targeted sequencing of MCL tumors, we identified recurrent mutations in HNRNPH1 (heterogeneous nuclear ribonucleoprotein H1). These mutations are largely intronic or silent and are associated with a putative cis regulatory region involving a single exon. In RNA-seq data from matched cases, we identified variable representation of two distinct HNRNPH1 isoforms. Based on the reading frame of the affected exons, canonical splicing is predicted to produce a functional protein, while alternative splicing introduces a premature termination codon leading to nonsense-mediated decay. We observed a significantly higher proportion of canonical transcripts in MCL tumors bearing HNRNPH1 mutations, leading us to conclude that mutations in HNRNPH1 significantly alter its splicing. Furthermore, increased canonical splicing results in higher HNRNPH1 protein abundance as determined by immunohistochemical analysis of an MCL tissue microarray. HNRNPH1 mutation status and splicing ratio are associated with shorter survival of MCL patients. We developed an in vitro reporter minigene and introduced three specific mutations corresponding to the patient-identified mutations in HNRNPH1. These mutations lead to an increase in canonical splicing and translation of the HNRNPH1 minigene-derived peptide. Additionally, when HNRNPH1 is overexpressed, we observe a decrease of this peptide, implicating HNRNPH1 in the regulation of its own splicing. This is supported by preliminary data indicating that HNRNPH1 binds its own RNA. Beyond its own splicing, HNRNPH1 is likely also involved in the splicing of additional splicing factors. Overexpression of HNRNPH1 affects the splicing of SRSF3 and HNRNPDL, and many other targets are likely to be identified. This work elucidates a functional role for recurrent noncoding HNRNPH1 mutations and implicates HNRNPH1 expression and splicing of its downstream targets in lymphomagenesis. We continue to explore trans regulatory targets of HNRNPH1 using in vitro and cell-based models. While splicing is a growing field of interest in lymphoma biology, the unique pattern and consequences of these largely silent mutations specifically implicate alternative splicing as an oncogenic mechanism in MCL.

PO-05 TET2 deficiency alters the epigenome of germinal center B cells, contributing to lymphoma formation. Pilar M. Dominguez[1], Wojciech Rosikiewicz[2], Xiaowen Chen[2], Hussein Ghamlouch[3], Said Aoufouchi[3], Olivier A. Bernard[3], Ari M. Melnick[4], Sheng Li[2], Ricky W. Johnstone[1]. [1]Peter MacCallum Cancer Centre, Melbourne, VIC, Australia, [2]The Jackson Laboratory for Genomic Medicine, Farmington, CT, [3]Gustave Roussy, Villejuif, France, [4]Weill Cornell Medicine, New York, NY.

Diffuse large B-cell lymphomas (DLBCLs) are aggressive tumors derived from germinal center (GC) B cells. Despite progress in the treatment of DLBCL, approximately 40% of patients relapse or are refractory to the treatment, which usually leads to fatality. DLBCLs are characterized by aberrant DNA methylation and this feature correlates with poor clinical outcome. We and others have shown that deregulated epigenetic mechanisms contribute to lymphoma formation. Particularly, we have demonstrated that TET2, an enzyme that converts methylcytosine (mC) into hydroxymethylcytosine (hmC) and is mutated in ~10% of DLBCLs, is a B-cell tumor suppressor. GC-specific deletion of TET2 (Cg1Cre/Tet2-/-) resulted in accelerated lymphomagenesis in DLBCL mouse models driven by BCL6 overexpression, with 100% Cg1Cre/Tet2-/-;ImBcl6 mice developing lymphoma at 7 months compared to only 50% in ImBcl6 control mice. In addition, TET2 deletion in hematopoietic stem cells (VavCre/Tet2-/-) induced GC B-cell hyperplasia (B220+GL7+CD95+ cells; 10% VavCre/Tet2-/- vs 5% VavCre/Tet2+/+), promoting malignant transformation. Further analysis of the GC reaction revealed that TET2-deficient GC B cells displayed anomalous patterns of DNA methylation. GC B cells from VavCre/Tet2-/- mice presented 1) focal loss of hmC—using hMeDIPseq—with 25,000 differentially hydroxymethylated regions (DHMR) lost compared to VavCre/Tet2+/+ GC B cells and 2) increased mC—using RRBS—with almost 11,000 differentially methylated cytosines (DMCs), 84% hypermethylated, compared to VavCre/Tet2+/+ GC B cells. TET2-mediated reduction of hmC and hypermethylation affected enhancers and promoters, respectively, of genes mediating GC exit and terminal differentiation of GC B cells, especially those regulated at enhancers by the opposing functions of CREBBP and HDAC3. We are currently investigating the potential cooperative role between TET2-mediated hmC and CREBBP-mediated H3K27Ac, supported by reduced H3K27Ac at enhancers activated by CREBBP in VavCre/Tet2-/- GC B cells and mutual exclusion between TET2-mutant and CREBBP-mutant primary DLBCL. RNA sequencing analysis revealed that the genes epigenetically regulated by TET2 were aberrantly repressed in VavCre/Tet2+/+ GC B cells, explaining the observed GC hyperplasia in TET2-deficient GC B cells since these genes control the differentiation of GC B cells into plasma cells. Importantly, TET2-mutant DLBCL primary samples display a similar repressive transcriptional signature associated with GC B-cell terminal differentiation. Our data show how TET2-induced epigenetic changes

contribute to lymphoma development and highlight the multilayered nature of the epigenome, which can be therapeutically exploited. We are evaluating the therapeutic potential in TET2-mutant DLBCL of a combinatorial therapy consisting of DNA methylation inhibitors (DNMTi), to revert hypermethylation at promoters, plus specific HDAC3 inhibitors, to compensate for the loss of hmC at enhancers.

PO-06 Ultradeep sequencing of classical Hodgkin lymphoma (cHL) identifies recurrent somatic mutations and demonstrates the production of reproducible data from rare malignant cells. Felicia Gomez, Matthew Mosior, Zachary Skidmore, Alina Schmidt, Fernanda Rodrigues-Martins, Kilannin Krysiak, Cody Ramirez, Eric Duncavage, Grace Triska, Lee Trani, Nancy Bartlett, Amanda Cashen, Neha Mehta-Shah, Friederike Kreisel, Malachi Griffith, Todd Fehniger, Obi Griffith. Washington University School of Medicine, St. Louis, MO.

Purpose/Background: cHL patients who receive standard therapy have a high rate of event-free and overall survival. However, some patients (~10%) will be refractory to initial therapy and up to 1/3 will relapse. Thus, improved methods of prognostication and new treatment targets are needed. High-throughput sequencing can identify recurrent somatic mutations that drive lymphomagenesis and impact treatment response. However, Hodgkin-Reed-Sternberg (HRS) cells have a low (~1%) abundance in cHL biopsies, creating a challenge for comprehensive and accurate detection of somatic mutations in bulk lymphoma biopsies. Genomic studies of cHL have characterized HRS somatic mutations through the analysis of malignant cells obtained using purification techniques, cell-free DNA, or DNA amplified through whole-genome amplification. We hypothesized that ultradeep sequencing of bulk lymphoma biopsies provides a more accessible approach to HRS characterization while also creating robust and reproducible data.

Methods: We performed exome sequencing on 32 fresh frozen samples from 31 cHL patients obtained prior to treatment (27) or after relapse (4) with paired normal skin samples (31). The Illumina HiSeq platform (2 x 150bp reads) was used with multiple independent library constructions and a 1,000X median coverage goal. Sequence data were aligned to GRCh38. SNVs and INDELs were called using multiple algorithms. We employed several variant filtering strategies, including manual review, to remove common polymorphisms and false positives. Because we discovered mutations with VAFs close to the platform error rate (~1%), we used an orthogonal sequencing strategy (Haloplex) to validate all somatic variants.

Results: We observed 4,020 somatic variants. On average, we observed 32 protein-coding mutations/case, excluding one hypermutated case in which 3,084 variants were observed. We identified a potential loss-of-function insertion in *MSH6* that could explain the hypermutated phenotype. We achieved a 99% validation rate across the cohort for somatic variants discovered in exomes. We confirmed known recurrently mutated cHL genes (e.g., *SOCS1* [43%], *STAT6* [20%], *TNFAIP3* [40%]). We identified several significantly recurrent mutated genes not well characterized in cHL, including *IGLL5* [26%] and *IL4R* [13%]. All *IL4R* mutations are potential loss-of-function mutations that could result in greater activation of STAT6 through ablation of ITIM negative modulation. We identified an enrichment

of *SOCS1* and *IGLL5* mutations that is likely the result of aberrant somatic hypermutation. Pathway analysis also identified an enrichment of mutations in MAPK pathways.
Conclusion: These data suggest that cHL somatic mutations can be confidently identified via ultradeep exome sequencing without cell purification. We show that cHL genomes harbor somatic variation that inform new targets for treatment and prognostication.

PO-07 The FLI1 direct target ASB2 promotes NF-KB pathway activation in diffuse large B-cell lymphoma of the germinal center B-cell type. Giulio Sartori[1], Sara Napoli[1], Luciano Cascione[1], Elaine Y.L. Chung[1], Valdemar Priebe[1], Alberto J. Arribas[1], Andrea Rinaldi[1], Michela Dall'Angelo[1], Mattia Forcato[2], Silvio Bicciato[2], Margot Thome[3], Francesco Bertoni[1]. [1]Institute of Oncology Research, Faculty of Biomedical Sciences, USI, Bellinzona, Switzerland, [2]University of Modena and Reggio Emilia, Modena, Italy, [3]Department of Biochemistry, University of Lausanne, Epalinges, Switzerland.

Introduction: Gains affecting chromosome 11 are recurrent events in lymphomas. The 11q24.3 gain occurs in 25% of diffuse large B-cell lymphoma (DLBCL), and it is associated with the overexpression of two ETS transcription factors, ETS1 and FLI1 (Blood 2013). Here, we have focused on the latter to identify the network of FLI1 regulated genes in GCB DLBCL.

Methods: GCB and ABC cell lines. Gene expression data were obtained from public datasets GSE98588, phs001444.v2.p1, GSE95013, GSE10846, and EGAS00001002606. Anti-FLI1 antibody - ChIP Grade (ab15289). ChIP-Seq for FLI1 paired with transcriptome analysis (RNA-Seq) after FLI1 silencing (siRNA) was performed. Sequencing was carried out using the NextSeq 500 (Illumina). Detection of peaks was analyzed using HOMER (v2.6); differential expressed genes were identified using moderated t-test (limma R-package) and functionally annotated with g:Profiler.

Results: The analysis of DLBCL cell lines showed that FLI1 protein levels were higher in GCB (n=12) than ABC (n=8) cell lines and was more commonly expressed at high levels in GCB (n= 414) than ABC (n= 518) DLBCL clinical specimens. Integration of identified binding sites from ChIP-Seq with RNA-Seq from GCB DLBCL cell lines (OCI-Ly1 and VAL) with genetically silenced FLI1 allowed the identification of putative FLI1 direct targets. The FLI1 negatively regulated genes included tumor-suppressor genes involved in negative regulation of cell cycle and p53 cascade. Among the FLI1 positively regulated targets we found genes annotated for immune response, MYC targets and B cell receptor, TNF-alpha and IL2 signaling pathways. Of note, direct targets of FLI1 overlapped with those genes regulated by ETS1, the other transcription factor co-gained in DLBCL, suggesting a functional convergence within the ETS family. ASB2 was downregulated after FLI1 silencing and had FLI1 binding sites in both promoter region and distal enhancer regions. Furthermore, ASB2 is known to promote NF-kB activation in T-cell acute lymphoblastic leukemia and might be an essential gene in DLBCL cells according to a genetic screening. Consistently, ASB2 gene silencing was toxic in GCB DLBCL lines. We observed inhibition of NF-kB pathway by a strong protein downregulation of RELB, along with increased IκBα upon ASB2 and FLI1 silencing, although with no differences in NF-kB2 levels. Only FLI1 silencing caused downregulation of NF-kB1 and RELA protein levels, but no

effect on these two proteins was observed upon ASB2 silencing. These results indicate that FLI1 regulates either the classic NF-kB pathway at transcriptional level or the alternative pathway, via ASB2, in GCB DLBCL.
Conclusions: FLI1 is expressed at higher levels in GCB than ABC DLBCL and directly regulates a network of biologically crucial genes and processes in DLBCL, contributing to the regulation of NF-kB pathway in GCB DLBCL. ASB2, a subunit of a multimeric E3 ubiquitin ligase complex, is a novel FLI1 direct target, and its inhibition might represent a therapeutic approach for GCB DLBCL.

PO-08 Understanding the properties and oncogenic mechanisms of EZH2 Y641 mutations in lymphoma. Samantha J. Nixon[1], Sarah Zimmerman[1], Jeremy M. Simon[2], George P. Souroullas[1]. [1]Washington University School of Medicine, St. Louis, MO, [2]University of North Carolina in Chapel Hill, Chapel Hill, NC.

EZH2 (Enhancer of Zeste Homolog 2) is the catalytic component of the Polycomb Repressive Complex group 2 (PRC2), which establishes the repressive epigenetic mark histone 3 lysine 27 trimethylation (H3K27me3), resulting in silencing of gene expression. EZH2 was originally identified as a tumor suppressor since loss-of-function events were observed in myelodysplastic syndrome, acute myeloid, and T-cell leukemias. However, next-generation sequencing identified recurrent activating point mutations at tyrosine residue 641 (Y641) in 25% of germinal center diffuse large B-cell lymphoma and 15% of follicular lymphomas, suggesting that EZH2 also functions as an oncogene in a cell type-dependent manner. Using a faithful genetically engineered mouse model, we previously demonstrated that the Ezh2Y641F mutation drives formation of B-cell lymphoma as a single event and in cooperation with Bcl2 amplification or p53 loss. Unexpectedly, at the chromatin level, expression of Ezh2Y641F did not monotonically increase abundance of global H3K27me3 but rather resulted in redistribution of this mark across the genome, exhibiting loss of H3K27me3 at many loci, with direct effects on transcription. Others have shown that EZH2 mediates germinal center proliferation via repression of p21 and cooperation with Bcl6; however, given the global effect of Ezh2Y641F on chromatin, the oncogenic mechanisms of these mutations remain underexplored. These include the timing of the mutation during hematopoietic development, its role during differentiation, and the oncogenic activity of downstream targets. In order to investigate the effect of Ezh2Y641 mutations on hematopoietic development and differentiation, we used Cre alleles to drive expression of the mutant protein at different stages of hematopoietic development. Our data demonstrate that expression of Ezh2Y641 in early B cells is sufficient to drive oncogenic transformation. However, expression of Ezh2Y641 in hematopoietic stem cell (HSC) is not sufficient to drive oncogenic transformation despite the presence of mature healthy B cells. Additionally, expression of Ezh2Y641F in HSCs results in loss of self-renewal, differentiation bias towards the lymphoid lineages, and a partial block at the pre-pro B-cell stage. With regards to direct downstream targets, we identified upregulation of several HoxC cluster genes in Ezh2Y641F-mutant B cells prior to transformation. Upregulation of these genes is mediated via loss of H3K27me3 at the HoxC cluster and focal gains of H3K27 acetylation (H3K27ac). Overall, our findings underline the complicated biology and oncogenic activity of EZH2Y641 mutations in lymphoma, shed light on the effect of these mutations during hematopoietic development, and underscore some of the consequences of the paradoxical loss of H3K27me3 at certain loci.

PO-10 Histone 1 deficiency drives lymphoma through disruption of 3D chromatin architecture. Nevin Z. Yusufova[1], Matt Teater[1], Andreas Kloetgen[2], Alexey Soshnev[3], Adewola Osunade[4], Christopher Chin[1], Ashley Doane[1], Louis Staudt[5], David Scott[6], Neil Kelleher[7], Aristotelis Tsirigos[2], Marcin Imielinski[1], Yael David[4], David Allis[3], Ethel Cesarman[1], Ari Melnick[1]. [1]Weill Cornell Medicine, New York, NY, [2]New York University, New York, NY, [3]Rockefeller University, New York, NY, [4]Memorial Sloan Kettering Cancer Center, New York, NY, [5]National Cancer Institute, Bethesda, MD, [6]British Columbia Cancer Agency, Vancouver, BC, Canada, [7]Northwestern University, Chicago, IL.

Linker histone H1 binds to nucleosomes and causes chromatin compaction, yet little is known about their biologic function. Somatic missense mutations in histone 1 genes (HIST1H1B-E) occur in ~30% of follicular lymphomas and DLBCL, with significant mutual co-occurrence among these alleles, most frequently involving H1C and H1E. Examining whole exomes in 547 DLBCL patients, we found significant enrichment for H1 gene SNVs and copy number loss in the MCD class of ABC-DLBCLs as compared to other subtypes. Next we performed a genetic driver analysis in 101 DLBCLs with matched germline control to identify mutations significantly enriched over background somatic variation. We find that lymphoma-associated H1 alleles H1C and H1E are true genetic driver mutations in lymphomas. Lymphoma H1 mutations affect the H1 globular domain or C-terminus. We found that the globular domain mutants fail to bind to chromatin whereas C-ter mutants fail to compact chromatin as shown by atomic force microscopy, in vitro assembled nucleosome arrays, and FRAP assays in live cells. Hence both types of mutation confer loss of function. Constitutive H1C/E knockout mice are healthy and have no overt phenotype. However, immunization with T cell-dependent antigen caused significant GC hyperplasia ($p=0.013$) and disruption of polarity due to expansion in the number of centrocytes. Notably, H1C/EDKO GC B-cells readily outcompeted WT GC B-cells in mixed chimera experiments, indicating that they have superior fitness ($p=0.0086$). We performed RNA-seq in H1C/EDKO GC B-cells, which revealed an aberrant gene derepression signature. Strikingly, these same genes are upregulated during induced pluripotency (iPS cell) reprogramming and are normally silenced during early development by the PRC2 complex ($p <0.05$ to 1e-05). Indeed, histone mass spectrometry showed reduced H3K27me3 ($p=0.0003$) and increased H3K36me2 ($p =0.001$) in H1C/EDKO GC B cells. This prompted us to characterize the epigenome of purified H1C/EDKO vs WT GC B cells using Hi-C, ATAC-seq, and ChIP-seq for multiple histone marks. H1 deficiency causes profound architectural remodeling of the genome characterized by large-scale yet focal compartment shifts from compartment B to compartment A. The degree of decompaction results in distinct epigenetic states, primarily due to gain of H3K36me2 and/or loss of repressive

H3K27me3. Strikingly, the H1C/EDKO primitive stem cell gene expression signature was highly significantly enriched (NES 1.24, FDR<0.001) in the RNA-seq profiles of independent cohorts of DLBCL patients with H1C and H1E mutations. We crossed H1C+/-H1E+/- mice with VavP-Bcl2 transgenic mice and observed significant acceleration of lymphomagenesis (p=0.0001). Consistent with acquisition of stem cell characteristics, H1C+/-H1E+/-;VavP-Bcl2 but not VavP-Bcl2 primary lymphoma cells manifested lymphoma-initiating functionality after secondary transplantation into recipient animals.

PO-11 Targeting scavenger receptor type B1 in cholesterol-addicted lymphomas abolishes glutathione peroxidase 4 expression and results in ferroptosis. Jonathan S. Rink, Adam Lin, Kaylin M. McMahon, Andrea E. Calvert, Shuo Yang, Amir Behdad, Reem Karmali, Colby S. Thaxton, Leo I. Gordon. Northwestern University, Chicago, IL.
Introduction: In B-cell lymphomas, disparate molecular aberrations often produce common onco-metabolic phenotypes, including a dependency upon cholesterol uptake from serum lipoproteins rather than de novo synthesis. Recent reports have linked cancer cell cholesterol to ferroptosis, a newly appreciated form of programmed cell death characterized by the accumulation of oxidized membrane polyunsaturated fatty acids (PUFAs), facilitated by reduced glutathione peroxidase 4 (GPX4) activity. Diffuse large B-cell lymphoma (DLBCL) and anaplastic large T-cell lymphoma (ALCL) were identified as potentially sensitive to ferroptosis. We have developed high-density biomimetic lipoprotein nanoparticles (HDL NPs), a first-in-class platform that specifically targets scavenger receptor type B1 (SCARB1) and inhibits cholesterol uptake. In DLBCL, HDL NPs potently induces cell death. Accordingly, we hypothesized that HDL NPs induce ferroptosis in DLBCL and would be potent therapy for other cholesterol-addicted malignancies.
Methods: Burkitt's lymphoma (Ramos), DLBCL (SUDHL4), ALCL (SR-786, SUDHL1), and histiocytic lymphoma (U937) cell lines were used. RNA, protein, and cholesterol were isolated from cells treated with HDL NPs and analyzed using RT-qPCR, Western blot, and total cholesterol assays, respectively. Oxidized PUFA accumulation was quantified using the C11-BODIPY dye and flow cytometry. The lipophilic antioxidant ferrostatin-1 (Fer-1) and iron chelator deferoxamine (DFO) were used to inhibit ferroptosis. Following informed consent, primary B-cell lymphoma samples were isolated from patients and assayed for SCARB1 expression and viability following HDL NP treatment.
Results: HDL NPs reduced cholesterol levels in Ramos and SUDHL4 cells, leading to upregulation of cholesterol biosynthesis genes and downregulation of GPX4. This correlated with an increase in accumulation of oxidized PUFAs, and eventually cell death by ferroptosis. Fer-1 and DFO rescued Ramos and SUDHL4 cells from HDL NP-induced cell death. Oxidized PUFA accumulation and GPX4 downregulation were confirmed in vivo using HDL NP-treated SUDHL4 xenografts, which resulted in reduced tumor burden compared with controls. These results were replicated in other ferroptosis-sensitive malignancies, including SCARB1+ ALCL (SUDHL1, SR-786) and histiocytic lymphoma (U937) cell lines. Finally, primary B-cell lymphoma cells isolated from patients with follicular lymphoma (n=4) and DLBCL (n=2) demonstrated SCARB1 expression and were sensitive to HDL NP-induced cell death.
Conclusion: HDL NP binding to SCARB1 results in inhibition of cholesterol uptake and triggers a metabolic compensatory response, namely upregulation of cholesterol

biosynthesis genes and downregulation of GPX4, obligating cells to ferroptosis. As this onco-metabolic profile is observed across a range of cancers, HDL NPs represent a unique platform for metabolic therapy for cholesterol- and GPX4-dependent malignancies.

PO-12 Activity of ASTX660, a small-molecule IAP antagonist, reveals a novel, immune-based therapeutic approach in T-cell lymphoma. Simone Jueliger, Nicola Ferrari, George Ward, Joanne Munck, Tomoko Smyth, Matthew Davis, Christina Gewinner, Roberta Ferraldeschi, John Lyons, Martin Sims. Astex Pharmaceuticals, Cambridge, United Kingdom.

Introduction: ASTX660 is a potent, non-peptidomimetic antagonist of the cellular and X-linked inhibitors of apoptosis proteins (cIAP1/2 and XIAP), which is currently being tested in a first-in-human phase I-II study in patients with advanced solid tumors and lymphomas (NCT02503423). IAP antagonists are known regulators of cell death and have been reported to modulate immune responses (1). Herein, we describe the profile of ASTX660 in preclinical models with a specific focus on ASTX660's ability to harness a particular form of regulated cell death in T-cell lymphoma that directs an effective immune response and leads to sustained tumor regressions.

Methods: ASTX660 was profiled across a panel of T-cell lymphoma lines, assessing apoptosis, necroptosis, and immunogenic cell death (ICD). ASTX660 was tested in vitro with or without caspase-8/RIPK1 inhibitors to demonstrate mechanism of action. Target engagement along with induction of apoptosis, necroptosis, and ICD markers were analyzed by Western blotting and flow cytometry. Murine tumor models in immunocompetent and immunocompromised mice were utilized to evaluate the efficacy of ASTX660 in the presence or absence of an adaptive immune response.

Results: ASTX660 antagonized IAPs in cell lines, as indicated by a decrease in cIAP1 protein levels. In certain murine T-cell lymphoma cell lines, ASTX660 treatment was associated with an increase in cell death and release of biomarkers of ICD. Use of caspase and RIPK1 inhibitors indicated the cell death was via necroptosis. In a syngeneic model of T-cell lymphoma, administration of ASTX660 resulted in complete and sustained tumor regressions which were not seen in mice deficient in T and B cells. Mice cured of T-cell lymphoma by ASTX660 were resistant to a second inoculation of the same lymphoma.

Conclusion: These data add to the description of ASTX660's mode of action and our ongoing understanding of the preliminary clinical efficacy in T-cell lymphoma (2). We describe a new role for ASTX660 as an immunomodulatory molecule with potential to deliver a novel immune-based therapeutic approach in T-cell lymphoma.

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PO-13 Structural requirements for GRK2-mediated inhibition of the MALT1 proto-oncoprotein. Lisa M. Maurer[1], Matthew Trotta[1], Jing Cheng[1], Beibei Bill Chen[1], Peter C. Lucas[2], Linda M. McAllister-Lucas[1]. [1]University of Pittsburgh, Pittsburgh, PA, [2]UPMC Hillman Cancer Center, Pittsburgh, PA.

Stimulation of the B-cell or T-cell receptor triggers assembly of an intracellular complex composed of three proteins, CARMA1, BCL10, and MALT1 (CBM), which then induces activation of the NF-κB transcription factor and lymphocyte proliferation. Dysregulated CBM signaling, which occurs as a result of several gain-of-function mutations, underlies a variety of lymphoid malignancies including activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL). Multiple studies established that inhibiting MALT1 reduces growth and viability of ABC-DLBCL. We recently identified G-protein-coupled receptor kinase 2 (GRK2), one of seven members of the GRK family, as a novel MALT1-binding protein and demonstrated that GRK2 inhibits MALT1-dependent NF-κB activation. Further, knockdown of GRK2 in ABC-DLBCL enhances tumor growth, suggesting that GRK2 may act as a tumor suppressor by binding and inhibiting MALT1. As a first step toward elucidating how GRK2 regulates MALT1, we sought to identify the specific site(s) within GRK2 that interacts with MALT1. We created a series of expression constructs encoding specific GRK2 fragments and tested the ability of these fragments to inhibit CBM-dependent NF-κB activation. “GRK2 N-term” (AA1-173) includes the N-terminal helix (αN) and the regulator of G-protein signaling homology (RH) domain while “GRK2 ΔαN” (AA 30-C) lacks αN and retains all other domains. Using an NF-κB reporter assay, we found that GRK2 N-term and GRK2 ΔαN each block CBM-dependent NF-κB activation. These results suggest that RH, the only domain retained in both GRK2 fragments, is sufficient to inhibit MALT1. Since the GRK2 RH domain is known to interact with the alpha subunit of guanine-nucleotide binding proteins (Gα), we hypothesized that this protein interaction site may also mediate GRK2 RH binding to MALT1. However, we found that mutations that abrogate GRK2 binding to Gα do not affect GRK2’s ability to inhibit CBM-dependent NF-κB activation, suggesting that GRK2 interacts with MALT1 via a different site. We next compared the ability of GRK family members, which each have structurally homologous RH domains, to inhibit CBM-dependent NF-κB activity. We found that, like GRK2, GRK3 inhibits CBM-dependent NF-κB activity while GRK1 does not. We are now comparing RH domain structures for GRK2, GRK3, and GRK1 to identify structural requirements for RH interaction with MALT1. Also, we performed in silico modeling, which predicts that a short stretch of amino acids within GRK2 RH, designated “P1-P2,” interacts with the MALT1 Death Domain. Together, our data suggest that the GRK2 RH domain is sufficient to inhibit MALT1. Ongoing work aims to narrow down the region(s) within the RH that mediates GRK2 binding and inhibition of MALT1. We hope to utilize these analyses to inform the

development of a novel class of MALT1 inhibitors for treatment of MALT1-dependent leukemia and lymphoma.

PO-17 Microphysiologic model of ALK+ anaplastic large cell lymphoma and vascular interactions predicts drug efficacy in a 3D microfluidic chip. Marco Campisi[1], Claudia Voena[2], Ines Mota[3], Enrico Patrucco[2], Roger Dale Kamm[4], Roberto Chiarle[5]. [1] Dept. of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy, [2]Dept. of Molecular Biotechnology and Health Sciences, University of Torino, Turin, Italy, [3]Dept. of Pathology, Boston Children's Hospital and Harvard Medical School , Boston, MA, [4]Dept. of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, [5]Dept. of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, MA.

Anaplastic large-cell lymphoma (ALCL) initially responds well to anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitor (TKI) therapies (e.g., crizotinib); however, resistance appears once the treatment is concluded and persistent cells are not eradicated. ALCL preferentially grows around blood and lymphatic vessel in the lymph node, and our preliminary data have shown that CCL19/21-CCR7 chemokine-receptor signaling axis might be involved in TKI resistance of ALCL cells. To investigate this mechanism, we developed a microphysiologic model of ALCL interacting with a 3D vasculature using a microfluidic chip. Microfluidic technology has the potential to impact cancer diagnosis and therapy by overcoming the limitations of 2D culture. These ex vivo models are able to uncover the continuous interactions and chemokine signaling that exist between cells of the tumor microenvironment (TME) and evaluate the preclinical efficacy of novel and personalized cancer therapeutics. Two ALK+ ALCL cell lines (COST and Karpas299) were knocked out by CRISPR/Cas9 of the CCR7 receptor, transduced with GFP for microscopic visualization. A commercial microfluidic chip with a central gel channel, flanked by two fluidic media channels, was used to develop the model. A collagen hydrogel (2.5 mg/mL) was injected into the central region of the 3D chip, incubated in sterile humidity chambers, and channels were hydrated with RPMI. 50 µL of $3x10^6$ cells/mL human umbilical vein endothelial cells (HUVECs) suspension was injected twice on the fluidic channel, and the chip was rotated twice to create a confluent hollow-lumen 3D macrovessel. Then, $2x10^5$ cells/mL ALCL cells were added inside the 3D macrovessel. Medium was refreshed daily, supplemented +/- 300 nM crizotinib (ALK TKI). Image analysis was performed using a confocal microscope. The model consisted of a well-formed perfusable macrovessel with ALCL cells flowing inside and interacting with HUVECs. ALCL viability was evaluated with a luminescent readout assay after 3 days of interactions with HUVECs. Strikingly, ALCL cells cultivated within the vasculature showed marked resistance to treatment with ALK TKI compared to cells cultivated in the absence of vessels. CCR7 KO cells showed decreased viability and decreased perivascular localization compared to wild-type control cells. The results suggest that the 3D ALCL-vascular microphysiologic model is a feasible

platform with great potential to unveil the molecular mechanisms of drug resistance in a complex TME. The presence of macrovessels was sufficient to induce resistance to ALK TKI, which was decreased in CCR7 KO cells. These data indicate that CCL19/21 and possibly other cytokines produced by vessels generate survival signals in ALCL cells treated with ALK TKI. This physiologically relevant ALCL-vascular model offers a tool to genetically dissect the TME contribution to drug resistance, predict more reliably therapeutic vulnerabilities, and recapitulate patient-specific cell-cell interactions.

PO-18 Comprehensive correlation between genetic alterations in DLBCL and deregulated activation of the PI3K-AKT pathway isolates unique players in lymphoma dissemination and inferior outcome. Shannon Healy[1], Daisuke Ennishi[2], Elena Viganò[1], Aixiang Jiang[1], Anja Mottok[3], Stacy Hung[1], Olga Kutovaya[1], Gerben Duns[1], Bruce Woolcock[1], David Scott[1], Christian Steidl[1]. [1]British Columbia Cancer, Vancouver, BC, Canada, [2]Okayama University Hospital, Okayama, Japan, [3]Ulm University and Ulm University Medical Center, Ulm, Germany.

Diffuse large B-cell lymphoma (DLBCL) is characterized by deregulation of numerous signaling pathways that direct cell proliferation and survival during B-cell maturation in the germinal center reaction. The subtype of germinal center B-cell DLBCL (GCB-DLBCL) demonstrates addiction to tonic B-cell receptor (BCR) signaling in part through the PI3K-AKT pathway. However, a comprehensive catalogue of the somatic genetic alterations leading to this deregulation is currently lacking and represents a limitation in advancing precision medicine options. In this study, we present a comprehensive analysis in 347 DLBCL biopsies uniformly treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), describing recurrent mutations, copy number alterations, and clinical parameters that are associated with AKT activation (pAKT) as determined by immunohistochemistry. We observed a significantly enhanced pAKT population including players in the Gα13 signaling (GNA13, P2RY8, ARHGEF1, RHOA, and S1PR2) and PI3K activation pathways (NFATC1, USP7, PTEN, INPP4B). Following this correlation, we observed a co-occurrence of BCL2 protein overexpression and mutation in Gα13 signaling associated with advanced clinical stage (P = .008) in 183 GCB-DLBCL cases, as well as activation of cell migration and cell-cell adhesion pathways, indicating their contribution to lymphoma dissemination. This co-occurrence was shown to be associated with shorter time to progression in GCB-DLBCL (P = .005). We furthermore uncovered recurrent deletions of INPP4B, a lipid phosphatase involved in AKT deactivation, to be associated with increased pAKT in GCB-DLBCL. We observed that INPP4B loss is similarly associated with shorter time to progression (P = .036) in GCB-DLBCL, and demonstrated altered AKT phosphorylation in a GCB-DLBCL-derived cell line with a monoallelic INPP4B deletion. We present here a useful comprehensive tool, through a positive correlation to enhanced pAKT staining, to uncover novel prognostic indicators related to genetic alteration status in DLBCL. Our findings provide novel insight into somatic genomic alterations underlying prosurvival signaling of GC-derived lymphomas, and present new avenues for therapeutic targeting.

PO-19 Sequential inverse dysregulation of the RNA helicases DDX3X and DDX3Y facilitates MYC-driven lymphomagenesis. Chun Gong[1], Joanna A. Krupka[1], Jane Gao[1], Nicholas F. Grigoropoulos[2], Francesco Cucco[1], Sharon Barrans[3], De Los Mozos Igor[4], Zhou Peixun[5], Forde Sorca[1], Matthews Jamie[1], Burke Amos[1], Sze Siu Kwan[6], Beer Philip[7], Burton Cathy[3], Campbell Peter[7], Rand Vikki[5], Turner Suzanne[1], Ule Jernej[4], Roman Eve[8], Tooze Reuben[9], Oellerich Thomas[10], Du Ming[1], Samarajiwa Shamith[1], Daniel J. Hodson[1]. [1]University of Cambridge, Cambridge, United Kingdom, [2]Singapore General Hospital, Singapore, Singapore, [3]St. James Institute of Oncology, Leeds, United Kingdom, [4]The Francis Crick Institute, London, United Kingdom, [5]Teesside University, Middlesbrough, United Kingdom, [6]Nanyang Technological University, Singapore, Singapore, [7]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [8]University of York, York, United Kingdom, [9]University of Leeds, Leeds, United Kingdom, [10]Frankfurt Cancer Institute, Frankfurt, Germany.
DDX3X is a ubiquitously expressed RNA helicase involved in multiple stages of RNA biogenesis. DDX3X is frequently mutated in Burkitt lymphoma, but the functional basis for this is unknown. Here, we show that loss-of-function DDX3X mutations are also commonly found in MYC-translocated diffuse large B-cell lymphoma. We reveal functional cooperation between mutant DDX3X and MYC in ex vivo cultured human germinal center B cells. By integrating results of iCLIP, ribosome profiling, and proteomics, we show WT DDX3X promotes the translation of mRNAs encoding components of the core translational machinery, thereby driving global protein synthesis. Loss-of-function DDX3X mutations act to buffer the effect of abrupt MYC expression and moderate MYC-driven global protein synthesis, thereby buffering MYC-induced proteotoxic stress during early lymphomagenesis. Established lymphoma cells subsequently restore full protein synthetic capacity by aberrant expression of DDX3Y, a Y-chromosome homologue that is normally expressed exclusively in testis. These findings show how sequential dysregulation of DDX3X and DDX3Y acts to titrate global protein synthesis to suit the stage-specific needs of developing lymphoma cells and identify DDX3Y as an attractive, male-specific therapeutic target absent from normal human B cells but required for the survival of male lymphomas.

PO-20 Molecular characterization of a mouse model of peripheral T-cell lymphoma with Tfh and Th2 features. Elizabeth Kuczynski[1], Giulia Morlino[2], Alison Peter[1], Dinis Calado[2], Charles Sinclair[1], Larissa Carnevalli[1]. [1]AstraZeneca, Cambridge, United Kingdom, [2]Francis Crick Institute, London, United Kingdom.

Peripheral T-cell lymphomas (PTCLs) comprise a heterogeneous group of malignancies with an aggressive clinical course. Molecular and immunophenotypic analysis have linked PTCL subgroups to derivation from differentiated T cells, including T follicular helper (Tfh), Th1, and Th2 cells. Effective therapies for these PTCLs are undefined and tumor models are limited. We developed a novel mouse model of PTCL and applied it to evaluate responses to therapy. An in vivo transplantable cell line was isolated by passaging a splenic lymphoma arising from a mouse with B cell-specific Blimp1-deletion (Cγ1-Cre Blimp1$^{fl/fl}$) into C57bl/6J mice. Lymphomas were profiled by flow cytometry, immunohistochemistry, RNA, and exome sequencing. Mice intravenously inoculated with lymphoma cells developed disseminated disease affecting primarily lymphoid tissues and the liver. Model latency to welfare endpoint was 3-4 weeks. Transplantable cells expressed multiple T-cell lineage markers, were CD8 negative and CD4 positive, but gradually downregulated CD4 with passaging. Lymphoma cells ("mPTCL") also expressed a single TCRvβ chain, suggesting origin from a T helper cell clone. Immunophenotypically, mPTCL stably expressed Tfh markers ICOS, PD-1, CD40L, and low BCL6, and thus was potentially classifiable as a surrogate PTCL of Tfh phenotype. However, the transcriptome of purified mPTCL or spleens indicated a resemblance to Th2 cells and corresponding PTCL-GATA3 subtype (a PTCL-not otherwise specified). In accord with Tfh and Th2 helper function, B cells in the tumor microenvironment had an activated phenotype but were nonexpansive. Investigation into oncogenic pathways in mPTCL revealed a predicted deleterious mutation in Ctnnb1 (β-catenin) and enrichment for Myc-activated, cell cycle regulation, and DNA damage response genes. In vivo, mPTCL progression was significantly and potently delayed by monotherapy inhibition of ataxia telangiectasia and Rad3-related (ATR), a kinase central to resolving replication stress. Taken together, we established a novel model of PTCL with overlapping Tfh and Th2 features. Our findings in this model suggest the use of a DNA damage response inhibitor as a potential treatment strategy for aggressive PTCL-GATA3 lymphomas.

PO-21 Investigating the role of exosomes derived from chemotherapy-resistant leukemia cells as mediators of cellular plasticity. Taismara Garnica[1], Jéssika Lesbon[1], Arina Rochetti[1], Chantell Payton[2], Rhona Muirhead[2], Lisa Pang[2], David Argyle[2], Juliano da Silveira[1], Heidge Fukumasu[1]. [1]University of São Paulo, Pirassununga, Brazil, [2]University of Edinburgh, Edinburgh, United Kingdom.

Adult T-cell leukemia/lymphoma (ATLL) is a type of cancer with aggressive and rapid progression and has been linked to infection with the human T-cell lymphotropic virus type 1 (HTLV-1). Standard treatment consists of chemotherapy, radiation therapy, and stem-cell transplant, but the development of chemoresistance is common. Despite major advances in understanding the pathogenesis of the disease, patients who do not respond or achieve only transient responses remain a challenge for clinicians. Exosomes are small extracellular vesicles (30-150 nm in diameter) involved in intercellular communication and biologic material transference (including mRNA, miRNA, lipids, and metabolites) between the cells. Exosomes are an emerging key to understanding cancer cell communication and signaling pathway activation during the chemoresistance process. Our hypothesis is that exosomes secreted by chemoresistant (CR) ATLL cells can drive phenotypic changes in nonresistant ATLL cells. For this purpose, we induced chemoresistance using the CHOP protocol (dexamethasone, doxorubicin, and vincristine cocktail) in Jurkat cells (human acute T-cell leukemia/lymphoma). We determined IC50 values as 1 nM of dexamethasone, 0.05 μM of doxorubicin, and 1 nM of vincristine. Acquisition of chemoresistance was demonstrated by increased cell viability after treatment using IC50 of drugs cocktail. We found that Jurkat-CR have slower doubling time compared to respective naïve cells, 26 hours and 21 hours to CR-Jurkat and naïve, respectively. Exosomes derived from the putative chemoresistant were isolated and used to treat their respective naïve cells. There was an increase in cell proliferation after the exosome's treatment comparing to control for Jurkat ($P<0.0035$). However, there was no difference in proliferation comparing the treatment using exosomes derived from naïve cells and those derived from CR cells ($P=0.91$). The cell proliferation was higher after 78 hours of exosomes' treatment ($P<0.001$). Those results are preliminary data. Despite the differences in proliferation related to exosomes treatment, further experiments are required to prove our initial hypothesis.

PO-22 Compromised counterselection by FAS creates a lethal subtype of germinal center lymphoma. Raud Razzaghi[1], Shreya Agarwal[1], Nikita Kotlov[2], Olga Plotnikova[2], Krystle Nomie[2], Da Wei Huang[1], George W. Wright[1], Moyi Li[1], Katsuyoshi Takata[3], Chen Yao[4], John J. O'Shea[4], James D. Phelan[1], Stefania Pittaluga[1], David W. Scott[3], Jagan R. Muppidi[1]. [1]National Cancer Institute, Bethesda, MD, [2]BostonGene, Waltham, MA, [3]British Columbia Cancer Agency, Vancouver, BC, Canada, [4]National Institute of Arthritis and Musculoskeletal and Skin Disease, Bethesda, MD.
Fas is highly expressed on germinal center (GC) B cells, and mutations of *FAS* have been reported in diffuse large B-cell lymphoma (DLBCL); however, the function of Fas in the GC remains controversial. Although GC-derived DLBCL has better overall outcomes to therapy than other DLBCL types, some cases are refractory and the molecular basis for this is often unknown. We show that Fas is a strong cell-intrinsic regulator of GC B cells that promotes B-cell death in the light zone likely via T follicular helper (Tfh) cell-derived Fas ligand. In the absence of Fas, GCs were more clonally diverse due to an accumulation of cells that did not demonstrably bind antigen. We found that *FAS* alterations occurred most commonly in the GC-derived genetic subtype of DLBCL, EZB. FAS alterations in EZB were associated with inferior outcomes and an enrichment of Tfh cells. Alterations in FAS co-occurred with deficiency in HVEM and PD-1 ligands that regulate the Tfh-B cell interaction and were associated with increased diversity of B-cell receptor variable genes across samples. This work shows that Fas is critically required for GC homeostasis and suggests that loss of Tfh-mediated counterselection in the GC contributes to lethality in a distinct subtype of GC-derived lymphoma.

PO-23 Somatic hypermutation is perturbed in ABC-DLBCL lymphoma cell lines expressing high levels of activation-induced deaminase. Huseyin Saribasak, Ravi Dinesh, Lizhen Wu, David G. Schatz. Yale University School of Medicine, Department of Immunobiology, New Haven, CT.

Somatic hypermutation (SHM) is a B cell-specific process that supports the affinity maturation of antibodies. The reaction targets the immunoglobulin loci ("on-target" activity) and is initiated by activation-induced cytidine deaminase (AID). AID is known to be able to mutate the genome more broadly ("off-target" activity) and hence was found to be linked to various types of B-cell malignancies. However, the mechanism by which AID is recruited and exerts its function is partially known. To unravel this puzzle, we have analyzed two types of diffuse large B-cell lymphoma (DLBCL) cell lines: germinal B cell like (GCB-DLBCL), in which AID is expressed and introduces mutations, and activated B cell like (ABC-DLBCL), where AID levels are typically increased but paradoxically, SHM is much reduced. To begin to accomplish this goal, we have taken advantage of a Diversification Activator (DIVAC)-GFP reporter assay to measure targeted SHM activity. We first confirmed that AID is expressed in ABC-DLBCL cell lines at levels up to fourfold higher than in GCB-DLBCL cell lines by RT-qPCR. In contrast to the high levels of AID seen in ABC-DLBCL cells, we have observed that SHM is reduced by as much as 10-fold in ABC versus GCB lines using the DIVAC–GFP assay. As this assay should provide a good surrogate for SHM activity at Ig loci, our data support the possibility that the Ig-locus-specific SHM targeting program is defective in ABC-DLBCL. To search for factors that might explain the differential activity of the SHM program in GCB versus ABC cells, we performed RNA-seq using cell lines from each group. Initial analysis revealed that expression of two cell surface markers, CD27 and CD38, is highly correlated with the high mutation phenotype. Flow cytometry analysis for these two markers along with the pan B cell marker CD20 revealed various subpopulations in some DLBCL lines. Isolation, characterization, and measurement of SHM activity using the DIVAC-GFP assay of these subpopulations further strengthened the correlation between CD27/CD28 expression and SHM activity. These analyses also uncovered SHM-permissive subpopulations in ABC lines and SHM-resistant subpopulations in GCB lines. This observation explains residual SHM activity in ABC lines and argues for heterogeneity in the activity of the SHM program in DLBCL cell lines and perhaps within tumor populations in patients. Future studies will include deletion and introduction of specific factors from GCB or ABC lines to validate phenotype and identify regulators of the SHM program. Finally, sequence-analysis will be done to quantify mutations with the broad goal of understanding how AID targeting and mis-targeting are regulated.

PO-24 Modeling marginal zone lymphomagenesis. Victor Yazbeck, Ian McConnell, Emily Harris, Joseph Lownick, Ariel Sindel, Roy Sabo, Alden Chesney, Guanhua Lai, Adolfo Mauro, Chad Cain, Fadi Salloum, Jamal Zweit, Steven Grant, Jolene Windle, Steven Grossman. Massey Cancer Center, Richmond, VA.

Introduction: Indolent B-cell non-Hodgkin's lymphomas (NHL) represent a heterogeneous group of lymphoproliferative malignancies that remain largely incurable. Marginal zone lymphomas (MZL) are the second most common subtype of indolent NHL and lack a unique cytogenetic identifying abnormality. The B-cell receptor signaling pathway is activated in B-cell malignancy and mediates its activity mainly through the phosphoinositide 3-kinase (PI3K) pathway. Furthermore, novel PI3K inhibitors, such as copanlisib and parsaclisib, have shown impressive clinical activity in several indolent lymphomas including MZL. This further supports the important role of the PI3K pathway in the pathogenesis of this tumor. Therefore, we hypothesized that the PI3K-mTOR pathway is sufficient for driving the pathogenesis of MZL.

Methods: In order to test our hypothesis, we generated a genetically engineered mouse model carrying heterozygous global knockout alleles of both the tumor suppressor genes Phosphatase and Tensin Homolog (PTEN) and Liver Kinase B1 (LKB1). This led to overactivation of the PI3K-mTOR pathway in all mouse tissues. We closely monitored these mice for tumor formation via weekly physical examinations for several months. Upon tumor detection, the mouse was sacrificed, and tumors were sectioned for histologic characterization. In order to generate a more specific model of B cells, and more accurately mimic the underlying human disease, we used the Cre-LoxP system to create the CD19-Cre-PTENfl/fl-LKB1fl/fl.

Results: Thirty mice of global KO PTEN+/- LKB1 +/- died or were sacrificed due to disease progression, defined as either lymph node enlargement and/or splenomegaly. All mice showed either abnormal lymphadenopathy or splenomegaly. By Kaplan-Meier analysis, we saw a steady decrease in both tumor-free and overall survival after 3 months of age. Utilizing the product limit method, the median survival time was 6 months (95% CI: 6, 8). A total of 51 lymph nodes were sent for immunohistochemistry and pathologic characterization. Of the 51 nodes, 61.5% (N=32) showed indolent non-Hodgkin's lymphoma, 25% (N=13) were atypical, and 11.5% (N=6) were reactive. All lymph nodes with indolent NHL were of MZL subtype. Compared to wild type (n=3), the new CD19-Cre-PTENfl/fl -LKB1fl/fl (n=3) showed an overall increase in spleen mass (120 vs. 196 mg, p=0.0564), % B1 cells (4% vs. 59%, p= 0.0075), % MZ cells (5% vs. 30%, p=0.0547), % plasma cells (1% vs. 12%, p=0.0729), and decrease in % FO cells (80% vs. 12%, p=0.0003) by flow cytometry. Further characterization of the new model is currently under way.

Conclusion: Marginal zone lymphoma remains an incurable lymphoma that lacks reliable preclinical models. Our data provide, for the first time, a proof of concept on the role of the PI3K-mTOR pathway in the pathogenesis of marginal zone lymphoma and pave the way for future studies understanding the biology of this disease and developing rational therapies for this incurable malignancy.

PO-25 CLR 131 demonstrates 100% overall response rate in relapsed or refractory lymphoplasmacytic lymphoma (LPL)/Waldenstrom's macroglobulinemia (WM): initial results from ongoing phase 2 trial, CLOVER-1 study. Sikander Ailawadhi[1], Jarrod Longcor[2], Kate Oliver[2], Igor D. Grachev[2]. [1]Mayo Clinic Florida, Jacksonville, FL, [2]Cellectar Biosciences, Florham Park, NJ.

Introduction: CLR 131 (I-131-CLR1404) is a novel targeted radiotherapeutic that exploits the selective uptake and retention of phospholipid ethers (PLEs) by malignant cells. Based on preclinical and clinical experience and the radiosensitivity of MM, CLR 131 is being examined in relapsed or refractory B-cell malignancies through an open-label, phase 2 trial, CLOVER-1 (NCT02952508); initial results in pts with LPL/WM are reported here.

Procedures: The primary objective of this study is to determine the efficacy and safety of CLR 131 in select B-cell malignancies. Eligibility criteria include adult pts with relapsed or refractory chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), lymphoplasmacytic lymphoma (LPL)/Wadenstrom's macroglobulinemia (WM), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), or diffuse large B-cell lymphoma (DLBCL). Pts with LPL/WM must have received at least two prior treatment regimens unless ineligible to receive standard agents and have measurable disease: either a nodal lesion > 15 mm, an extranodal lesion > 10 mm, or measurable IgM. Prior external beam radiation therapy is allowed (< 20% of total marrow irradiated). CLR 131 is administered intravenously up to 30 minutes at total body doses (TBD) of <50 mCi, ~50 mCi, and ~75 mCi. Adverse events (AEs) are graded by NCI-CTCAE v4.03; responses are assessed by the 2012 WM Criteria for Response Assessment.

Results: Four pts with LPL/WM have been enrolled in the study with data presented as of 1 Mar 2020. The median age was 70 (range 54-81) and included 2 females and 2 males who had a median of two prior regimens (range 1-5). The overall response rate was 100%, including one pt with a complete response, one pt with a partial response, and one pt with a minimal response; one pt remains under evaluation. The primary treatment-emergent AEs across all doses in pts with LPL/WM included neutropenia, thrombocytopenia, nausea, fatigue, anemia, and decreased white blood cell count, in line with experience to date with CLR 131 in other B-cell malignancies. Median time to resolution of cytopenias was 21 days. One pt received two cycles of CLR 131 with a TBD of ~100 mCi. The pt was determined to be a partial response 43 days post initial dose and a complete response at approximately 200 days; this was confirmed radiologically 406 days post initial dose. The duration of response for this pt is currently at 25 months and is ongoing. One pt received ~ 50 mCi CLR 131 and saw an 89% decrease in IgM at 64 days post infusion. One pt received ~ 75 mCi CLR 131 and had a 45% decrease in IgM at 64 days post infusion.

One pt received two cycles of CLR 131 with a TBD of ~150 mCi, experiencing a 48% reduction in IgM to date and has not yet completed the evaluation period.
Conclusions: CLR 131 is a unique, first-in-class targeted radiotherapeutic in development for multiple B-cell hematologic malignancies. The observed positive signals of efficacy and safety from this preliminary data in relapsed or refractory LPL/WM pts is encouraging and will be further explored.

PO-26 Prognostic significance of Fc gamma receptor IIB expression in the response of previously untreated diffuse large B-cell lymphomas to anti-CD20 monoclonal antibodies: Differing impact of rituximab and obinutuzumab. Laura K. Hilton[1,2]*, Malgorzata Nowicka[3]*, Margaret Ashton-Key[4,5], Chantal E. Hargreaves[4,6], Chern Lee[5], Russell Foxall[7], Matthew J. Carter[7], Stephen A. Beers[7], Kathleen N. Potter[4], Christopher R. Bolen[8], Christian Klein[9], Andrea Knapp[3], Farheen Mir[10], Matthew Rose-Zerilli[4], Cathy Burton[11], Wolfram Klapper[12], David W. Scott[1,13], Laurie H. Sehn[1,13], Umberto Vitolo[14], Maurizio Martelli[15], Marek Trneny[16], Graham W. Slack[1], Pedro Farinha[1], Jonathan C. Strefford[4#], Mikkel Z. Oestergaard[17#], Ryan D. Morin[2,18#], Mark S. Cragg[4,7#]. [1]BC Cancer Centre for Lymphoid Cancer, Vancouver, BC, Canada, [2]Canada's Michael Smith Genome Sciences Centre, Vancouver, BC, Canada, [3]F. Hoffmann-La Roche Ltd, Basel, Switzerland, [4]School of Cancer Sciences, University of Southampton, Southampton, United Kingdom, [5]Southampton University Hospitals NHS Foundation Trust, Southampton, United Kingdom, [6]Nuffield Department of Medicine, John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom, [7]Antibody and Vaccine Group, Centre for Cancer Immunology, School of Cancer Sciences, University of Southampton Faculty of Medicine, Southampton, United Kingdom, [8]Genentech Inc., South San Francisco, CA, [9]Roche Innovation Center Zurich, Schlieren, Switzerland, [10]Royal Marsden Hospital, Sutton, United Kingdom, [11]St. James's Institute of Oncology, Leeds, United Kingdom, [12]University of Kiel, Kiel, Germany, [13]University of British Columbia, Vancouver, BC, Canada, [14]Candiolo Cancer Institute, FPO-IRCCS, Candiolo (TO), Italy, [15]Department of Translational and Precision Medicine, Hematology, Sapienza University, Rome, Italy, [16]Charles University General Hospital, Prague, Czech Republic, [17]F. Hoffmann-La Roche Ltd., Basel, Switzerland (currently, Novo Nordisk, Zurich, Switzerland), [18]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada. *Shared first authorship, #shared senior authorship.

Background: The anti-CD20 monoclonal antibody (mAb), obinutuzumab (G), has shown improved outcomes versus rituximab (R) in indolent lymphomas; however, no improvement was seen in diffuse large B-cell lymphoma (DLBCL), and the molecular basis is unclear. The inhibitory Fc gamma receptor IIB (Fc γRIIB), expressed on lymphoma cells, can impair the effects of direct targeting mAbs, such as R, by binding and internalizing them, reducing opsonization and limiting FcγR-mediated killing. We hypothesized that FcγRIIB expression on cellular effectors and/or the lymphoma confers treatment resistance in some patients (pts) and evaluated if outcomes differ for therapies involving a non-internalized mAb (G).

Methods: We evaluated correlates between FCGR2B mRNA and/or FcγRIIB protein expression and pt outcomes in two discovery cohorts of de novo DLBCL treated with R-CHOP (Arthur et al. 2018, n=372; Schmitz et al. 2018, n=234), and the phase III

GOYA trial (NCT01287741; n=552), which compared R-CHOP with G-CHOP in pts with previously untreated DLBCL. FCGR2B mRNA expression was assessed by RNA-Seq, and protein expression was assessed in evaluable cohorts by immunohistochemistry, using tissue microarrays with macrophages identified by CD68. FCGR2B expression was also measured by a NanoString assay (Arthur cohort). Cox regression analyzed the impact of FcγRIIB/FCGR2B expression on progression-free survival (PFS), with univariate and multivariate models adjusted for International Prognostic Index (IPI), cell of origin (COO), and BCL2 protein expression.

Results: In the discovery cohorts, a higher FCGR2B expression was significantly associated with shorter PFS (Arthur: HR 1.09 [95% CI: 1.01–1.19], P=0.036; Schmitz: HR 1.13 [95% CI: 1.02–1.26], P=0.0243). Expression by NanoString strongly correlated with RNA-Seq, confirming the association with shorter PFS (HR 1.13 [95% CI: 1.04–1.23], P=0.0048). In GOYA, a significant association between PFS and FCGR2B was observed in the R arm (HR 1.26 [95% CI: 1.00–1.58], P=0.0455), with no prognostic effect observed for G (HR 0.91 [95% CI: 0.69–1.20], P=0.5). Pts with high FCGR2B expression appeared to benefit more from G than R (HR 0.67 [95% CI: 0.44–1.02], P=0.0622), in contrast to pts with low FCGR2B expression (HR 1.58 [95% CI: 1.00–2.50], P=0.0503). In both Arthur and GOYA cohorts, FCGR2B expression by RNA-Seq was associated with FcγRIIB on the tumor, which correlated with a shorter PFS for R (HR 2.17 [95% CI: 1.04–4.50], P=0.03), but not G (HR 1.37 [95% CI: 0.66–2.87], P=0.4). This prognostic effect on PFS was independent of established prognostic biomarkers, IPI, COO and BCL2.

Conclusion: High FcγRIIB/FCGR2B expression in pts with DLBCL has prognostic value in those treated with R and may confer differential responsiveness to R or G.

PO-27 Clonotypic cell-free DNA (cfDNA) in the cerebrospinal fluid (CSF) of patients with aggressive lymphomas. Adam J. Olszewski[1], Anna Chorzalska[2], Habibe Kurt[1], Thomas A. Ollila[1], Diana O. Treaba[1], Andrew Hsu[2], Adam Zayac[2], John L. Reagan[1], Ilyas Sahin[1], William Rafelson[2], Pamela C. Egan[1], Jordan Robison[3], John Vatkevich[3], Chelsea D. Mullins[4], Max Petersen[1], Patrycja M. Dubielecka[2]. [1]Alpert Medical School of Brown University, Providence, RI, [2]Rhode Island Hospital, Providence, RI, [3]Lifespan Oncology Cancer Research, Providence, RI, [4]Adaptive Biotechnologies, Seattle, WA.
Current methods for detection of CNS involvement in lymphoma (CSF cytology, flow cytometry) have very limited sensitivity, particularly in cases of parenchymal brain involvement. Early detection is critical to institute CNS-directed therapy and avert dismal outcomes of overt CNS recurrence. Clonotype-specific cfDNA can be detected in the plasma of patients with lymphoma using next-generation sequencing (NGS), and cfDNA-based minimal residual disease (MRD) assay can predict impending recurrence (Roschewski, Lancet Oncol 2015). cfDNA has not been systematically evaluated in the CSF, yet it may hold promise as a sensitive and specific method to detect CNS invasion. To evaluate the ability of an NGS-MRD assay in CSF to detect CNS invasion, we examined CSF and plasma samples from patients with aggressive lymphomas who either had overt CNS disease or who were without known CNS invasion, but at high clinical risk. Genomic DNA from primary tumors was analyzed for tumor-specific clonotype using NGS of rearranged IGK, IGH (VJ or DJ), or IGL loci (Adaptive Biotechnologies; Carlson, Nat Commun 2013). Tumor-specific clonotypes from each case were selected for subsequent tracking by NGS-MRD in CSF and plasma samples. Clonotype copy numbers are expressed per mL for acellular CSF, and clonotype frequency per all B cells. NGS identified median 3 (range, 2-7) dominant immunoglobulin sequences in each primary lymphoma (N=16), with median dominant clonotype frequency 50.3% (range, 26.8-9.28%). In the CSF, the NGS-MRD assay detected the dominant clonotype in 9 out of 16 samples, including all (N=4) with overt CNS invasion (sensitivity=100%), of which 2 had parenchymal disease only with negative CSF cytology, flow cytometry, or IGH PCR. Median detectable cfDNA clonotype in the CSF was 1,077 copies per mL (range, 2-5,620), with median clonotype frequency of 28.4% (range, 0.1-98.5%). cfDNA copy counts were significantly higher in cases with positive CSF cytology than those with parenchymal or clinically occult disease (P=.0016). We observed no significant correlation between the red blood cell count in the CSF and the cfDNA clonotype concentration (P=0.73) or frequency (P=0.62), suggesting that the presence of cfDNA in the CSF was not due to contamination by blood plasma. There was also no evident correlation between cfDNA in plasma and CSF. Within median 11 months of follow-up, 1 of 4 patients (25%) with a positive CSF NGS-MRD assay and no CNS disease developed a fatal CNS recurrence. Our results suggest that NGS-MRD assay for cfDNA

in the CSF can identify intraparenchymal or leptomeningeal CNS invasion with high sensitivity, including cases not identifiable by traditional methods. Prognostic significance of detecting lymphoma-specific cfDNA in the CSF of high-risk patients without overt CNS disease will be explored in a larger sample. Pretreatment NGS-MRD assay could be prospectively tested to predict the risk of CNS recurrence and potentially enable more accurate selection of patients for CNS prophylaxis therapy.

PO-28 Antitumor effects of cannabinoids against B-cell lymphoma. Saba Omer, Dawn Boothe, Mohammedohammed Mansour, Muralikrishnan Dhanasekaran, Satyanarayana Pondugula. Auburn University, Auburn, AL.

Introduction: In the last two decades, cannabinoids have been studied extensively for their potential use in various fields of medicine including oncology. Aggressive B-cell lymphoma or non-Hodgkin lymphoma (NHL) is the fifth leading cause of human cancer death and is the second fastest growing cancer with regard to mortality in people. Lymphomas are generally characterized by a high rate of initial remission following conventional CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)-based therapies; however, 30% of humans will succumb to drug-resistant relapse. To date, lymphoma is still a serious condition for which there are unmet medical needs. The purpose of this study was to demonstrate the antitumor effects of cannabinoids in B-cell lymphoma using canine as a model due to striking similarities b/w canines and human B-cell lymphoma in histology, biology, and gene expression. Another advantage of studying B-cell lymphoma in canines will be to study a spontaneous tumor in future clinical trials instead of using genetically engineered animal models of cancer.

Methodology: Canine B-cell lymphoma cell lines (1771, CLBL1) and lymphocytes from healthy dogs (control) were cultured in RPMI. Expression of cannabinoid receptors was studied using qPCR. Based on receptor expression 17-71 cells were treated with receptor agonists (AEA, 2AG, CBD, THC, WIN, and HU-210,) and antagonists (S16 and S28); normal lymphocytes were treated with CBD. Cell viability was assessed using MTT assay. Biochemical analysis was performed using spectrofluorometry to evaluate apoptotic makers involved in inducing cell death. Data were analyzed using ordinary one-way ANOVA on Prism software.

Results: All B-cell lymphoma cell lines showed positive expression of CB1 and CB2 receptors. Cell viability assay demonstrated a dose-dependent decrease in cell proliferation with all cannabinoid receptor agonists. Out of two antagonists used, S28 did not affect cell proliferation; however, S16 showed antiproliferative effects like agonists. No significant effect on cell proliferation was found on normal lymphocytes. Biochemical analysis showed a decrease in nitrite and caspase activity in treated cells as compared to control untreated cells.

Conclusion: Canine lymphoma cells express both cannabinoid receptors like human B-cell lymphoma, and activating cannabinoid receptors with agonists induces cancer cell death in canine B-cell lymphoma. Our results suggest that cannabinoids have an antiproliferative and proapoptotic effect on canine lymphoma cells and support the need for further studies providing evidence of efficacy against both human and canine B-cell lymphomas.

Acknowledgments: We are grateful to Dr. Steven Suter, North Carolina State University, for sharing canine lymphoma cell lines.

PO-29 Conserved and unique transcriptional programs in human and canine non-Hodgkin lymphomas inform the judicious applications for the spontaneous canine model of disease. Aaron L. Sarver, Jaime F. Modiano. University of Minnesota, Minneapolis, MN.

Non-Hodgkin lymphomas (NHL) represent a diverse group of tumors that are common in both humans and dogs. Extensive work has documented driver genes and inferred the cell of origin for many subtypes of human NHL, and emerging data suggest that the driver events are distinct for the corresponding subtypes of canine NHL. This raises important questions about how and when spontaneous canine NHLs can or should be considered as models for human NHLs. Here, we show the application of bioinformatics tools that allow us to systematically reduce the dimensionality of complex transcriptional patterns across independent datasets, platforms, and species, and that are capable of identifying conserved and unique traits, as well as outcome associations. The application of these tools to human and canine NHL datasets shows that, for example, the gene expression cluster that defines the germinal center-B-cell (GCB)-like subtype is exclusively present in human samples, but not in dog samples. Conversely, we confirm that non-GCB subtypes (activated B-cell [ABC] or ABC-like) include a heterogeneous group of subtypes defined by expression of shared and species-specific gene clusters, including resident or infiltrating cells that mold the stromal and immune microenvironments. Moreover, we are able to expand this approach to other subtypes of NHL to discern the contributions from stromal transcriptional programs and from lymphocyte transcriptional programs by including samples in the analyses where stromal cells were depleted. Overall, our comparative genomic approach will enhance our understanding of the conserved and species-specific events that underlie the molecular etiology of NHL, informing when spontaneous canine NHL subtypes should be considered as independent, unrelated conditions and improving and refining when and how they can be used as a relevant spontaneous model of human NHL.

PO-30 Is it more explanatory to integrate the leukocyte/lymphocyte ratio (LLR) and prognostic nutritional index (PNI) to international prognostic systems (IPS) in cases with Hodgkin lymphoma (HL)? Semra Paydas[1], Sahin Lacin[2], Mutlu Dogan[3], Ibrahim Barista[2], Birol Yildiz[4], Gulsah Seydaoglu[1], Nuri Karadurmuş[4], Sinem Civriz[5], Muhammed Ali Kaplan[6], Munci Yagci[7], Emel Gurkan[1], Vehbi Ercolak[1]. [1]Cukurova University, Adana, Turkey, [2]Hacettepe University, Ankara, Turkey, [3]Ankara Numune Hospital, Ankara, Turkey, [4]Gulhane Hospital, Ankara, Turkey, [5]Ankara University, Ankara, Turkey, [6]Dicle University, Diyarbakir, Turkey, [7]Gazi University, Ankara, Turkey.

Aim: To determine the power of the International Prognostic Scoring systems (IPS-7 and IPS-3) and to obtain more explanatory indexes by integrating LLR and PNI as prognostic indicators to IPS in cases with HL.

Patients and Methods: 1,060 patients with HL were included in this study from 8 centers in Turkey. IPS-7 using seven parameters (age, sex, stage hemoglobin, albumin, lymphocyte count, and white cell count) and the modified IPS-3 score with three parameters (stage, age, and hemoglobin level) were calculated. An alternative IPS-4 score with four parameters (stage, age, hemoglobin level, and LLR or PNI) was also calculated. LLR and PNI were integrated to IPSs.

Statistical Analyses: Chi square test or Student t test was used to compare the groups. The cut-off points of this population for the LLR and PNI were evaluated using AUC (area under ROC curve) statistics and median values of the distributions of the variables. Possible alternative cut-off points for the LLR and PNI were evaluated using AUC statistics and median values of the distributions of the variables. For LLR, 5.7 was detected as cut-off point based on ROC analysis and 5.1 based on median. Cut-off for PNI was 45.2 based on ROC analysis and 47.5 based on median. The Kaplan-Meier method and Cox proportional regression model were used to estimate the mean-median overall survival (OS), failure-free survival (FFS) rates, and hazard ratios (HRs). Log-rank test was used to compare the survival distributions between groups. The prognostic ability of parameters was evaluated for OS and FFS in both univariate and multivariate Cox regression models. p value < 0.05 was considered as significant. The analyses were performed using the statistical package SPSS v 22.0.

Results: Female/male ratio was 396/664; mean age was 37.6±15.8 (15-88). About 75% of the patients had stage II-III disease and two thirds of the patient had nodular sclerosis subtype. All factors of IPS-7 and IPS-3 scoring systems were found to be significant factors that related with OS and FFS according to univariate analyses. PNI and LLR were also found to be prognostic factors for OS and FFS. PNI ≤45.2 and LLR ≥5.8 were found to be poor prognostic indicators. Among 7 factors of IPS-7, gender and albumin were not found to be significant according to multivariate Cox regression model. Hence albumin and gender were excluded from the models and

PNI and LLR as prognostic factors were added to obtain easier and more explanatory indexes to the model, respectively. Two different Cox regression models were obtained for OS and FFS. Model 1 showed LLR ≥5.8 as the highest risk for OS (OR: 2.7) and Hb <10.5g/dL as the highest risk for FFS (OR: 2.3). Model 2 showed PNI ≤45.2 as the highest risk for OS (OR: 3.2) and Hb <10.5g/dL as the highest risk for FFS (OR: 2.9). Goodness of fit and agreement between IPS-7, IPS-3 groups and IPS-4 groups were found to be acceptable.
Conclusion: IPS-4 score obtained by integrating LLR or PNI to IPS systems can be used as an explanatory index for prognostic indicator in HL.

PO-31 HIV-1 transactivator of transcription deregulates key Burkitt lymphoma associated oncogenes at both the transcriptional and post-transcriptional level. Leonardo Alves de Souza Rios, Nontlantla Mdletshe, Shaheen Mowla. University of Cape Town, Cape Town, WC, South Africa.

Burkitt lymphoma (BL) is one of the most common HIV-associated lymphomas. This cancer represents one of the most frequent causes of mortality among HIV+ people in Southern Africa, which has the highest incidence of HIV/AIDS worldwide. As is the case for Kaposi sarcoma, recent reports associate an oncogenic function with HIV in lymphoma development. This study explores the oncogenic potential of HIV-1 protein Transactivator of transcription (Tat) in BL via its ability to manipulate the expression of c-MYC and AID, two key drivers of disease in BL. The ability of HIV-1 Tat to influence the activity of the full-length (FL) (2861 bp) *c-MYC* promoter was assessed using Dual Luciferase Reporter (DLR) assays. Using sequential deletions, the minimal promoter region mediating the effect was identified. Site-directed mutagenesis (SDM) was used to identify promoter elements mediating the response. Co-immunoprecipitation assays (Co-IP) were used to assess interactions between Tat and the AP-1 factor JunB. Chromatin Immunoprecipitation (ChIP) assays were done to confirm that JunB bound the promoter in vivo. To assess the ability of Tat to mediate the expression of miRNA-181b-5p, a known repressor of *AICDA*, Ramos cells were transfected with Tat followed by qRT-PCR. Corresponding changes in AID protein levels were determined using Western blot. To assess whether miRNA-181b-5p targets *AICDA* in B cells, the FL *AICDA* 3'UTR was cloned into the pGL3-Promoter vector and DLR assays were performed in the presence of miRNA-181b-5p mimic. Our data reveal that Tat enhances *c-MYC* promoter activity in a dose-dependent manner, with a maximum activity of 2.6-fold recorded when 500ng of pcDNA-Tat was used on 400ng of pGL3-c-MYC plasmid harboring the FL promoter. A loss of 35% activity was observed [1.7 (±0.05) fold] upon promoter deletion and two AP-1 sites (positions -1128 bp and -1376 bp) within the deleted region were found to mediate the Tat-dependent increase. A loss in activity of 20.6% was recorded when AP-1 site 1 was mutated, and 25% when AP-1 site 2 was mutated. A double mutant decreased the activity by 29%. Co-IP revealed a strong interaction between Tat and AP-1 factor JunB, and ChIP data showed that JunB was strongly recruited to those two AP-1 sites in the presence of Tat. Our result also reveals that the expression of the *AICDA* specific miRNA181b-5p is significantly reduced in the presence of Tat, which correlates with an increase in AID protein levels. We also confirm that the *AICDA* FL 3'UTR, which contains putative miRNA-181b-5p binding sites, is partially repressed by a miRNA-181b-5p mimic. Our study reveals that Tat potentiates oncogenesis by enhancing the expression of c-MYC and AID, two key lymphoma drivers. We show that Tat can enhance *c-MYC* expression by enhancing

the recruitment of cellular TFs to the promoter. We also show that the same HIV protein can enhance the expression of AID by modulating the expression of miRNA-181b-5p.

PO-32 NFKBIZ 3' UTR mutations confer selective growth advantage and affect drug response in diffuse large B-cell lymphoma. Sarah E. Arthur[1], Nicole Thomas[1], Jeffrey Tang[1], Christopher K. Rushton[1], Miguel Alcaide[1], Adèle Telenius[2], Shannon Healy[2], Anja Mottok[2], David W. Scott[2], Christian Steidl[2], Ryan D. Morin[1]. [1]Simon Fraser University, Burnaby, BC, Canada, [2]BC Cancer Research Centre, Vancouver, BC, Canada.

Introduction: The activated B cell-like (ABC) molecular subgroup of diffuse large B-cell lymphoma (DLBCL) is characterized by activation of NF-κB signaling. Recurrent mutations affecting genes such as MYD88, CD79A/B, and TNFAIP3 contribute to this in some cases, but there remain tumors with no known genetic basis for this pathway activation. This suggests that our understanding of ABC DLBCL drivers remains incomplete. Previously, NFKBIZ was shown to be amplified in 10% of ABC DLBCLs and to contribute to activation of NF-κB signaling. We recently described a novel pattern of mutations affecting the 3' UTR of NFKBIZ resulting in an overall mutation rate of 30% (UTR or AMP) in ABC DLBCL. These NFKBIZ UTR mutations are mutually exclusive with MYD88 mutations, thus suggesting they may also lead to activation of NF-κB signaling. The NFKBIZ protein interacts with NF-κB transcription factors and is thought to regulate canonical NF-κB signaling. We hypothesized that NFKBIZ UTR mutations affect the normally rapid degradation of this mRNA by disrupting secondary structures recognized by RNA-binding proteins such as ribonucleases. The resulting elevated NFKBIZ mRNA levels would lead to accumulation of protein and may be a novel mechanism to promote cell growth and survival in ABC DLBCL.

Methods: NFKBIZ 3' UTR mutations were introduced into a DLBCL cell line using the CRISPR-Cas9 system. A competitive growth assay with wild-type (WT) and CRISPR-mutant lines was performed to assess whether UTR mutations provide a growth advantage in culture (in vitro) and in mouse xenografts (in vivo). RNA-sequencing was then performed on WT and a subset of CRISPR-mutant lines and analyses were performed to identify genes upregulated by IκB-ζ in mutant lines. The IC50 of relevant drugs was determined by WST-1 assays after drug treatment on WT and mutant lines.

Results: Introduction of NFKBIZ mutations into a DLBCL cell line confirmed that UTR deletions lead to increased mRNA and protein levels. NFKBIZ UTR deletions give DLBCL cells a selective growth advantage over WT both in vitro and in vivo. RNA-sequencing of mutant and WT lines revealed possible transcriptional targets of NFKBIZ, including NF-κB targets and genes commonly overexpressed in ABC DLBCL. Novel candidate NFKBIZ targets were also discovered through this analysis, including CD274, the gene encoding PD-L1. Mutant cell lines had significantly higher IC50 compared to WT for the drugs ibrutinib, idelalisib, and masitinib, but not

bortezomib, suggesting that NKFBIZ UTR mutations make cell lines more resistant to specific NF-κB pathway-targeted drugs.
Conclusions: This work directly establishes a role for NFKBIZ amplifications and 3' UTR mutations in driving ABC DLBCL through NF-κB signaling. We demonstrate that these mutations can lead to overexpression of NFKBIZ and provide a selective growth advantage to cells both in vitro and in vivo. In addition, we found that these mutant lines were more resistant to some targeted lymphoma drugs but not others.

PO-34 Subcutaneous panniculitis-like T-cell lymphoma with HAVCR2 mutation shows unique clinicopathologic features and gene expression profile. Jiwon Koh[1], Insoon Jang[1], Seungchan Mun[2], Cheol Lee[1], Hee-Jung Cha[3], Young Ha Oh[4], Jin Man Kim[5], Young Hyeh Ko[6], Jae Ho Han[7], Heounjeong Go[8], Jooryung Huh[8], Kwangsoo Kim[1], Yoon Kyung Jeon[9]. [1]Seoul National University Hospital, Seoul, Republic of Korea, [2]Seoul National University Cancer Research Institute, Seoul, Republic of Korea, [3]Ulsan University College of Medicine, Ulsan, Republic of Korea, [4]Hanyang University College of Medicine, Guri, Gyeonggi-do, Republic of Korea, [5]Chungnam National University College of Medicine, Daejeon, Republic of Korea, [6]Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea, [7]Ajou University School of Medicine, Suwon, Gyeonggi-do, Republic of Korea, [8]Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea, [9]Seoul National University College of Medicine, Seoul, Republic of Korea. Recent studies identified germline HAVCR2 (TIM-3) mutations as the specific genetic predisposition to subcutaneous panniculitis-like T-cell lymphoma (SPTCL). However, distinction between HAVCR2-mutated and HAVCR2-wildtype SPTCLs remains elusive. We studied the prevalence of HAVCR2 mutation in a nation-wide cohort of SPTCL and investigated clinical and molecular distinction between HAVCR2-mutated and HAVCR2-wild-type SPTCLs. A multicenter nationwide cohort of 53 SPTCL cases was established; patients were diagnosed at seven Korean institutions between 2003 and 2020. Histologic features were reviewed by experienced hematopathologists and clinical features were retrospectively reviewed. Whole-exome sequencing (WES) and RNA-seq were performed using formalin-fixed, paraffin-embedded (FFPE) samples of 8 patients diagnosed in Seoul National University Hospital (SNUH), with matched non-neoplastic tissue samples from two patients. Direct sequencing of HAVCR2 exon 2 was successfully carried out using FFPE samples from 33 of the remaining 45 patients. Among 41 patients with available HAVCR2 mutation status, 28 (68.3%) were women, and the median age at diagnosis was 30 years (range 11–74). Ten patients (10/40; 25.0%) suffered hemophagocytic syndrome (HPS) or HPS-like systemic illness during the clinical course, and 14 patients (14/40; 35.0%) progressed during the follow-up. Six patients (6/41; 14.6%) died of disease progression or HPS. We found 18 patients (18/41; 43.9%) with HAVCR2 mutation; 15 patients harbored biallelic HAVCR2 Y82C mutation and 3 patients were noted for heterozygous HAVCR2 Y82C mutation. HAVCR2-mutated SPTCLs occurred in younger patients (median age 26.5 versus 37; Mann-Whitney p-value = 0.003), and were more often complicated by HPS or HPS-like systemic illness (10/18 versus 0/22; Fisher's exact p-value < 0.001), compared to HAVCR2-wild-type SPTCLs. Survival analysis using log-rank test revealed that HAVCR2-mutated SPTCLs had shorter progression-free survival, though statistical significance was not achieved (p-value = 0.081) WES results did not show

recurrent genetic alteration other than HAVCR2 Y82C in 4 out of 8 patients. Mutations in genes involved in T/NK cell-associated inflammation (PVRL1, PVRL2, TICAM1, GZMA), epigenetic modification (BAZ2A, KMT2C, KMT2D, SETD1A), JAK-STAT signaling (IFNL2, PIAS3), and NF-kB pathway (PDCD11) were observed in individual cases. Gene set enrichment analyses (GSEA) using RNA-seq results showed significant enrichment of pathways involving TNF-alpha signaling via NF-kB (FDR q-value = 0.008), hypoxia (FDR q-value = 0.009), IL6-JAK-STAT3 signaling (FDR q-value = 0.026), apoptosis (FDR q-value = 0.121), and MTORC1 signaling (FDR q-value = 0.188) in HAVCR2-mutated SPTCLs. HAVCR2 Y82C hotspot mutation frequently occurs in Korean patients with SPTCL, which was characterized by unique clinicopathologic features. SPTCL with HAVCR2 Y82C was enriched with distinct cellular pathways, which remains to be further validated.

PO-35 Prognostic significance of MYC, BCL2, and BCL6 colocalization at single-cell resolution in DLBCL. Michal Hoppe[1], Shuangyi Fan[2], Patrick Jaynes[1], Phuong Mai Hoang[1], Liu Xin[3], ,Sanjay De Mel[3], Li Mei Poon[3], Esther Chan[3], Joanne Lee[3], Yen Lin Chee[3], Choon Kiat Ong[4], Tiffany Tang[5], Soon Thye Lim[4], Nicholas Francis Grigoropoulos[6], Sheng-Tsung Chang[7], Shih-Sung Chuang[7], Joseph Khoury[8], Hyungwon Choi[9], Wee Joo Chng[10], Siok-Bian Ng[11], Claudio Tripodo[12], Anand D. Jeyasekharan[10]. [1]Cancer Science Institute of Singapore, National University of Singapore, Singapore, Singapore, [2]Department of Pathology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore , [3]Department of Haematology-Oncology, National University Health System, Singapore , Singapore, [4]Division of Cellular and Molecular Research, National Cancer Centre Singapore, Singapore, Singapore, [5]Division of Medical Oncology, National Cancer Centre Singapore, Singapore, Singapore, [6]Department of Haematology, Singapore General Hospital, Singapore, Singapore, [7]Department of Pathology, Chi-Mei Medical Center, Tainan City, Taiwan, [8]Department of Hematopathology, Division of Pathology/Lab Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, [9]Saw Swee Hock School of Public Health, National University of Singapore, Singapore, , Singapore, [10]Cancer Science Institute of Singapore, National University of Singapore; Department of Haematology-Oncology, National University Health System, Singapore, Singapore, [11]Cancer Science Institute of Singapore, National University of Singapore; Department of Pathology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, [12]Tumor Immunology Unit, University of Palermo School of Medicine, Palermo, Italy.
MYC, BCL2, and BCL6 are commonly used markers for immunohistochemistry of Diffuse large B-cell lymphomas (DLBCL). Coexpression of MYC and BCL2 in particular constitutes a subgroup of “double expressor lymphomas” (DEL) with a distinct poor clinical outcome. However, it is not known if MYC and BCL2/BCL6 coexpression occurs in the same cell or in different cells within the tumor, as traditional immunohistochemistry (IHC) is limited by the number of markers that can be simultaneously assessed within formalin-fixed, paraffin-embedded (FFPE) samples. We set out to discover the clinical significance of MYC, BCL2, and BCL6 colocalization at single-cell resolution using multiplexed quantitative immunofluorescence (qIF) based on sequential OPAL-TSA staining and spectral microscopy on the Vectra platform. The initial discovery cohort comprised 90 cases of DLBCL from NUH Singapore with adequate clinical follow-up after R-CHOP therapy. We stratified each DLBCL tumor into 8 “clonal fractions” based on the possible permutations of MYC (M), BCL2 (2), and BCL6 (6) colocalization: M+2+6+, M+2+6-, M+2-6+, M+2-6-, M-2+6+, M-2-6+, M-2+6-, and M-2-6-. Interestingly, even within cases that fit traditional IHC criteria for “positivity” of MYC, BCL2, and BCL6, only a subset of cells within each

case expressed multiple markers concurrently. Using the fraction of each of these clones as a continuous variable, Cox regression analysis revealed that the percentage of M+2+6- cells in a case was most predictive of poor survival. Importantly, the same clonal fraction (M+2+6-) was a significant poor prognostic feature in 2 smaller validation cohorts from SGH Singapore (n=41) and MD Anderson Cancer Centre USA (n=36). The single-cell staining pattern of these markers revealed a stark contrast between healthy tonsil tissue and DLBCL tissue. In the tonsil, colocalization of each marker was nonrandom (mutually exclusive BCL2 positivity in B cells outside the germinal center and BCL6 positivity inside the germinal center), whereas in DLBCL samples the mutual exclusivity pattern noted in the tonsil was lost, leading to a random distribution of colocalization of MYC, BCL2, and BCL6. The random nature of this colocalization allowed us to mathematically predict the “extent” of these 8 subclones from any data set with quantitative data of each single marker (MYC, BCL2, and BCL6). We therefore attempted to evaluate this model in RNA expression datasets of DLBCL cases with clinically annotated data. Remarkably, in concordance with our IF data, the “predicted” M+2+ 6- subgroup consistently was associated with an unfavorable prognosis in 3 independent mRNA datasets (GSE10846 n=233, GSE117556 n=469, GSE32918 n=140). In summary, we have for the first time assessed the expression of MYC, BCL2, and BCL6 at the single-cell level in DLBCL. These results may explain the apparent protective function of BCL6 expression in prior cohort studies of DEL, and provide a quantitative tool for the identification of DLBCL cases with poor survival on R-CHOP.

PO-36 Functional bypass of cell cycle entry checkpoints by MYC T58A mutation in germinal center-derived lymphomas. Jongkuen Lee, David Dominguez-Sola. Icahn School of Medicine at Mount Sinai, New York, NY.

Somatic missense mutations targeting MYC's coding sequence are found in >50% of Burkitt lymphomas and in a small fraction of diffuse large B-cell lymphomas. These mutations typically arise in translocated MYC alleles and cluster at specific protein residues, particularly Threonine 58 (T58). Previous studies in non-B cell models suggested that MYC mutations enhance oncogenic properties by escaping MYC-induced apoptosis. However, the functional impact of these mutations on the biology of germinal center (GC) B cells, where these mutations arise, has never been investigated. In GC B cells, MYC expression is induced by positive selection signals during cognate interactions with T follicular helper cells, concurrent with activation of PI3K and mTOR signaling. PI3K and mTOR are activated upon CD40 and B-cell receptor co-engagement and are critically required for positive selection and clonal expansion of GC B cells. Using newly generated MYC T58A knock-in mouse models, we found that MYC T58A mutation allows B cells to bypass a cell cycle entry checkpoint dependent on PI3K and mTOR activities. Exposure of wild-type (WT) B cells to rapamycin or, less markedly, to PI3K inhibitors, prevented the proliferation upon CD40 and IL4 stimulation ex vivo (a cytokine combination mimicking T-cell help) whereas MYC T58A mutant B cells divided multiple times and maintained adequate rates of active biosynthesis. Similarly, administration of rapamycin to immunized WT mice collapsed formed GCs while MYC T58A GCs maintained normal size and proliferation rates in vivo. These phenotypes were in part explained by the effects of PI3K/mTOR signaling on GSK3-beta, a kinase that normally phosphorylates MYC T58 to control protein turnover. Differences in metabolic and gene expression profiles between MYC WT and T58A cells offered additional insights into the mechanisms by which this mutation bypasses PI3K/mTOR checkpoint. Finally, we found that GC B cell-specific overexpression of MYC T58A, resulted in abnormally enlarged GCs upon immunization, a phenotype common to lymphoma models. Altogether, these results indicate that MYC somatic mutations confer competitive advantages to B cells by bypassing a strict requirement for upstream signals during cell cycle entry, which may support the clonal expansion of MYC mutant B cells during GC responses. These results have important translational implications, particularly given the increasing interest in the potential use of PI3K and/or mTOR inhibitors in the treatment of lymphomas.

PO-38 Genomic subtypes correlate with the risk of central nervous system (CNS) recurrence in diffuse large B-cell lymphoma (DLBCL). Thomas A. Ollila[1], Habibe Kurt[2], Jozal Waroich[3], John Vatkevich[3], Ashlee Sturtevant[3], Nimesh Patel[2], Diana O. Treaba[1], Patrycja M. Dubielecka[1], Adam J. Olszewski[1]. [1]Warren Alpert Medical School of Brown University, Providence, RI, [2]Molecular Pathology, Rhode Island Hospital, Providence, RI, [3]Rhode Island Hospital, Providence, RI.

Clinicopathologic features are poor at predicting the risk of CNS relapse after initial treatment in DLBCL, or at guiding CNS prophylactic therapy. However, mutational profiles may correlate with primary extranodal disease, including primary CNS lymphoma (Ollila T, Curr Treat Options Oncol 2018). We hypothesized that specific genomic profiles in DLBCL would also correlate with CNS relapse or with systemic-only (non-CNS) relapse. We examined 26 DLBCLs with CNS-only (n=13) or systemic-only relapse (n=13) using a 592 gene next-generation sequencing (NGS) assay (Illumina NextSeq, average coverage depth >750x; Caris Life Sciences) performed on formalin-fixed tissue. We also determined cell of origin (COO) by IHC; MYC/BCL2/BCL6 rearrangements by FISH, and karyotype by cytogenetics. We used the novel LymphGen genomic DLBCL classifier (Wright GW, Cancer Cell 2020) to group tumors into the MCD, ST2, EZB, BN2, and A53 subtypes. We then constructed a simplified hierarchical classifier (hc) usable with common clinical multigene NGS panels to identify 3 relevant subtypes: (1) hc-MCD subtype (defined as MYD88L265P or >2 mutations in CD79B, PIM1, ETV6, BTG1, TBL1XR1, or PRDM1), (2) hc-TP53 subtype characterized by TP53 mutations +/- complex karyotype, and (3) hc-GCB subtype with ≥2 mutations in BCL2, CREBBP, EZH2, KMT2D, TNFRSF14, GNA13, MEF2B, or PTEN. We compared prevalence of these subtypes between our groups and a reference dataset of unselected DLBCL tumors combining data from Chapuy (Nat Medicine 2018; n=135; dbGaP phs000450) and Reddy (Cell 2018; n=1001; EGA: EGAS00001002606). There was no difference between DLBCL with CNS-only or systemic recurrence in any standard clinicopathologic characteristics, including COO, FISH, or karyotype. The LymphGen MCD subtype was significantly more frequent in DLBCL with CNS-only relapse compared with reference datasets (39% vs. 7%, P=.001), but not in DLBCL with systemic relapse (P=.05). Conversely, the ST2 subtype was more frequent in DLBCL with systemic relapse (23% vs. 3%, P=.010) and completely absent in CNS relapse (0%). The A53 or EZB subtypes did not significantly differ between any groups. The simplified NGS classifier identified 96% of LymphGen MCD cases and 100% of A53 cases. The hc-MCD subtype was also significantly associated with CNS relapse (46% vs. 18%, P=.018) but not with systemic relapse (P=.26), whereas hc-TP53 and hc-GCB subtypes did not differ from the reference dataset (P=.15 and P=.70, respectively). Our data demonstrate that the MCD DLBCL subtype is specifically associated with the risk of CNS relapse. Full molecular

classification of DLBCL is not yet incorporated into current clinical practice, as it requires integration of single nucleotide, structural chromosomal variants, and copy alterations. We show that a meaningful molecular predictor signature for the MCD subtype can be obtained from clinically validated multigene NGS assays. If validated, this signature may prove useful for selecting patients for CNS-directed prophylactic therapy.

PO-40 FOXO1 mutations mimic positive selection signals to promote germinal center B-cell expansion and lymphomagenesis. Mark P. Roberto[1], Gabriele Varano[1], Rosa Viñas-Castells[1], Antony B. Holmes[2], Rahul Kumar[2], Pedro Farinha[3], David W. Scott[3], David Dominguez-Sola[1]. [1]Icahn School of Medicine at Mount Sinai, New York, NY, [2]Columbia University Medical Center, New York, NY, [3]Center for Lymphoid Cancer, Vancouver, BC, Canada.

The transcription factor FOXO1 directs germinal center (GC) polarity and supports affinity maturation by modulating immune activation programs in GC B cells. Recurrent somatic mutations targeting FOXO1 in GC-derived B-cell non-Hodgkin lymphomas (B-NHL) are thought to disrupt its negative regulation by PI3K and function as gain-of-function, constitutively active alleles. Herein, we demonstrate that B-NHL FOXO1 mutants are instead hypomorphic alleles encoding proteins with altered transcriptional activities. Analysis of Foxo1 mutant mouse models, engineered cell lines, and primary samples shows that B-NHL FOXO1 mutations induce simultaneous hyperactivation of Stress Activated Protein Kinase -SAPK/JNK and Phosphoinositide 3-kinase -PI3K/AKT signaling pathways and gene expression programs characteristic of GC B cells undergoing positive selection. These alterations confer mutant B cells with a competitive advantage in response to key immune signals, leading to abnormal amplification of GC responses. Moreover, we find that FOXO1 mutant-driven transcriptional programs are prevalent in human B-NHL and predict poor clinical outcomes. These results imply the frequent co-option of GC positive selection programs in the pathogenesis of GC-derived lymphomas.

PO-41 Expression of Vav1-Myo1F fusion affects T-cell differentiation and induces T-cell lymphoma. Jose Rodriguez Cortes[1], Robert Albero[1], Ioan Filip[2], Juan Angel Patino[2], Anisha R. Cooke[1], Wen-Hsuan Lin[3], Anouchka P. Laurent[1], Bobby B. Shih[1], Aidan S. Quinn[1], Raul Rabadan[2], Adolfo Ferrando[1], Teresa Palomero[1]. [1]Institute for Cancer Genetics, Columbia University, New York, NY, [2]Department of Systems Biology, Columbia University, New York, NY, [3]Department of Pathology and Cell Biology, Columbia University, New York, NY.

Peripheral T-cell lymphomas (PTCL) are highly aggressive, malignant hematologic tumors that arise from clonal proliferation of mature T cells. Among these, angioimmunoblastic T-cell lymphoma (AITL), and peripheral T-cell lymphomas not otherwise specified (PTCL, NOS) account for >45% diagnoses, show limited response to intensified chemotherapy treatment, and are associated with dismal survival. Genomic studies from our group have uncovered recurrent mutations and novel cancer-associated gene fusions involving the guanine nucleotide exchange factor VAV1 in AITL and PTCL, NOS. Interestingly, mutation co-occurrence analysis in AITL and PTCL, NOS showed significant mutual exclusivity of VAV1 genomic alterations and the highly prevalent RHOA mutations (p-value 0.0142), supporting a common mechanism of action. Gene fusions involving VAV1 are characterized by the substitution of their auto-inhibitory C-terminal SH3 domains by different domains from their fusion partners, leading to increased activation of VAV1-dependent signaling pathways. Among them, the VAV1-MYO1F fusion shows the strongest increase in VAV1 activity and activation of the mitogen-activated protein kinase (ERK1/2), c-Jun N-terminal kinase (JNK), and nuclear factor of activated T-cells (NFAT) pathways. To study the role of VAV1-MYO1F, we engineered a conditional knock-in mouse that expresses the Vav1-myo1f fusion in CD4+ T-cells. Expression of Vav1-myo1f in CD4 T cells induces cell activation and alterations in T-cell specification, associated with upregulation of master transcription factors involved in helper T-cell cell differentiation. Moreover, Vav1-Myo1f increased CD4+ T-cell survival upon cytokine withdrawal and enhanced Vav1 phosphorylation and activation of the MAPK pathway, resulting in increased cell activation and proliferation both in vivo and in vitro in response to TCR engagement. Notably, expression of Vav1-Myo1f fusion in CD4+ T cells is sufficient to induce development of fatal malignant lymphomas with a latency of 6-14 months. Histologic examination showed disrupted splenic architecture associated with clonal expansion of CD4+ cells indicative of T-cell lymphoma with PTCL, NOS phenotype. Similar results were obtained in a genetic model that combined the expression of Vav1-myo1f with the deletion of the Tet2 epigenetic regulator. In both genetic models, tumor cells specifically present a memory cell-associated immunophenotype (CD44+ CD62L-) and characteristic Th2-like features including increased expression of the transcription factors Gata3 and c-

Maf and the IL4 and Il10 cytokines. Overall, these results demonstrate a direct oncogenic role for Vav1-Myo1f in the pathogenesis of PTCL, associated with deregulation of T-cell specification and of signaling programs critical for the control of T-cell proliferation.

PO-42 TBL1XR1 mutations drive extranodal lymphomagenesis by inducing a protumorigenic memory B-cell fate. Leandro Venturutti[1], Matt Teater[1], Andrew Zhai[2], Amy Chadburn[3], Leena Babiker[3], Daleum Kim[1], Louis Staudt[4], Michael Green[5], Pedro Farinha[6], Andrew Weng[6], Christian Steidl[6], Ryan Morin[6], David Scott[6], Gil Privé[2], Ari Melnick[1]. [1]Weill Cornell Medicine, New York, NY, [2]University of Toronto, Toronto, ON, Canada, [3]New York-Presbyterian, New York, NY, [4]National Cancer Institute, Bethesda, MD, [5]The University of Texas MD Anderson Cancer, Houston, TX, [6]BC Cancer Agency, Vancouver, BC, Canada.

The most aggressive B-cell lymphomas frequently manifest extranodal distribution and carry somatic mutations in the poorly characterized gene TBL1XR1. Here, we show that TBL1XR1 mutations skew the humoral immune response towards generating abnormal immature memory B-cells (MB), while impairing plasma cell differentiation. At the molecular level, TBL1XR1 mutants co-opt SMRT/HDAC3 repressor complexes towards binding the MB cell transcription factor (TF) BACH2, at the expense of the germinal center (GC) TF BCL6, leading to pre-memory transcriptional reprogramming and cell-fate bias. Upon antigen recall, TBL1XR1 mutant MB cells fail to differentiate into plasma cells and instead preferentially reenter new GC reactions, providing evidence for a cyclic re-entry lymphomagenesis mechanism. Ultimately, TBL1XR1 alterations lead to a striking extranodal immunoblastic lymphoma phenotype that mimics the human disease. Both human and murine lymphomas feature expanded MB-like cell populations, consistent with a MB-cell origin and delineating an unforeseen pathway for malignant transformation of the immune system.

PO-43 Targeting DNA repair in EZH2 gain-of-function diffuse large B-cell lymphoma.
Danielle Johnson, Sneha Patel, Srividya Bhaskara. Huntsman Cancer Institute, Salt Lake City, UT.

An FDA-approved HDAC inhibitor for B-cell lymphoma is currently not available. While pan-HDAC inhibitors (HDIs) hold much promise, they display unwanted toxic side effects, because they hit multiple enzymes belonging to both Class I and/or Class II HDAC families. Class I HDAC (HDAC 1, 2, 3, and 8) are the key targets of many pan-HDIs, including the FDA-approved drugs SAHA and depsipeptide. Diffuse large B-cell lymphoma (DLBCL), a type of non-Hodgkin's lymphoma, is the most common lymphoid malignancy of all adult lymphomas. These lymphoma cells acquire chemoresistance and consequently have a high relapse rate, making it a hard-to-treat disease. About 30% of germinal center (GC)-derived DLBCLs have a recurrent somatic gain-of-function mutation (GOF) in the polycomb-group oncogene EZH2. EZH2 codes for the methyltransferase that catalyzes histone H3 lysine-27 trimethylation (H3K27me3) and promotes lymphomagenesis. Increased levels of EZH2 are purported to also contribute to chemoresistance in EZH2GOF DLBCL cells. EZH2-catalyzed H3K27me3 localizes to double-strand breaks (DSBs) and participates in DNA repair. Our published and recent unpublished results showed that selective inhibition of HDAC1,2 decreases H3K27me3 at DSBs during DSB repair, alters the H3K27me3/H3K27ac switch specifically at DSB sites, and impairs EZH2-mediated downstream repair signaling. In addition to H3K27me3, we found that EZH2GOF DLBCL cells overexpress B-lymphoma and BAL-associated protein (BBAP) enzyme that also confers chemoresistance to EZH2GOF DLBCL cells. BBAP is an E3 ubiquitin ligase and catalyzes H4K91ub1, which is also involved in DNA repair. Our published results showed that selective inhibition of HDAC1,2 decreases H3K27me3 at DSBs and H4K91ub1 during DSB repair by increasing H3K27ac and H4K91ac. This alteration of H3K27me3/H3K27ac and H4K91ub1/H4K91ac switches results in decreased DSB repair, an induction of DNA damage response, and cytotoxic effects, providing a novel mechanism by which HDAC1,2 inhibition can overcome survival advantage in chemoresistant DLBCL cells. The consequences of the altered H3K27me3/H3K27ac switch on DNA repair events are still not understood. How HDAC1,2 acts in concert with enzymes that modulate the chromatin dynamics during DSB repair in EZH2GOF DLBCL is not clear. We hypothesize that HDAC1,2 inhibition specifically impairs DSB repair used by EZH2GOF DLBCL cells at various steps (local transcriptional repression at DSBs during active repair and the chromatin remodeling required for DNA repair). We will present our recent results obtained from the mechanistic studies performed to address the above-mentioned questions. We are also investigating how HDAC1,2 functions in normal germinal center functions to complement our lymphoma studies.

PO-44 Past infection and risk of adolescent/young adult HL. Maryam Saleh[1], Amie Hwang[1], Julia Simon[2], Thomas M. Mack[1], Tim Waterboer[2], Wendy Cozen[1]. [1]Keck School of Medicine of USC, University of Southern California, Los Angeles, CA, [2]German Cancer Research Center, Heidelberg, Germany.

Background: Adolescent/young adult (AYA) Hodgkin lymphoma (HL) risk is increasingly linked to a deficit of microbial exposure in early life, especially from fecal-oral transmission.

Methods: In this cross-sectional study, we examined past exposure to 15 infections in serum samples from 291 AYA HL cases and 194 of their unaffected family members. We measured antibodies to HSV1, HSV2, VZV, EBV, HHV7, HCMV, HHV6, KSHV, *S. gallolyticus, F. nucleatum, T. gondii, H. pylori*, Rubella virus, and Parvovirus B19 using a multiplex serology bead array (Luminex). A positive history was determined by a titer threshold specific for each infection. A logistic regression model was used to calculate the odds ratio (OR) of AYA HL risk for each infection separately adjusted for sex and age at sampling. We then applied the same logistic regression model, adding an adjustment for family.

Results: Evidence of past HHV6 infection was associated with an increased risk of AYA HL (OR= 1.80; 95% CI= 1.06-3.07). Having antibodies to *H. pylori* was associated with a decreased risk (OR= 0.49; 95% CI= 0.25-0.98). An increasing number of fecal-oral infections (*H. pylori, T. gondii*) was associated with a decreasing risk of AYA HL (ptrend<0.001), while an increasing number of respiratory-transmitted infections (Rubella virus, Parvovirus) was not significantly associated (ptrend=0.062). After adjusting for family, infection with Parvovirus was the only significant risk factor for AYA HL (OR=3.8,1.06-13.6).

Conclusions: We show preliminary evidence for an inverse association between fecal-oral transmitted agents and risk of AYA HL, supporting the hypothesis that a deficit of early life fecal-oral transmitted microbes may be associated with increased risk. HHV6 and Parvovirus were associated with an increased risk, possibly due to a subclinical immune deficiency or an unknown mechanism. In the final presentation, we will add data from 40 additional unaffected family members currently missing age, and results by histology and EBV status.

PO-45 Robust detection of translocations in lymphoma FFPE samples using Targeted Locus Capture-based sequencing. Amin Allahyar[1], Mark Pieterse[1], Joost Swennenhuis[2], Tjitske Los-de Vries[3], Mehmet Yilmaz[2], Roos Leguit[4], Ruud Meijers[4], Nathalie Hijmering[3], Daphne de Jong[3], Bauke Ylstra[3], Robert van der Geize[5], Joost Vermaat[6], Arjen Cleven[7], Tom van Wezel[7], Arjan Diepstra[8], Leon van Kempen[8], Karima Hajo[2], Harma Feitsma[2], Marieke Simonis[2], Max van Min[2], Erik Splinter[2], Wouter de Laat[1]. [1]Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, Netherlands, [2]Cergentis, Utrecht, Netherlands, [3]Amsterdam UMC-Vrije Universiteit Amsterdam, Department of Pathology and Cancer Center Amsterdam, Amsterdam, Netherlands, [4]University Medical Centre Utrecht, Department of Pathology, Utrecht, Netherlands, [5]Laboratorium Pathologie Oost-Nederland, Hengelo, Netherlands, [6]Leiden University Medical Centre, Department of Hematology, Leiden, Netherlands, [7]Leiden University Medical Centre, Department of Pathology, Leiden, Netherlands, [8]University Medical Centre Groningen, Department of Pathology and Medical Biology, Groningen, Netherlands.

Chromosomal translocations with immunoglobin (IG) loci are the classic drivers in a large subset of B-cell lymphomas. Detection of these translocations is important for confirmation of diagnosis and for prognosis and therapy decisions. Currently, molecular diagnosis of translocations in lymphomas is not addressed well by next-generation sequencing (NGS). The standard method for detection of translocations is fluorescence in situ hybridization (FISH), which is labor intensive and can be difficult to interpret. There is a need for a robust technology that can be standardized. Targeted Locus Capture (TLC) selectively enriches and sequences entire genes based on the crosslinking of physically proximal sequences, and thereby enables complete sequencing of genes of interest, including detection of large structural variants. Because the technology is based on the crosslinking and fragmenting of DNA, it has particular advantages in the analysis of formalin-fixed, paraffin-embedded (FFPE) samples in which DNA is inherently crosslinked and fragmented. In order to validate the FFPE-TLC technology as a novel approach for translocation detection in lymphoma samples, we have developed a panel assay containing genes with frequent translocations (MYC, BCL2, BCL6, IG loci). With this assay we have analyzed >140 lymphoma and control FFPE samples of variable input amounts and qualities that had previously been analyzed with FISH, and a subset also with standard targeted NGS. Good concordance with FISH results was observed for both translocation-positive and -negative samples. In 10 cases for which FFPE-TLC analysis resulted in a different finding than FISH, discordance could be explained by higher sensitivity of FFPE-TLC or by inconclusive FISH results. In a specific case, FFPE-TLC detected a small-distance rearrangement on chromosome 3 that caused a BCL6 fusion but led to insufficient and therefore undetectable break-apart with FISH.

Secondly, the FFPE-TLC approach was tested on a set of 19 B-cell lymphoma FFPE samples that had previously been analyzed using standard targeted NGS and FISH and was enriched for discordant results between these methods. FFPE-TLC-based NGS enables more robust translocation calling as the detection relies on broad sequencing coverage across the translocation partner rather than on breakpoint sequences only. In 3 cases, FFPE-TLC could proof false negative calls in standard targeted NGS due to breakpoints located in regions difficult to capture or to sequence. In 1 case, standard targeted NGS had made a false positive call on a breakpoint sequence that was shown to be caused by a small insertion rather than a genuine translocation. This study shows that FFPE-TLC promises to be a robust alternative for FISH analysis and standard targeted NGS procedures in lymphoma diagnostics and in other cancers with frequent structural variants. The FFPE-TLC approach enables a single, DNA-based NGS test detecting both small mutations and translocations.

PO-46 Mechanisms of resistance to the PI3K inhibitor copanlisib in marginal zone lymphoma. Alberto J. Arribas[1], Sara Napoli[1], Luciano Cascione[1], Eugenio Gaudio[1], Roberta Bordone-Pittau[2], Marilia Barreca[3], Giulio Sartori[1], Chiara Tarantelli[1], Filippo Spriano[1], Andrea Rinaldi[1], Anastasios Stathis[2], Georg Stussi[2], Davide Rossi[2], Emanuele Zucca[2], Francesco Bertoni[1]. [1]Institute of Oncology Research, Faculty of Biomedical Sciences, USI, Bellinzona, Switzerland, [2]Oncology Institute of Southern Switzerland, Bellinzona, Switzerland, [3]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy.
Background: PI3Kδ is expressed in B cells and has a central role in the B-cell receptor signaling. Copanlisib is a highly selective PI3Kδ and PI3Kα inhibitor, and it is currently under clinical development in indolent lymphomas including marginal zone lymphoma (MZL). Copanlisib is Food and Drug Administration (FDA) approved for the treatment of patients with relapsed or refractory follicular lymphoma. Nevertheless, a subset of patients can eventually relapse due to acquired resistance. A better understanding of resistance mechanisms could help to design improved therapies; hence, we generated MZL cell lines resistant to copanlisib.
Materials and Methods: Cells were kept on copanlisib (IC90) until acquisition of resistance (RES) or with no drug (parental, PAR). Stable resistance was confirmed by MTT assay after 3 weeks of drug-free culture. Multidrug resistance phenotype was ruled out by confirming sensitivity to vincristine. Cells underwent transcriptome profiling (RNA-Seq) and immunophenotypic analysis.
Results: The RES models were obtained from VL51 cell line with over 50-fold times higher IC50s than PAR counterparts. Of note, the copanlisib-resistant lines showed decreased sensitivity to other PI3K inhibitors such as duvelisib (50-fold) and idelalisib (5-fold) and to the BTK inhibitor ibrutinib (15-fold), suggesting that the mechanism observed here might drive resistance to other downstream B-cell receptor inhibitors. Gene expression profiles of RES showed the upregulation of cytokine signaling (IL1A, IL1B, CXCR4), NFkB (LTA, TNF), MAPK (RASGRP4, RASGRP2), and JAK-STAT (STAT3, JAK3) signaling pathways and negative regulators of apoptosis (CD44, JUN). Conversely, repressed genes in RES were involved in cell adhesion (ITGA4, ITGB1), antigen presentation (HLAs), and IFN response (PARP12, GBP6). Consistent with the overexpression of antiapoptotic signaling genes, RES cells exhibited also resistance to the BCL2-inhibitor venetoclax, either as a single as in combination with copanlisib. Flow cytometry confirmed the CXCR4 upregulation and the downregulation of CD49d (ITGA4), paired with reduced CD20 and CD81 surface expression. In accordance, addition of a CXCR4 inhibitor overcame resistance to copanlisib.
Conclusions: We created a model of secondary resistance to the PI3K inhibitor copanlisib, derived from an MZL cell line. This model will help in clarifying mechanisms of resistance to the drug and to evaluate alternative therapeutic

approaches. Indeed, we already identified novel potential targets, such as IL1 and CXCR4, that might be exploited in overcoming resistance to copanlisib and are worthy of further investigation.

PO-47 BTM-3566 induces complete tumor regression in diffuse large B-cell lymphoma: Regulation by the eIF2α kinase "heme-regulated inhibitor" and the mitochondrial protein FAM210B. Matthew Kostura, Jedd Levine, Alan Cooper, Andy Anantha, Michael Stocum, Michael Luther. Bantam Pharmaceutical, New York, NY. BTM-3566 is a novel small-molecule compound with broad anticancer activity in hematologic and solid cancers, with exceptional activity against diffuse large B-cell lymphoma (DLBCL). The compound's mechanism of action (MoA) is dependent on activation of the ATF4 integrated stress response (ISR) by the eIF2 kinase heme-regulated inhibitor (EIF2AK1, HRI) resulting in translational repression, increased expression of ATF4, and apoptosis. To evaluate HRI's role in mediating the ISR and apoptosis by BTM-3566 in DLBCL, homozygous HRI knockout BJAB cells were generated. HRI gene deletion resulted in inhibition of BTM-3566 induction of ATF4 expression, reduction of cell growth inhibition, and abrogation of apoptosis. Expression of the antiapoptotic proteins Mcl1 and Bcl6 was reduced following treatment with BTM-3566 in wild-type BJAB cells but not in HRI KO BJAB cells. To further elucidate BTM-3566 MoA, an activity screen was performed across 407 hematologic and solid cancer cell lines. Expression of FAM210B, a mitochondrial membrane protein, emerged as a key predictor of response to BTM-3566. FAM210B expression was *negatively* correlated with response to compound; the cell lines most responsive to BTM-3566 were the B-cell malignancies, notably DLBCL, which have the lowest expression of FAM210B among all cancers. The relevance of FAM210B to BTM-3566 activity was confirmed by overexpression of FAM210B in the DLBCL cell line BJAB and the Burkitt lymphoma cell line RAMOS. Overexpression of FAM210B abrogated BTM-3566 antiproliferative activity in these cell lines. The specificity of BTM-3566 activity regulation by FAM210B was demonstrated by comparison to treatments known to activate the ATF4 ISR through eIF2α kinases; tunicamycin (PERK), amino acid deprivation (GCN2), ONC201, and bortezomib (HRI) were tested in FAM210B-overexpressing BJAB cells. FAM210B overexpression inhibited BTM-3566-mediated induction of ATF4 protein expression but had no effect on ATF4 induction by the other treatments. The antitumor activity of BTM-3566 in DLBCL was evaluated in patient-derived DLBCL xenograft (PDX) mouse models. Eight models (n=3/model) were tested, which included GCB and ABC subtypes, and as typical for DLBCL, all had low FAM210B expression. Treatment was initiated with BTM-3566 at 20 mg/kg po daily after tumor volume reached 200 mm^3. Complete tumor regression was observed in 79% of the mice with no evidence of tumor progression during the 21-day treatment period. Treatment was well tolerated with only mild weight loss noted. In summary, BTM-3566 induces robust complete regressions in DLBCL PDX models via a unique MoA characterized by apoptosis mediated by HRI induction of the ATF4 ISR in an FAM210B-regulated manner. BTM-3566 has the potential to

meaningfully address unmet clinical needs in DLBCL and other B-cell malignancies with low FAM210B expression.

PO-48 Cytotoxic mechanism of a novel transferrin receptor-targeting chemotherapeutic nanocarrier for use in diffuse large B-cell lymphoma. Artavazd Arumov, Piumi Y. Liyanage, Asaad Trabolsi, Evan R. Roberts, Braulio Ferreira, Daniel Bilbao, Roger M. LeBlanc, Jonathan H. Schatz. University of Miami Miller School of Medicine, Miami, FL.

Diffuse large B-cell lymphoma (DLBCL), the most common hematologic malignancy, is an aggressive form of non-Hodgkin lymphoma. Approximately 60% of DLBCL patients achieve long-term disease-free survival from frontline R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone), but patients with relapsed or refractory disease have poor prognosis. Doxorubicin (Dox) remains the most active anti-lymphoma agent, but its use is limited by well-characterized toxicities, including potentially irreversible cardiac damage. Targeted and specific delivery of Dox to tumors to overcome these limitations has been an area of active research. Targeted delivery of Dox via the transferrin receptor (TFR1) has preclinical efficacy in solid malignancies but has yet to be explored in lymphoma. We have uncovered for the first time a significant association between TFR1 expression and poor prognosis in DLBCL. Carbon-nitride dots (CNDs) are third-generation nanocarriers with inherent photoluminescence, which are easily conjugated with a wide variety of substrates. We conjugated Dox and transferrin (TF), the ligand for TFR1, to CNDs to develop CND-Dox-TF (CDT). CDT is uniquely designed to enhance Dox delivery to TFR1-expressing DLBCL tumors while limiting effects on nonmalignant tissues. BJAB and Farage cell lines treated with CDT for 24 hours underwent apoptosis at a significantly lower concentration when compared to Dox (50 nM vs. 1000 nM). Overall, CDT was 1-2 Log10 more potent than Dox against DLBCL cell lines. Co-incubation with 250 uM of antagonist holo-transferrin significantly decreased BJAB and Farage sensitivity to CDT, confirming activity through TFR1. Confocal microscopy of BJAB cells incubated up to 24 hours with 250 nM Dox and 30 nM CDT reveled enhanced nuclear colocalization of Dox at a significantly lower concentration. Prior dose-finding experiments in non-tumor bearing mice identified a CDT safe working dose of 33.0 mg/kg containing 16% moles of Dox in comparison to Dox maximum-tolerated dose (MTD) 3.3 mg/kg. We engrafted 2 groups of 10 NSG mice with a Dox-sensitive high-TFR1 expressing patient-derived xenograft (PDX) tumor. Mice were dosed with CDT and Dox on day 0, 14, and 24. CDT demonstrated similar anti-lymphoma efficacy with reduced toxicity: CDT-treated mice displayed no significant decline in body weight, which is characteristic and was observed following each single-agent Dox treatment. Histology analyses revealed little to no obvious damage to CDT-treated nonmalignant tissues, including myocardium. With a working dose of CDT identified, we now have under way clinically relevant R-CHOP vs. R-nanoCHOP (CDT substituted for Dox) assessments in our PDX mouse model. Capitalizing on the unique design, we

expect to observe improved anti-lymphoma efficacy, with decreased toxicities and an improvement in overall survival. In sum, we provide mechanistic insight for novel DLBCL nano-chemotherapy and illustrate preclinical efficacy for a promising new therapeutic approach.

PO-49 Discovery of JNJ-67856633: A novel, first-in-class MALT1 protease inhibitor for the treatment of B-cell lymphomas. Ulrike Philippar[1], Tianbao Lu[1], Lorena Fontan[2], Nele Vloemans[1], Mariette Bekkers[1], Luc van Nuffel[1], Marcello Gaudiano[1], Katarzyna Wnuk-Lipinska[1], Bas-Jan Van Der Leede[1], Katie Amssoms[1], Kristof Kimpe[1], Bart Medaer[1], Tony Greway[1], Yann Abraham[1], Max Cummings[1], Emanuele Trella[1], Greet Vanhoof[1], Weimei Sun[1], Jan Willem Thuring[1], Peter Connolly[1], Haopeng Rui[1], Sriram Balasubramanian[1], John Gerecitano[1], Ari Melnick[2], Ricardo Attar[1]. [1]Janssen R&D, Beerse, Belgium, [2]Weill Cornell Medicine, New York, USA.

Introduction: Constitutive activation of the classical nuclear factor kappa-light-chain-enhancer of activated B cells (NF κB) pathway is a clear driver of B-cell lymphomas, especially the aggressive activated B-cell (ABC) subtype of diffuse large B-cell lymphoma (DLBCL). Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is a key mediator of the classical NF-κB signaling pathway downstream of B-cell receptor and T-cell receptor. MALT1 possesses two functions: a scaffolding function to recruit NF-κB signaling proteins and a protease function to cleave and inactivate inhibitors of the NF-κB signaling pathway.

Methods: Using a high-throughput screen followed by iterative structure-activity relationship (SAR) analyses, the MALT1 inhibitor JNJ-67856633 was identified. JNJ-67856633 was evaluated using biochemical, cellular in vitro, in vivo tumor efficacy and safety models.

Results: JNJ-67856633 is a potent, selective, allosteric inhibitor of MALT1 protease activity as measured by biochemical assays or downstream cellular cytokine readouts (IL6/10) or direct MALT1 substrate cleavage (RelB, BCL10). The compound inhibits proliferation of activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL) cell lines bearing CD79b or CARD11 mutations as well as models mimicking resistance to covalent Bruton's tyrosine kinase (BTK) inhibitors. Furthermore, combination effects were observed in CD79b cellular ABC-DLBCL models when JNJ-67856633 was combined with a BTK inhibitor. JNJ-67856633 showed activity in organoid cultures derived from ABC-DLBCL patients. JNJ-67856633 leads to potent in vivo pharmacodynamic shutdown in CD79b- as well as CARD11-mutant ABC-DLBCL models as measured by serum IL10 or uncleaved BCL10 levels in tumors. JNJ-67856633 exhibits potent tumor growth inhibition in two human DLBCL xenograft models, OCI Ly3 and OCI Ly10. In addition, >5 patient-derived DLBCL xenografts were evaluated and activity in mutation selected models was observed. To address the role of MALT1 inhibition in T cells, primary human T cells derived from normal healthy volunteers were treated with JNJ-67856633 in vitro. Dose-dependent inhibition of the generation of Tregs (CD4+CD25+FoxP3+) following CD3/28 stimulation was observed upon treatment with JNJ-67856633, suggesting a potential immune-modulatory role of MALT1 inhibition.

Conclusions: Phase 1 clinical trials assessing the safety and efficacy of JNJ-67856633 initiated in 2019. JNJ-67856633 is a combination partner for BTK inhibitors and a promising treatment option for BTKi-resistant tumors, with demonstrated preclinical activity in CARD11 mutant tumors. In addition to ABC-DLBCL, a MALT1 inhibitor is a promising treatment option for patients with CLL, MCL, WM, and FL whose tumors have been shown to be sensitive to inhibition of BTK. MALT lymphomas, characterized by MALT1 and BCL10 translocation, represent another attractive target for MALT1 inhibition.

PO-50 Phophatases modulate resistance to ALK inhibitors in anaplastic large-cell lymphoma. Elif Atabay[1], Qi Wang[1], Taek-Chin Cheong[1], Nina Prokoph[2], Chiara Ambrogio[3], Ines Mota[1], Achille Pich[3], Enrico Patrucco[3], Claudia Voena[3], Roberto Chiarle[1]. [1]Department of Pathology, Boston Children's Hospital, Harvard Medical School, Boston, MA, [2]University of Cambridge, Cambridge, United Kingdom, [3]Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy.
Introduction: Anaplastic large-cell lymphomas (ALCL) frequently carry oncogenic fusions involving the anaplastic lymphoma kinase (ALK) gene. The ALK fusions activate several signaling pathways, promoting cell growth, migration, and survival. Chemotherapy is the standard treatment for ALCL patients, but about 30% of patients relapse. Targeting ALK using tyrosine kinase inhibitors (TKIs) has shown promising results, and the FDA recently granted breakthrough therapy designation to crizotinib for use in patients with relapsed/refractory ALK+ ALCL. However, resistance to crizotinib develops in ALK+ ALCL patients secondary to ALK mutations or unknown mechanisms. In this study, we aimed at elucidating unknown bypass mechanisms of crizotinib resistance in ALK+ ALCL.
Methods: We used Genome-wide CRISPR-Cas9 Knockout Screening (GeCKO.v2) to identify candidate genes that contribute to resistance to crizotinib. Four different ALCL cell lines were infected with Lenti-GeCKO libraries. After treatment with crizotinib for 14 days to select for resistant cells, next-generation sequencing was performed on crizotinib-resistant cells to identify candidate resistance genes. Top candidates were selected for validation assays and further analyses.
Results: Genomic loss-of-function screens identified two phosphatases, PTPN1 and PTPN2, in all ALCL cell lines as consistent top hits driving resistance to crizotinib. Functional validation of these candidate genes showed that single loss of either PTPN1 or PTPN2 generates immediate resistance to crizotinib and other ALK TKIs such as alectinib and lorlatinib. Consistently, RNA-seq in patients who developed resistance to crizotinib showed downregulation of PTPN1 or PTPN2 expression. By multiple assays, we demonstrate that PTPN1 and PTPN2 are phosphatases that de-phosphorylate ALK, thereby regulating its overall phosphorylation levels and activity. In addition, we found that PTPN1, but not PTPN2, is also a phosphatase of SHP2, a key mediator of oncogenic ALK signaling. Downstream signaling analysis showed that deletion of PTPN1 or PTPN2 induces resistance to crizotinib by hyperactivating the MAPK and JAK/STAT pathways. A treatment that combined crizotinib and a recently developed SHP2 inhibitor (SHP099) completely blocked the sustained MAPK activation and reverted crizotinib resistance in vitro and in vivo.
Conclusions: We discovered that PTPN1 and PTPN2 are ALK phosphatases that control sensitivity to ALK TKIs in ALCL. Combined inhibition of SHP2 is a potential therapeutic approach to overcome resistance to ALK TKIs in ALCL.

PO-51 Drug combination analytics platform for accurate prediction of treatment response in refractory and relapsed lymphomas. Jasmine Goh[1], Sanjay de Mel[2], Anand D. Jeyasekharan[2], Edward K.H. Chow[1]. [1]National University of Singapore, Singapore, Singapore, [2]National University Hospital, Singapore, Singapore.

While 5-year survival rates for non-Hodgkin lymphoma have improved with effective first-line therapies, refractory and relapsed lymphomas continue to suffer from poor response rates and low median overall survival. The increased availability of targeted therapies has been critical to improving response rates in lymphomas. As the number of therapeutic options increases, however, identifying the most appropriate patient-specific drug combination from among a range of available drug sets is virtually impossible by conventional methods due to the large search space. Further complicating this clinical decision is the diversity in interpatient therapeutic responses due to lymphoma patient heterogeneity. Math and AI-based analytical tools are beginning to positively impact all facets of drug development and personalized medicine. Rather than aggregating large datasets from other sources (data repositories, text mining, etc.) to develop predictive models, we developed an experimentally driven small dataset analytics platform, Quadratic Phenotypic Optimization Platform (QPOP), to identify and rank drug combinations from a specific drug set search space. QPOP identifies optimal drug combinations from queried drug sets against specific biologic systems of interests, including patient-derived primary cancer cells. Utilizing small datasets built from drug combination tests designed by orthogonal array composite design, QPOP analyzes drug combination sensitivity data to identify and rank possible drug combinations. Recently, we applied QPOP towards patient-specific clinical decision support applications in refractory and relapsed non-Hodgkin lymphoma. Utilizing 1 million patient-derived primary lymphoma cells for 155 drug combination tests, QPOP ranked 531,441 possible therapeutic options from a 12-drug search set within 6 days of patient sample biopsy. Across a series of refractory and relapsed T-cell lymphoma patients, effective treatment options were accurately predicted that included both standard salvage regimens, such as gemcitabine-dexamethasone-cisplatin, as well as targeted regimens, such as bortezomib-panobinostat. For a hepatosplenic T-cell lymphoma case that had previously progressed following 6 lines of treatment, analysis by QPOP was able to accurately identify patient-specific bortezomib-panobinostat treatment that resulted in a complete response. Furthermore, QPOP-predicted drug combination sensitivity results from our study were compared to historical outcomes of a phase 2 trial of bortezomib-panobinostat in peripheral T-cell lymphoma (NCT00901147), with 20% predicted response in our QPOP-analyzed cohort mirroring 21.7% complete response observed in the previous trial. These results provide evidence that ex vivo drug combination sensitivity platforms may be

useful tools for clinical decision support, for both personalized medical applications as well as enhanced clinical trial patient selection and parallel outcome tracking.

PO-52 Durable and excellent response in patient with Richter's transformation and Del 17p treated with ibrutinib and venetoclax. John McKay, Jonathan Soray, Roger Riley, Victor Yazbeck. Virginia Commonwealth University, Richmond, VA.

Background: Richter syndrome (RS) is defined as the transformation of chronic lymphocytic leukemia (CLL) into an aggressive lymphoma, most often diffuse large B-cell lymphoma (DLBCL). It carries a poor prognosis and is often unresponsive to conventional chemo-immunotherapy. Furthermore, TP53 disruptions have been universally associated with poor prognosis and worse response to conventional chemotherapy. The activated B-cell-like (ABC) subtype of diffuse large B-cell lymphoma is more common in Richter syndrome and is dependent on chronic activation of the B-cell that is mediated by Bruton's tyrosine kinase (BTK), leading to activation of nuclear factor kappa B. Ibrutinib is the first-in-class BTK inhibitor currently approved in several B-cell malignancies. The BCL-2 inhibitor venetoclax has shown activity in a wide array of malignant hematologic processes. Additionally, preclinical data have suggested synergistic activity between these two medications that work in a p53-independent manner. The activity of ibrutinib and venetoclax in RS remains poorly characterized.

Case Presentation: An 82-year-old gentleman, diagnosed with Richter transformation from small lymphocytic lymphoma (SLL), was started on a clinical trial with R-CHOP and brentuximab vedotin with partial response at end of therapy. Repeat biopsy confirmed RS with ABC-subtype and deletion 17p. He was started on single-agent ibrutinib with excellent clinical response. However, the patient experienced transient neutropenia, atrial fibrillation, and gastrointestinal bleeding, but continued ibrutinib given clinical benefit. Patient eventually progressed on single-agent ibrutinib after over three years of therapy. Upon progression, venetoclax was added to ibrutinib therapy and patient has again achieved CR for nearly two years. His most recent PET scan shows no evidence of recurrent disease with a tolerable side effect profile.

Discussion: Richter's syndrome patients carry a poor prognosis with a median survival of 19 months and less durable responses to traditional chemo-immunotherapy regiments. TP53 disruptions are found in 47.1% of patients and are independently associated with worse outcomes. Additionally, there are little to no data on optimal management of patients with RS, especially those with evidence of poor prognosis such as del17p as in this patient. In this case, we present an elderly male who has attained a CR over 5 years with the novel combination of ibrutinib and venetoclax.

Conclusion: This case report demonstrates that the novel combination of ibrutinib and venetoclax represents an important treatment option for patients with RS and TP53 disruptions as it can induce excellent and durable response with tolerable side

effects. This supports further evaluation of novel treatment options in patients with limited proven effective treatment strategies and suboptimal responses to current standard of care.

PO-53 Combined EZH2 and BCL2 inhibitors as precision therapy for genetically defined DLBCL subtypes. Hanna Scholze[1], Regan E. Stephenson[2], Raymond Reynolds[3], Shivem Shah[2], Rishi Puri[4], Matthew R. Teater[5], Herman van Besien[3], Destini Gibbs-Curtis[3], Hideki Ueno[6], Salma Parvin[7], Anthony G. Letai[7], Susan Mathew[3], Ankur Singh[8], Ethel Cesarman[3], Ari Melnick[5], Lisa Giulino-Roth[9]. [1]Department of Pediatrics, Weill Cornell Medical College, New York, NY, [2]School of Biomedical Engineering, Cornell University, Ithaca, NY, [3]Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY, [4]School of Mechanical Engineering, Cornell University, Ithaca, NY, [5]Department of Medicine, Weill Cornell Medical College, New York, NY, [6]Department of Microbiology, Mount Sinai School of Medicine, New York, NY, [7]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, [8]School of Biomedical Engineering, Cornell University; School of Mechanical Engineering, Cornell University, Ithaca, NY, [9]Department of Pediatrics, Weill Cornell Medical College; Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY.

Molecular alterations in the histone methyltransferase EZH2 and the antiapoptotic protein BCL2 frequently co-occur in diffuse large B-cell lymphoma (DLBCL). We hypothesized that EZH2 inhibition and BCL2 inhibition would be synergistic in DLBCL. To test this, we evaluated the EZH2 inhibitor tazemetostat and the BCL2 inhibitor venetoclax in DLBCL cells, 3D lymphoma organoids, and patient-derived xenografts (PDXs). We found that tazemetostat and venetoclax are synergistic in DLBCL cells that harbor both an *EZH2* mutation and a *BCL2/IGH* translocation, as demonstrated by CI values <1 (CI: 0.034, 0.259 and 0.074 in SUDHL-6, WSU-DLCL2, and OCI-Ly1 respectively), but not in wild-type cells. Since cell lines in suspension do not reflect lymph node architecture, we developed a 3D lymphoma organoid culture system that consists of extracellular matrix, lymphoma cells, and stromal cells (Tian et al., Biomaterials 2015). We observed synergy between the two agents in two organoid model systems: 1) OCI-LY1; 2) PDX derived from a DLBCL with *BCL2/IGH* translocation and *EZH2* mutation. To investigate mechanisms of synergy, we evaluated previously published RNA-seq profiles of DLBCL cell lines (n=26) treated with vehicle or tazemetostat to investigate changes in BCL2 family members (Brach et al., Mol Can Ther 2017). Tazemetostat-treated cells showed enhanced expression of proapoptotic BCL2 family members including *BCL2L11* ($p=0.012$), *BMF* ($p<0.001$), and *BCL2L14* ($p=0.002$), suggesting that these may be direct or indirect EZH2 target genes that are de-repressed upon EZH2 inhibition. To assess mitochondrial priming to apoptosis as a result of EZH2 inhibition, we performed BH3 profiling of DLBCL PDX organoids treated with vehicle vs. tazemetostat. Tazemetostat-treated cells had increased priming as evidenced by cytochrome c release in response to general apoptotic signaling peptides BIM and PUMA ($p<0.0001$) and to the BCL2 specific

peptide BAD ($p<0.0001$), suggesting that pretreatment with tazemetostat increases mitochondrial sensitivity to BCL2 inhibition. We next evaluated combination therapy in vivo. In SUDHL-6 xenografts, the combination resulted in attenuation of tumor growth compared to either drug alone (combination vs. venetoclax $p<0.0001$, combination vs. tazemetostat $p=0.0004$) and improved overall survival. In DLBCL PDXs, combination therapy resulted in complete resolutions of tumors, which were durable over time and associated with improved overall survival. Strikingly, after 197 days of follow-up there was no detectable disease in any combination-treated animal. In summary, we demonstrate that combined BCL2 and EZH2 inhibition results in synergistic anti-lymphoma effects. We expect this combination to be especially effective as precision therapy for the newly identified cluster 3/EZB DLBCL subtype, which frequently harbors both *EZH2* and *BCL2* alterations. A clinical trial of this combination is currently in development.

PO-54 Mechanistic consequences of histone-deacetylase inhibition towards sensitizing PD1 blockade-resistant B-cell lymphomas. Xiaoguang Wang, Brittany C. Waschke, Rachel A. Woolaver, Zhangguo Chen, Gan Zhang, Anthony D. Piscopio, Xuedong Liu, Jing H. Wang. University of Colorado, Anschutz Medical Campus, Aurora, CO.

PD1 blockade is effective in a subset of B-cell lymphoma patients (e.g., classical Hodgkin lymphomas); however, most patients do not respond to anti-PD1 therapy. To overcome PD1 resistance, we employ a newly developed isoform-selective histone-deacetylase-inhibitor (HDACi) (OKI-179) and a novel mouse mature B-cell lymphoma, G1XP lymphoma, that resembles immunosuppressive features of human B-cell lymphomas including downregulation of major histocompatibility complex (MHC) class I and II, exhaustion of CD8 and CD4 tumor-infiltrating lymphocytes (TILs), and PD1-blockade resistance. Using multiple lymphoma models, we show that combined treatment of OKI-179/anti-PD1 significantly inhibited growth of B-cell lymphomas refractory to PD1 blockade; furthermore, sensitivity to single or combined treatment required tumor-derived MHC class I, and positively correlated to MHC class II level. We conclude that OKI-179 sensitizes lymphomas to PD1 blockade by enhancing tumor immunogenicity. Additionally, we found that different HDACi exhibited distinct effects on tumors and T cells, yet the same HDACi could differentially affect HLA expression on different human B-cell lymphomas. Thus, our study highlights the importance of immunologic effects of HDACi on antitumor responses and suggests that optimal treatment efficacy requires personalized design and rational combination based on prognostic biomarkers (e.g., MHCs) and unique profiles of HDACi.

PO-55 Single-center experience of chimeric antigen receptor T-cell (CAR-T) immunotherapy in relapsed/refractory large B-cell lymphoma identifies association of acute toxicities with inferior disease outcomes. Fahmin Basher, Caroline A. Coughlin, Deukwoo Kwon, Lazaros Lekakis, Jonathan Schatz. University of Miami Miller School of Medicine, Miami, FL.

Chimeric antigen receptor T (CAR-T) cells are an emerging approach for the treatment of hematologic and solid tumor malignancies. Axicabtagene ciloleucel (axi-cel) and tisagenlecleucel were the first FDA-approved CAR-T therapies targeting CD19 for patients with relapsed/refractory (r/r) large B-cell lymphoma. Pivotal studies showed complete response (CR) rates of 58% and 40%, respectively, and we sought to investigate if the data are similar to our single-center results. We carried out a retrospective analysis of patients diagnosed with r/r diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, or mantle cell lymphoma who were treated with axi-cel or tisagenlecleucel at Sylvester Comprehensive Cancer Center in Miami, FL between January 2016 and October 2019. Primary objectives were to identify clinical characteristics associated with improved overall and progression-free survival (OS and PFS). Secondary analyses included incidence of post-CAR-T toxicities, including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). Our analysis included 44 patients: 32 received FDA-approved commercial product and 12 received axi-cel through clinical trials. Median age at time of CAR-T therapy was 62 years, and 70% of patients were male. Median number of prior treatments was 4, and 14 patients had undergone prior hematopoietic stem cell transplantation (12 autologous, 2 allogeneic). By day-30 post-treatment PET scan, 25 patients (57%) achieved CR or partial response (PR), while 16 (36%) had progressive (PD) or stable disease (SD). The remaining 3 patients decompensated rapidly post-infusion. Overall, patients with a CR or PR at 30 days had significantly improved OS and PFS compared to patients with PD or SD (OS $p = 0.009$, PFS $p < 0.001$). Univariate analyses showed patients requiring aggressive supportive measures in the post-infusion period had decreased OS compared to those who did not: requirement for ICU care ($p = 0.018$), vasopressor use ($p = 0.01$), and steroid treatment ($p = 0.018$) were all associated with inferior survival. There was no survival difference in DLBCL patients classified as double expressor or double hit; however, patients with germinal center B-cell (GCB) DLBCL trended strongly towards improved OS ($p = 0.073$) compared to non-GCB patients. CRS affected 35 patients (80%), while 24 patients (55%) experienced ICANS. Incidence of toxicities did not vary significantly in patients who received CAR-T commercially or in clinical trials. Patients who did not experience CRS had improved OS ($p=0.061$), and of patients who had CRS or ICANS, SD/PD patients had significantly worse PFS ($p= <0.001$, $p= 0.024$). This single-center retrospective analysis of patients receiving CAR-T therapy

for r/r large B-cell lymphoma showed that incidence and management of toxicities and factors such as tumor subtype associate with treatment response. Further investigations into these factors may provide more insight into optimal management of patients undergoing CAR-T therapy.

PO-56 Identification of predicted neoantigen vaccine candidates in follicular lymphoma patients. Cody A. Ramirez[1], Felix Frenkel[2], Olga Plotnikova[2], Vladislav Belousov[2], Alexander Bagaev[2], Elena Ocheredko[2], Susanna Kiwala[1], Jasreet Hundal[1], Zachary L. Skidmore[1], Marcus Watkins[1], Michelle Becker-Hapak[1], Thomas B. Mooney[1], Jason Walker[1], Catrina Fronick[1], Robert Fulton[1], Robert Schreiber[1], Nancy L. Bartlett[1], Brad Kahl[1], Ravshan Ataullakhanov[2], Malachi Griffith[1], Obi Griffith[1], Todd A. Fehniger[1]. [1]Washington University School of Medicine in St. Louis, St. Louis, MO, [2]BostonGene, LLC, Waltham, MA.

Follicular lymphoma (FL) is the most common indolent non-Hodgkin's lymphoma; however, it remains incurable with conventional therapies and is poorly responsive to checkpoint blockade. Additionally, because FL develops so slowly (and often asymptomatically), a major research focus has been to avoid chemotherapy treatments to limit the potential development of treatment-related side effects and the risk of therapy-related second cancers. The mutational processes that lead to lymphomagenesis and progression also produce tumor-specific mutant antigens (TSMAs) that can be targeted by the immune system to control malignancies. Personalized cancer vaccines designed for these TSMAs represent a promising new strategy for treatment of FL. However, the feasibility of this approach and precisely how to optimize effective vaccine design, informed by next-generation sequencing data, are not fully understood. We hypothesize that (tumor/normal) whole-exome sequencing (WES) and (tumor) RNA sequencing (RNA-Seq) can be used to predict patient HLA typing and neoepitopes to engineer personalized cancer vaccines for FL. DNA and RNA from 58 patients' FL biopsies underwent WES and RNA-Seq. pVACtools and MiXCR predicted potential somatic and B-cell clonotype neoantigens, which were filtered to identify high-quality TSMAs. B-cell oligoclonality was determined by comparison to B-cell receptor (BCR) repertoire profiling of healthy individual lymph nodes. RNA-seq data allowed us to identify expressed TSMAs. Complementary in silico analysis based on mRNA-based peptide reconstruction and custom HLA affinity binding predictions were performed. An average of 52 somatic mutations per patient (range: 2-172) were identified. At least one high-quality TSMA was predicted for 57 of 58 patients. Five or more TSMA candidates were identified for 52 (90%) patients with a mean of 17 predicted peptides per patient (range: 0-45). 81% (813/1,004) of the total predicted TSMA peptides arose from missense mutations, 9% (94/1,004) from indels, and 10% (97/1,004) from BCR. 78% (45/58) of patients have both somatic and BCR vaccine candidates, while 21% (12/58) of patients had only somatic vaccine candidates. No fusion genes were identified within the cohort that could have been a source of neoepitope candidates. Predicted TSMAs were identified in multiple genes recurrently mutated in lymphoma (e.g., BCL2). There was a high prediction concordance with the orthogonal BostonGene Vaccine Module V1

pipeline. These preclinical results led to a first-in-human pilot trial of personalized TSMA vaccine combined with anti-PD-1 mAb for rel/ref FL patients (NCT03121677), with one response observed within 4 patients evaluable for response to date. TSMA peptides suitable for cancer vaccines were identified for most FL patients via next-generation sequencing, MiXCR and pVACtools. This preclinical study suggests that FL patients will be candidates for TSMA vaccine clinical trials, and pilot clinical results provide proof of concept for this approach.

PO-57 Association between cytokine levels and prolonged cytopenia after axicabtagene ciloleucel in patients with refractory large B-cell lymphoma. Paolo Strati, Nahum Puebla-Osorio, Guangchun Han, Jason R. Westin, Loretta J. Nastoupil, Sairah Ahmed, Felipe Samaniego, Nathan H. Fowler, Luis E. Fayad, Hun J. Lee, Christopher R. Flowers, Samer A. Srour, Grace Watson, Linghua Wang, Michael R. Green, Sattva S. Neelapu. The University of Texas MD Anderson Cancer Center, Houston, TX.

Introduction: Prolonged grade 3-4 (G3-4) cytopenias lasting beyond day 30 have been observed with various chimeric antigen receptor (CAR) T-cell products. Their biologic mechanism remains largely unknown, since it is unclear whether it is related to the effects of the conditioning regimen or due to CAR T-cell activity.

Methods: We measured 29 analytes associated with CAR-T cell activity by multiplex assays (V-Plex, Meso Scale Diagnostics) and CAR T-cell amplification by RT-PCR in serial plasma samples at baseline and days 7, 14, and 30 (D30) after standard-of-care axicabtagene ciloleucel (axi-cel) in patients with refractory large B-cell lymphoma (LBCL) treated at MD Anderson Cancer Center between March and August 2018. Patients with G3-4 cytopenia before conditioning, defined according to CTCAE v5, and those who progressed and/or died before D30 were excluded from the analysis. Unpaired t-test was used for area under the curve (AUC) and mean peak comparison, and the Benjamini-Hochberg method for adjustment of false discovery rate (FDR).

Results: Of 19 patients included in the analysis, 9 (47%) had ongoing G3-4 cytopenia at D30. Baseline clinical characteristics were not significantly different between the 2 groups. Patients with D30 G3-4 cytopenia had a significantly higher AUC for the following cytokine levels: IFN-ɣ, FLT3-L, G-CSF, GM-CSF, CXCL1, IL-1Rα, IL-3, IL-8, IL-10, M-CSF, CCL2 ($p<0.05$); IL-1β, IL-2Rα, IL-6, IL-15, TNF-α ($p<0.01$); and VWF ($p<0.001$). In contrast, a significantly lower AUC was observed for Ang-1 ($p=0.0002$) and EGF ($p=0.05$). After adjusting for FDR, the association was maintained only for the following cytokines: Ang-1, IL-10, IL-15, IL-1β, IL-2Rα, IL-6, IL-8, TNF-α, and VWF. As the majority of cytokines outlined above showed a peak at day 7, the association between day 7 peak and D30 G3-4 cytopenia was evaluated. Patients with D30 G3-4 cytopenia showed a significantly higher peak at day 7 for the following cytokines: IFN-ɣ, G-CSF, GM-CSF, CXCL1, IL-1β, IL-2Rα, IL-3, IL-6, IL-8, IL-10, M-CSF, CCL2 ($p<0.05$), and FLT3-L ($p<0.001$), and a significantly lower peak for EGF ($p=0.005$). After adjusting for FDR, the only cytokine maintaining its association was FLT3-L. Patients with D30 G3-4 cytopenia had a significantly higher AUC for CAR T-cell amplification (123794 vs. 71548 DNA copies/µg), though not statistically significant because of small sample size ($p=0.57$).

Conclusions: Development of prolonged cytopenia after axi-cel in patients with LBCL is associated with significant increase in the levels of multiple inflammatory

cytokines and with CAR T-cell amplification early after infusion, suggesting this is due to CAR T-cell activity rather than to the myelosuppressive effect of conditioning regimen. The majority of these cytokines appear to be derived from myeloid cells and peak at day 7, providing potential targets for prophylactic intervention in these patients.

PO-58 Obinutuzumab plus chemotherapy is cost effective versus rituximab biosimilars plus chemotherapy for previously untreated follicular lymphoma patients. David L. Veenstra[1], Gregory F. Guzauskas[1], Scott J. Spencer[1], Federico Felizzi[2], Aino Launonen[2], Keith Dawson[3], Anthony Masaquel[3]. [1]University of Washington Department of Pharmacy, Seattle, WA, [2]F. Hoffmann-La Roche Ltd., Basel, Switzerland, [3]Genentech, Inc., South San Francisco, CA.

Background: In the phase III GALLIUM study, obinutuzumab (GA101; G) plus chemotherapy (G+chemo) demonstrated superior progression-free survival (PFS) compared with rituximab (R) plus chemotherapy (R+chemo) for first-line treatment of patients with follicular lymphoma (FL) (Marcus et al., New Engl J Med 2017). G+chemo was subsequently shown to be highly cost effective vs. R+chemo, with a cost per quality-adjusted life year (QALY) of $2,300. Two R biosimilars, rituximab-abbs (Ra) and rituximab-pvvr (Rp), have now been FDA approved in this setting. The cost effectiveness of G+chemo vs Ra+chemo and Rp+chemo has not yet been estimated.

Methods: We adapted an existing Markov model comparing G+chemo vs. R+chemo, utilizing the GALLIUM trial's investigator-assessed PFS and post-progression survival to model overall survival. We assumed efficacy and safety equivalence for R biosimilars plus chemo and the R+chemo arm. Drug utilization, treatment duration, and adverse events (AEs) were based on GALLIUM trial data as of April 2018. Patients were randomized to either R+chemo or G+chemo; responders at end of induction received G or R maintenance for 2 yrs. We included costs for drugs, AEs, drug administration, and follow-up treatment upon disease progression. Health care costs were based on Medicare reimbursements; drug costs were April 2020 average sale prices for intravenous therapies or wholesale acquisition costs for oral therapies used post-progression; both R biosimilars cost less than R, and Rp cost less than Ra. Utility estimates were based on GALLIUM trial data and published literature and were used to estimate QALYs. Sensitivity analyses assessed key drivers of the model and uncertainty in the results.

Results: Treatment with G+chemo led to an increase in QALYs relative to R biosimilars+chemo of 0.93 (95% credible range [CR] 0.37–1.50). The total cost of G+chemo was $196,800, while those of Ra+chemo and Rp+chemo were $190,000 and $178,800, respectively, resulting in incremental costs of $6,800 (95% CR: -$1,200–$14,000) and $18,100 (95% CR: $10,700–$25,400), respectively. The average total cost was greater for G+chemo, primarily due to increased drug and administration costs ($128,100 for G+chemo vs. $114,600 for Ra+chemo and $103,400 for Rp+chemo); however, this was largely offset by cost savings for disease progression of -$7,800. AE costs were higher for G+chemo ($5,700) vs. R biosimilars+chemo ($4,400). The incremental cost-effectiveness ratios were $7,300

and $19,400 per QALY gained for G+chemo vs Ra+chemo and Rp+chemo, respectively. In probabilistic sensitivity analyses, G+chemo was cost effective at the $50,000 and $100,000 per QALY thresholds vs. both Ra+chemo (99% and 100% probabilities of cost effectiveness, respectively) and Rp+chemo (94% and 99%, respectively).

Conclusions: Despite the lower cost of R biosimilars compared with R, G+chemo is likely cost effective in the US as first-line treatment for FL, driven by delaying expensive progression treatments and increasing QALYs.

PO-59 Anti-membrane IgM monoclonal antibody, mAb4, is a novel, next-generation BTK inhibitor mediating B-cell leukemia and lymphoma cell death. Rachel Welt[1], Jonathan Welt[2], Virginia Raymond[3], David Kostyal[4], Sydney Welt[1]. [1]Welt Bio-Molecular Pharmaceutical, Briarcliff Manor, NY, [2]University of Michigan Medical School, Ann Arbor, MI, [3]Diligent Research, Armonk, NY, [4]ARDL, Barberton, OH.

Introduction: The concept that the B-cell receptor complex (BCRC) initiates a driver pathway in leukemia-lymphoma has been validated by clinical data. However, the BCRC has been overlooked as a therapeutic target due to homology between the BCRC's membrane IgM (mIgM) subunit and circulating serum IgM (sIgM). Previously, we reported the generation of a first-in-class antibody, mAb4, to a neoepitope specific to the BCRC, which inhibits cell growth (Welt et al. 2016; US Patent Nos. 9,926,381 and 10,227,419). Bruton's tyrosine kinase (BTK) inhibitors have emerged as valuable clinical therapies but can induce severe off-target effects. BTK is a downstream mediator of the BCRC's signaling pathway. We aim to characterize the impact of targeting the BCRC on BTK phosphorylation and its resulting biologic effects. We hypothesize that mAb4 binding to the BCRC initiates antitumor effects in part by inhibition of BTK phosphorylation.

Methods: Antibody reagents detecting phosphorylation of BTK at Tyr551 (clones A16064A, 797837, and M4G3LN), Tyr223 (clones A16128B and 720101), and Ser180 (clones 3i5 and 3D3), were used to compare inhibition of phosphorylation by a BTK inhibitor drug, and by mAb4, in Burkitt lymphoma cells (CA46) over 0-96 hours, by ELISA. Flow cytometry will be used to further confirm these biologic effects and examine cell cycle phase inhibition.

Results: We find that both mAb4 and a BTK inhibitor drug inhibit phosphorylation at Tyr551 and Tyr223, but not Ser180, across all Ab reagents used in ELISA. mAb4-treated leukemia and lymphoma cells undergo growth inhibition and apoptosis while the BTK inhibitor drug mediates only cell growth inhibition, as reported by others. Preliminary flow cytometry results suggest that mAb4 treatment induces a specific cell cycle arrest.

Conclusions: Here we show that phospho-tyrosine BTK inhibition is mediated upon mAb4 binding to the BCRC's mIgM. Though BTK exhibits identical patterns of phosphorylation inhibition following treatment with either mAb4 or a BTK inhibitor drug, each treatment results in different cell survival outcomes. While studies show that BTK inhibitors induce a cell growth inhibitory effect, we find that mAb4 mediates BCRC internalization, and in low-density cultures, cell growth inhibition, anticlonogenic activity, and apoptosis. Further studies will examine spleen tyrosine kinase (SYK) phosphorylation and regulation of CXCR4 (a chemokine receptor), as mobilization of tumor cells into the blood may additionally be an important clinical

parameter in BTK inhibitor drug efficacy. Due to the specific targeting of mAb4 to IgM-expressing B-cells, we expect that the toxicities associated with small-molecule BTK inhibitors upon interaction with nonlymphatic tissue, including bleeding, infection, cytopenia, arrhythmia, and secondary malignancies, will be avoided with mAb4 treatment. With increased antitumor activity and expected reduced toxicities, mAb4 represents a compelling candidate for clinical development.

PO-60 The stage-specific roles of radiotherapy and chemotherapy in nodular lymphocyte-predominant Hodgkin lymphoma patients: A propensity-matched analysis of the Surveillance, Epidemiology, and End Results database. Shijie Wang, Mingfang Jia, Jianglong Han, Rui Zhang, Kejie Huang, Yunfeng Qiao, Ping Chen, Zhenming Fu. Renmin Hospital of Wuhan University, Wuhan, China.

Background: The stage-specific roles of radiotherapy (RT) alone, chemotherapy alone, and chemoradiotherapy (CRT) for patients with nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) have not been adequately evaluated.

Methods: We analyzed patients with NLPHL in all stages enrolled from the Surveillance, Epidemiology, and End Results (SEER) registry from January 2000 to December 2015. Propensity score (PS) analysis with 1:1 matching was performed to ensure well-balanced characteristics between the comparison groups. Kaplan-Meier and Cox proportional hazards models were used to evaluate the overall survival (OS), cancer-specific survival (CSS), the hazard ratio (HR), and corresponding 95% confidence intervals (95% CI). Restricted mean survival times (RMST) were also used for the survival analyses.

Results: For early-stage patients, RT alone was found to be associated with the improved survival of 10 months to 15 months and the deceased HR of 60% to 80%. Chemotherapy alone also had survival benefits (improved OS = 12.1 months; improved CSS = 5.3 months). However, not all of these improvements were statistically significant. CRT was associated with the best survival: the mean OS was significantly improved by 20 months and the HR was reduced by more than 80%, both before and after PS matching (PSM) ($P < 0.05$). For advanced-stage patients, none of RT alone, chemotherapy alone, and CRT had a significant effect on survival. Chemotherapy alone and CRT might be more beneficial for long-term survival (RMST120m: neither RT nor chemotherapy vs. chemotherapy alone vs. CRT = 104m vs. 111m vs. 108m). Subgroup analysis showed that the efficacy of RT alone in patients with early-stage NLPHL was significantly different between younger people and older people (age < 40: HR, 95% CI = 5.70, 0.34 - 94.2, P = 0.224; age ≧ 40: HR, 95% CI = 0.06, 0.01 - 0.32, P = 0.001; P for interaction = 0.005).

Conclusions: The results from the large SEER database suggested that CRT was associated with the best survival of patients with the early-stage NLPHL. More effective treatment strategies for patients with advanced-stage NLPHL remain to be further studied.

PO-61 Rethinking radiation therapy in the modern era of advanced systemic treatments of malignant lymphoma. Lena Specht. Rigshospitalet, Copenhagen, Denmark.

Radiation therapy (RT) is the single most effective treatment modality for achieving local control in most types of lymphomas. RT causes DNA damage, which can be modulated very accurately in space and time. Modern advanced imaging (PET, MRI, image fusion) and treatment techniques (3-dimensional conformal RT, intensity modulated therapy [IMRT], volumetric arc therapy [VMAT], proton therapy, breathing adaptation, MR-accelerators) have changed lymphoma RT dramatically. The prescribed radiation dose is now delivered very precisely to the macroscopic lymphoma volume, and no more than that (1). This makes RT eminently suited for combination with systemic treatments, either as consolidation using spatial cooperation with cytotoxic systemic treatments, or as immune stimulators in combination with immunomodulatory treatments, the so-called abscopal effect. RT in the curative setting has always been given in many small fractions to protect the critical normal tissues from serious long-term effects. However, with modern conformal RT and very steep dose gradients around targets, the surrounding normal tissues get a much lower dose than before. Hence, even if large fractions to the tumor tissue are employed (so-called hypofractionation) the fraction size for the normal tissues will be in the desired low-dose range. During the recent COVID-19 pandemic RT resources became scarce, and the International Lymphoma Radiation Oncology Group (ILROG) published emergency guidelines for hypofractionation for lymphomas in order to reduce the pressure on RT departments (2). Preliminary experience has been encouraging. Delivering RT to lymphomas in a few large fractions may make it even more convenient for combination with systemic treatments. We and others have shown that the lymphocyte count decreases during a course of RT, most pronounced when many fractions are given, and that it does not reach its former level even a year after treatment (3). A low lymphocyte count increases the risk of infections and may also lead to an increased risk of long-term side effects. Hypofractionation may therefore prove not more but less toxic, challenging the radiobiologic paradigm that has hitherto formed the basis of RT in the curative setting. In conclusion, technological progress and innovative implementation of RT open new possibilities for combinations with systemic treatments to benefit patients with malignant lymphoma.

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associated with the end-of-radiation-therapy lymphocyte count, and risk of infection in patients with solid malignant tumors treated with curative-intent radiation therapy. IJROBP 2019;105:812-23.

PO-62 Overcoming venetoclax resistance in B-cell malignancies by antagonism of stromal TGF-beta-mediated drug resistance. Eugene Park[1], Jingyu Chen[1], Andrew Moore[1], Maurizio Mangolini[1], Joseph R. Byod[2], Hilde Schjerven[3], James C. Williamson[1], Paul J. Lehner[1], Michael Leitges[4], Alexander Egle[5], Marc Schmidt-Supprian[6], Seth Frietze[1], Ingo Ringshausen[1]. [1]University of Cambridge, Cambridge, United Kingdom, [2]University of Vermont, Burlington, VT, [3]University of California San Francisco, San Francisco, CA, [4]Memorial University of Newfoundland, St. John, NL, Canada, [5]Paracelsus Medical University, Salzburg, Austria, [6]Technical University Munich, Munich, Germany.

Novel targeted therapies have substantially improved the prognosis of patients with B-cell malignancies. However, a substantial fraction of patients relapse, even after initially achieving deep remissions. Many studies have characterized the interactions between tumor cells and their microenvironment as integral to leukemia/lymphoma homeostasis and for the provision of survival signals, also contributing to drug resistance (referred to as environment-mediated drug resistance [EMDR]). Therapeutic efforts to antagonize microenvironment-emanating survival cues have predominantly focused on perturbation of tumor cell adhesion enabling the physical displacement from protective niches. In an effort to address whether direct stromal targeting could more precisely mitigate EMDR, we antagonized stromal expressed PKC-beta, which we have previously shown to be a stroma-autonomous signaling pathway critical for the survival of malignant B cells (Lutzny et al., Cancer Cell 2013). The dependency on stroma PKC-b was uniformly found for acute (ALL) and chronic (CLL, MCL) B-cell malignancies. In particular, our data demonstrate that stroma PKC-b is of key importance for multidrug resistance of malignant B cells (Park et al., Science Trans Med 2020). Here we demonstrate novel mechanistic insights into stroma-mediated drug resistance in B-cell malignancies. We identified that stroma PKC-b drives a transcriptional program, activating TGF-b and BMP-signaling in tumor cells. Our data show that antagonizing stroma signals with TGF-b inhibitors abrogated upregulation of BCL-XL and overcomes stroma-dependent resistance to venetoclax. This activation operates in parallel to the activation of the transcription factor EB (TFEB) as a downstream target of PKC-b. Interference with these signaling pathways impairs plasma membrane integrity of MSCs by downregulation of numerous adhesion and signaling molecules (e.g., ADAM17), required for the reciprocal stabilization of BCL-XL in tumor cells. The significance of microenvironment PKC-b for drug resistance was demonstrated in vivo, using C57B/6 mice, diseased with EuTCL-1 driven B-cell tumors and treated with venetoclax in combination with or without enzastaurin (PKC-b inhibitor). Combined treatment significantly prolonged survival, based on PKC-b mediated impairment of lysosome biogenesis in vivo. Similarly, concurrent treatment of PKC-b inhibitors with

chemotherapy also improved survival in an ALL-PDx model. Our data demonstrate that mitigating EMDR with small-molecule inhibitors of PKC-b or TGF-b signaling enhances the effectiveness of both targeted and nontargeted chemotherapies and, moreover, has the ability to overcome venetoclax resistance in B-cell malignancies in vivo. A clinical trial to test the dual inhibition of stroma and tumor cells in lymphoma patients is in preparation.

PO-63 A phase 2a open-label study of MT-3724, a novel CD20-targeting engineered toxin body, in combination with lenalidomide (LEN) in subjects with relapsed or refractory B-cell non-Hodgkin lymphoma (NHL). Jason Tache[1], Deborah A. Katz[2], Amitabha Mazumder[3], David Peace[4], Christine Burnett[5], Thomas Strack[5], Elizabeth Bay[5], Roger Waltzman[5], Seung Lee[6]. [1]BRCR Medical Center, Plantation, FL, [2]Rush University Medical Center, Chicago, IL, [3]The Oncology Institute of Hope and Innovation, Glendale, CA, [4]University of Illinois at Chicago, Chicago, IL, [5]Molecular Templates, Inc., Jersey City, NJ, [6]University of Maryland, Baltimore, MD.
Background: Engineered toxin bodies (ETBs) are a distinct class of targeted immunotoxins in development by Molecular Templates as anticancer therapeutics. ETBs have a novel mechanism of action that drives a potent and targeted response mediated by antibody-like binding, cellular internalization, and enzymatic ribosomal inhibition via the delivery of a Shiga-like toxin subunit A (SLTA). MT-3724 comprises an anti-CD20 single chain variable fragment genetically fused to SLTA with an approximate molecular weight of 55 kDa and is being developed for the treatment of relapsed or refractory diffuse large B-cell lymphoma (r/rDLBCL). MT-3724 is currently being studied in three ongoing phase 1/2 studies for r/rDLBCL.
Methods: MT-3724 is being evaluated in this phase 2a study (NCT03645395) in combination with LEN in adult patients with histologically confirmed, relapsed, or refractory CD20+ B-cell NHL. The primary objectives of this study are to determine the safety and tolerability, including the maximum tolerated dose (MTD), of MT-3724+LEN. Secondary objectives include pharmacokinetics, pharmacodynamics, immunogenicity, and tumor response. The study is being conducted in two parts. Part 1 includes MT-3724 dose escalation over 5 dose cohorts according to the modified 3+3 design and will include up to 24 subjects with CD20+ NHL. Part 2 is designed to assess the safety and tolerability of MT-3724+LEN in the MTD Expansion Cohort, where the dose declared as MTD of MT-3724 in Part 1 will be given in combination with LEN in up to 40 subjects with CD20+ r/rDLBCL. Eligible subjects must have received at least one approved therapy for NHL and must have measurable disease by Lugano criteria. Subjects who have progressed following CAR T-cell therapy, autologous or allogeneic stem cell transplant are also eligible. Serum rituximab level must be negative (<500 ng/mL) at screening because it competes with MT-3724 for binding to CD20. In the first 2 dose cohorts (10 and 25 ug/kg/dose) of Part 1, subjects received MT-3724 IV infusions over 1 hour three times weekly for 2 weeks and LEN (20 mg daily) on Days 1-21 of each 28-day treatment cycle for Cycles 1 and 2. In subsequent cycles, MT-3724 was administered once weekly with continued LEN dosing on Days 1-21. Because of 2 dose-limiting toxicities (grade 2 capillary leak syndrome) in Cohort 2, the dose in Cohort 3 was reduced to 20 ug/kg/dose. The protocol was amended so that in future Cohorts 4 and 5 (25 and 50

ug/kg/dose), MT-3724 will be dosed biweekly for 2 weeks for Cycles 1 and 2 and then once weekly for subsequent cycles. The study is recruiting subjects at multiple study centers.