IA02 Immune exhaustion and suppression in lymphoma. Stephen M. Ansell. Mayo Clinic, Rochester, MN.

The tumor microenvironment in lymphoproliferative disorders, including lymphoma, shows a prevalence of intratumoral T cells suggesting an effective immune response to malignant B cells. This supposes that antigen-presenting cells present tumor-associated peptides to the immune system, resulting in T cells that are primed to target the malignant clone. It is anticipated that activated T cells will target the malignant cells and eradicate the tumor. However, while T cells appear plentiful in the tumor microenvironment in B-cell lymphoma, they are not able to eradicate the malignancy. Rather, the T cells appear inhibited, either due to immune suppression or immune exhaustion, and the lymphoma steadily progresses. An effective antitumor response requires presentation of tumor antigens to T cells in the context of major histocompatibility complex (MHC) proteins. The cells then receive a second activating signal resulting in proliferation of the T cells, expansion of a tumor-specific T-cell clone, and generation of cytotoxic proteins that lyse the lymphoma cell. To control this process, however, inhibitory receptors are typically upregulated upon T-cell activation that allow the process to be suppressed. This prevents damage to healthy tissue from T cells that remain inappropriately activated for protracted periods of time. In lymphoid malignancies, however, malignant B cells commonly overexpress the ligands for PD-1, namely PD-L1 and PD-L2. This results in suppression of the activated T cells and prevents the malignant clone from being eradicated. This leads to a subsequent problem, in which effector T cells are restimulated by the persistent presence of malignant cells that expressed tumor antigens which they are targeting. This repeat stimulation results in activation-induced exhaustion and upregulation of additional receptors that make the cells susceptible to apoptosis. These receptors, including TIM3, TIGIT, and LAG3, are then expressed on T cells whose effector function is severely impaired. The effector T cells expressing TIM3 and LAG3 have very little ability to proliferate or generate cytokines. Additional mechanisms that inhibit T-cell activation are present in lymphoma and include cytokine- and cell-mediated suppression. Among cytokines that mediate T-cell suppression, TGF-β plays a pivotal role in regulating immune responses by inhibiting the proliferation and differentiation in both CD4+ and CD8+ T cells. In B-cell malignancies, both malignant B cells and intratumoral T cells can synthesize and secrete TGF-β, and TGF-β inhibits intratumoral T-cell proliferation, upregulates Foxp3 expression, and suppresses the development of TH1 and TH17 cells. Similarly, IL-10 also inhibits the proliferation, cytokine production, and migratory capacities of T cells. IL-10 downregulates expression of MHC class II as well as costimulatory molecules CD80 and CD86 on dendritic cells, resulting in suboptimal stimulation of T cells. Furthermore, IL-10-expressing dendritic cells promote T regulatory cell

development. In B-cell NHL, it has been shown that serum IL-10 levels are elevated and correlate with a poor prognosis. IL-10 further affects monocytes by downregulating HLA-DR expression, and IL-10-induced CD14+HLA-DRlow/- monocytes inhibit T-cell activation and proliferation. In addition to regulatory cytokines, cell-mediated suppression plays a crucial role in T-cell suppression. Myeloid-derived suppressor cells (MDSCs) and CD4+ regulatory T cells are two major types of cells mediating T-cell suppression. In B-cell NHL, increased numbers of MDSCs may contribute to elevated absolute monocytosis at diagnosis that correlates with poor outcome in In DLBCL patients. Tumor-infiltrating MDSCs promote cross-tolerance in B-cell lymphoma by expanding or recruiting regulatory T cells, which supports tumor growth. Regulatory T cells also suppress immune function. In B-cell NHL, we have found that the frequency of regulatory T cells is increased in biopsy specimens. Regulatory T cells suppress proliferation and cytokine production of intratumoral CD4+ cells as well as granule secretion by CD8+ T cells, resulting in suppressed cytotoxicity of effector T cells. We found that both recruitment of naturally occurring regulatory T cells and induction of inducible regulatory T cells contribute to elevated frequency of these cells in the tumor microenvironment of B-cell NHL. Furthermore, malignant B cells play a role in the recruitment and induction of regulatory T cells as chemokine CCL22 secreted by lymphoma cells and CD70 expression by CD19+ lymphoma cells are involved in the process. All of these mechanisms that result in T-cell suppression and exhaustion prevent an effective antitumor immune response in lymphoma. Combination strategies that both activate T cells and prevent their inhibition and suppression are needed to rectify these issues.

IA03 Optimizing immunostimulatory antibodies for cancer immunotherapy. Xiaojie Yu, Anne White, Martin Glennie, Ivo Tews, Hayden Fisher, Chris Orr, Ruth French, Stephen Beers, Aymen Al'Shamkhani, Osman Dadas, Ali Roghanian, Jane Willoughby, Mark S. Cragg. University of Southampton, Southampton, United Kingdom.

Agonistic antibodies directed to immunostimulatory receptors are a currently untapped source for immunotherapy. Whereas checkpoint blockers have translated into the clinic, the rules for agonistic antibodies have been more difficult to discern and these reagents await further optimization. Here we discuss the salient properties of monoclonal antibodies (mAb) required to strongly agonize these receptors and discuss potential strategies for the future. We show that immunostimulatory mAb (ISA) can agonize key stimulatory receptors on the cell surface—many of which are from the TNFR family—and highlight the importance of isotype. Using CD40 mAb as a paradigm, we show that receptor clustering is key for ISA responses—probably mimicking ligand. This clustering can be achieved via Fc gamma receptor (FcgR) engagement or independently with the hIgG2(B) isotype. For CD40 this is highly epitope dependent, showing that epitope, isotype, and domain location all interact to drive mAb agonism. Finally, we show that isotype engineering can overcome "weak" epitopes. This can be completely independent of FcgR interaction in the case of hIgG2B, which may be of use in humans where the FcgR profile within the tumor microenvironment may vary.

IA04 The immune cell microenvironment of classical Hodgkin lymphoma by single-cell analysis. Christian Steidl. BC Cancer, Vancouver, BC, Canada.

Background: Over the past decade knowledge about the mechanisms by which the neoplastic Hodgkin and Reed-Sternberg (HRS) cells recruit and manipulate the extensive tumor microenvironment (TME) of classical Hodgkin lymphoma (cHL) has significantly expanded. While some findings support the concept that characteristics of the host immune system contribute to the composition of the TME, there is increasing evidence that somatically acquired genetic alterations and phenotypes of HRS cells influence disease evolution and underlie specific TME interactions. Integrating these concepts, the cHL TME might be the reflection of a symbiotic ecosystem containing the malignant cells and host immune cells. Dissection of this symbiotic relationship at single-cell resolution will be critical for a better understanding of cHL pathogenesis with relevance for improved therapeutic targeting.

Knowledge Gained: We present single-cell transcriptome sequencing data derived from cell suspensions of 22 Hodgkin lymphoma patients (representative of the nodular sclerosis and mixed cellularity subtype) that confirmed the previously identified predominance of Th1 polarized T regulatory cells in the TME. In comparison to 5 reactive lymph node samples, we describe a highly enriched, HL-specific T-cell subset that is characterized by high LAG3 expression and an immunosuppressive cytokine profile, consistent with type 1 regulatory (Tr1) T cells. Analyzing co-expression of inhibitory T-cell makers on the single-cell level, LAG3 expression occurred mostly in a mutually exclusive pattern to the canonical Treg marker FOXP3. Furthermore, using imaging mass cytometry and multicolor immunohistochemistry, we revealed a spatial pattern of rosetting LAG3+ cells in the direct vicinity of MHC-II negative HRS cells, suggesting differential microenvironment architecture dependent on malignant cell phenotypes. Comparative analysis of LAG3 expression across a spectrum of B-cell lymphomas confirmed the high abundance of these cells in cHL, but also suggest that LAG3+ CD4+ and LAG3+ CD8+ T-cell subsets play a role in the related entity of primary mediastinal large B-cell lymphoma (PMBCL) and diffuse large B-cell lymphoma (DLBCL). Expanding our studies to the lymphocyte-rich (LR) subtype of cHL, we also revealed subtype-specific differences in background B cell and T follicular helper (TFH) cell content, suggesting an important role of the CXCR5-CXCL13 axis in cHL.

Significance: Characterization of the TME using multiparametric single-cell technologies, including multicolor immunohistochemistry, flow-based mass cytometry, imaging mass cytometry, single-cell RNAseq, and proximity ligation

assays, has provided unprecedented insight into HL biology. These studies fuel hope for accelerated development of immunotherapies targeting the TME and predictive biomarker development that might guide the appropriate selection of checkpoint inhibitors.

IA05 Microenvironmental signatures reveal biologic and clinical subtypes of diffuse large B-cell lymphoma. Leandro Cerchietti. Weill Cornell Medicine, New York, NY.
Diffuse large B-cell lymphoma (DLBCL) is a biologically and clinically heterogenous disease almost invariably treated with combinatorial chemoimmunotherapy resulting in a ~65% cure rate in first line. Further improvement of treatment outcome relies on elucidating the biology that underlies the clinical behavior of this heterogenous disease. Transcriptomic and genetic characterization of DLBCL have increased our understanding of its pathogenesis and provided potential novel therapeutic targets. Over the past few years, the role of the microenvironment in DLBCL biology has been increasingly recognized. A guiding framework is necessary to effectively and safely translate research evidence into clinical practice. Towards this goal, we characterized the DLBCL microenvironment based on the transcriptional footprint of microenvironment cells and processes in a large cohort of patients. We first developed transcriptional signatures reflecting distinct cellular subtypes of the microenvironment, biologic processes, and canonical signaling pathway activation. We then used these signatures to virtually reconstruct the lymphoma microenvironment (LME) by applying an unsupervised community detection algorithm in 4,656 DLBCL cases. As a result, we described four classes of LMEs that reflect distinct enrichment of associated signatures. 1. “Germinal center-like” (GC-like) due to the presence of signatures from cell types commonly found in germinal centers including follicular dendritic cells, lymphatic endothelial cells, total T cells, and several CD4+ T-cell subpopulations. 2. “Mesenchymal” (MS) for the abundance of signatures from stromal cells such as cancer-associated fibroblasts, fibroblastic reticular cells, vascular endothelial cells, and extracellular matrix pathways. The MS-LME class was enriched in mutations affecting antigen presentation. These lymphomas also presented with high activity of the TGFB/SMAD and HIF pathways, previously associated with favorable prognosis in DLBCL. 3. “Inflammatory” (IN) for the presence of signatures from macrophages, neutrophils, NK cells, and cytotoxic T cells. The IN-LME class was enriched for immune suppressive/pro-lymphoma cytokines, expression of the immune checkpoint molecules PD-L1 and IDO1, and higher activity of the NF-kB, JAK/STAT, and TNF signaling pathways. 4. A “depleted” (DP) form that, contrasting with the other LMEs, was characterized by an overall lower presence of microenvironment-derived signatures. We analyzed the association of LME classes with overall survival (OS) and progression-free survival (PFS) in patient cohorts of, respectively, 2,646 and 2,189 DLBCLs treated with rituximab-based chemoimmunotherapy. Analysis of OS by LME indicated significant differences in prognosis from better to poor as follows: GC-like, MS ($p = 0.03$, vs. GC-like), IN ($p = 0.006$, vs. MS), and DP ($p = 0.008$, vs. IN) LMEs, whereas GC-like and MS have similarly favorable PFS curves ($p = 0.9$) followed by IN ($p < 0.01$) and DP ($p =$

0.03, vs. IN), which presented with the poorest PFS. In multivariate Cox-proportional hazard analysis controlled for C.O.O. and I.P.I., the LME subtypes remained informative with GC-like and MS LMEs associated with better outcome. When segregated by C.O.O., the DP-LME retained the poorest OS and PFS in both C.O.O. subtypes. However, in ABC-DLBCL, the LME with the best PFS and OS was MS, and in GCB-DLBCL, the best LME was GC-like, suggesting that the biologic impact of the microenvironment may be different depending on the lymphoma subtype. DHL and high-grade lymphomas (HGL) modified their outcome when LME was considered. DHL/HGL with the favorable prognosis LMEs GC-like and MS had significantly better PFS and OS than DHL/HGL with the unfavorable prognosis LMEs IN and DP. DLBCLs with a DP-LME were characterized by a minimal presence of LME signatures and tumor cells exhibited higher clonality, were enriched for mutations affecting TP53 and cell proliferation genes, as well as presented higher levels of aberrant hypermethylation of immune-related and TGFB/SMAD1 genes. Preclinical murine models and patient data demonstrated that some of these features can be pharmacologically reversed with hypomethylating agents, indicating that cytosine methylation patterning of lymphoma cells may contribute to a depleted LME. In summary, we categorized the DLBCL LME into four major transcriptionally defined subtypes with distinct biologic properties and clinical behavior, which complement genetically defined subtypes of DLBCL in guiding the development of rational therapeutic approaches for these patients.

IA06 Molecular classification of diffuse large B-cell lymphoma. Bjoern Chapuy. University Medicine Göttingen, Göttingen, Germany.

Key Points

• Sequencing-based approaches are readily used for molecular classifications of diffuse large B-cell lymphoma

• Genetically distinct diffuse large B-cell lymphoma provides insights into unique lymphomagenesis, prognosis prediction, and combination of targeted treatments

• Genetic classifiers identify novel vulnerabilities and inform clinical trial designs

Heterogeneity of Diffuse Large B-Cell Lymphoma. Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive B-cell non-Hodgkin lymphoma and is thought to arise from antigen-exposed B cells. Although the majority of patients with DLBCL are curable with combination immunochemotherapy consisting of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP), a substantial fraction of patients develop recurrent or progressive disease that is often fatal. The clinical heterogeneity of these tumors prompted the development of various classification schemes and included molecular classifiers that i.) improved the accuracy of diagnosis, ii.) identified relevant molecular subtypes with distinct biology, iii.) developed prognostic models for relevant clinical endpoints, and iv.) stratified patients for disease management.

The heterogeneity of DLBCL is partially captured in clinically and/or transcriptionally defined subtypes that provide insights into disease pathogenesis and candidate treatment targets. However, patient stratification for treatment-based transcriptional subtypes, including the activated B-cell type (ABC) and the germinal center B-cell type (GCB), has been largely unsuccessful thus far and suggested that additional heterogeneity had not been fully captured.

Technical advantages paved the way for next-generation sequencing (NGS)-based techniques to be included in clinical and/or low-throughput-based molecular classifiers. These technologies allowed detection and prioritization of all genetic alteration types—recurrent mutations, somatic copy number alterations (SCNAs), and structural variants (SVs) —followed by an integration and assessment of their temporal ordering and associated transcriptional profiles.

Genetic Heterogeneity of DLBCL. Recently, two independent comprehensive genomic efforts at base-pair resolution of hundreds of patients diagnosed with primary DLBCL have captured the complex intrinsic genetic heterogeneity of these lymphomas and have shed light on the genomic architecture of DLBCL. Integration of

significant genetic alterations composed of recurrent somatic mutations, SCNAs and SVs, resulted in the discovery of at least 5 genetically distinct DLBCL subsets that predicted outcome to state-of-the-art frontline treatment, suggesting new insights into the lymphomagenesis of DLBCLs and rational combination treatments. These 5 subsets included: 1) a high-risk ABC DLBCLs with near-uniform *BCL2* copy gain, frequent activating *MYD88* and *CD79B* mutations, and extranodal tropism (C5 DLBCLs); 2) favorable-risk ABC-DLBCLs with genetic features of an extrafollicular, possibly marginal zone origin (C1 DLBCLs); 3) poor-risk GCB-DLBCLs with *BCL2* SVs, inactivating mutations, and/or copy loss of *PTEN* and alterations of epigenetic enzymes (C3 DLBCLs); 4) a good-risk GCB-DLBCLs with distinct alterations in BCR/PI3K, JAK/STAT, and BRAF pathway components and multiple histones (C4 DLBCLs); and 5) an ABC/GCB-independent group of tumors with biallelic inactivation of *TP53*, *9p21.3/CDKN2A*, and associated genomic instability (C2 DLBCLs).

The biology of these 5 DLBCL subsets was largely confirmed by an independent non-overlapping large-scale study that followed a completely different analytical approach and discovered similar groups with shared pathogenetic mechanisms. In particular, C1 DLBCLs were similar to BN2 *(BCL6/NOTCH2)*, C3 DLBCLs were similar to EZB *(EZH2/BCL2)*, and C5 DLBCLs were similar to MCD *(MYD88/CD79B)*. In subsequent work, the biology of the remaining DLBCL subtypes (C2 and C4 DLBLCs) was recently independently validated by two other groups (C4 ~ ST2; C2 ~ A53 | C4 ~ SGK1; C2 ~ TP53).

These studies underscore that DLBCL is a genetically heterogenous disease with at least 5-7 different molecular subtypes. At the same time, they also highlight that despite having several hundreds of sequenced tumors, we still do not capture the full spectrum of DLBCL subsets, and for low-frequency subtypes in particular larger sample sizes are needed. More work is needed, to link genetically defined subtypes to actionable vulnerabilities, and eventually build molecular classifiers that link genetics to dependencies with the hope of using them for precision medical trials.

IA07 Molecular classification of T-cell lymphomas. Keisuke Kataoka. National Cancer Center, Tokyo, Japan.

Peripheral T-cell lymphomas (PTCLs) represent a clinically, histologically, and molecularly heterogeneous group of non-Hodgkin lymphomas derived from mature post-thymic T cells. Among them, the most common in Western countries is PTCL, not otherwise specified (NOS), accounting for approximately 30% of all PTCLs. Combining whole-exome and deep targeted-capture sequencing of 133 cases, we delineated the entire picture of genetic alterations in PTCL, NOS. Of note is the identification of a previously undescribed molecular subtype characterized by TP53 and/or CDKN2A mutations and deletions in PTCL, NOS without showing a T follicular helper cell phenotype. This subtype exhibited different prognosis and unique genetic features, including extensive chromosomal instability, which preferentially affected molecules involved in immune escape and transcriptional regulation. Among PTCLs, the most common entity in Japan is adult T-cell leukemia/lymphoma (ATL), which is an aggressive peripheral T-cell lymphoma associated with human T-cell leukemia virus type-1 (HTLV-1) infection. We previously carried out an integrated molecular study, in which whole-exome, transcriptome, and targeted resequencing, as well as array-based copy number analysis, were performed. We found recurrent genetic alterations in T-cell receptor/NF-κB signaling, T-cell trafficking, and other T cell-related pathways as well as immunosurveillance. Although our previous study discovered many driver mutations and copy number alterations, the whole-genome landscape of ATL still remains elusive. To this end, we have recently performed whole-genome sequencing (WGS) of 155 ATL samples, with a median depth of 95-fold for tumor samples. WGS presented a substantially different overview of driver alterations compared with WES: most driver genes were affected by more than one classes of somatic alterations, including structural variations (SV) and noncoding mutations. We integrated these genetic drivers using non-negative factorization clustering and identified several molecular subsets with distinct clinical features, including a leukemic subtype characterized by PLCG1 and STAT3 mutations. In addition, we newly identified several putative driver SVs and noncoding mutations (such as NOTCH1 truncations and mutations involving NFKBIZ 3'-untraslated region). Several types of SVs were linked to distinct alterations, suggesting their etiology, including associations of TP53 disruption with inversions and translocations, and inactivation of immune-related molecules with deletions. Our WGS analysis not only identifies novel somatic alterations, but also extends the overview of ATL genome, which can lead to future improvement of patient management. Taken together, our findings provide novel insights into genetic and molecular heterogeneity in PTCLs, which should help to devise a novel molecular classification and to exploit a new therapeutic strategy for these malignancies.

IA08 The role of gene expression in the classification of aggressive B-cell lymphoma. David W. Scott. BC Cancer Centre for Lymphoid Cancer, Vancouver, BC, Canada.

Aggressive B-cell lymphomas collectively make up half of all lymphoma diagnoses. The current classification system, the 2017 revision of the WHO 4th edition, assigns these tumors into groups based on morphology, immunophenotype, site of disease, and the presence of recurrent chromosomal rearrangements. Accurate and reproducible diagnosis is required for selection of optimal treatment, prognostication, and ongoing basic research and clinical trials aimed at improving outcomes. Ideally, the taxonomy would continue to evolve towards further defining homogeneous groups of tumors sharing targetable biology. Gene expression (GE) profiling of tumors supports the biologic validity of the entities in the current classification and, along with genomic sequencing, is driving the identification of new lymphoma subtypes. In the mid-2000s GE profiling studies identified specific signatures that distinguish aggressive B-cell entities from each other—namely, primary mediastinal large B-cell lymphoma (PMBL) from diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) from DLBCL. The increasingly divergent treatment of these entities makes reliable diagnosis important. Prior to those studies, GE profiling identified 2 distinct subtypes of DLBCL. This binary division of DLBCL into the cell-of-origin groups of germinal center B-cell-like (GCB) and activated B-cell-like (ABC) has been foundational to our understanding of the pathology of DLBCL. However, recent failure of clinical trials to improve outcomes by adding targeted agents to R-CHOP in upfront treatment of ABC-DLBCL highlights that this binary division may not be sufficiently granular to support precision medicine. These signatures have been translated onto tractable technology platforms, including nuclease protection assay, RT-MLPA, and NanoString, allowing potential integration into diagnostic workflows. More recently, GE signatures have been described that identify distinct molecular subtypes within GCB-DLBCL. Working from the standpoint of tumors that have GE profiles sitting between BL and DLBCL (the “molecular high grade” signature [MHG]) or the GE signature of tumors with rearrangement of MYC and BCL2 (the “double hit” signature [DHITsig]), a sizeable group of GCB-DLBCL can be identified with poor prognosis. Finally, a group of DLBCL without mediastinal involvement have been shown to display the PMBL GE signature. These tumors share perturbation of the hallmark pathways of PMBL but arrive at this biology through different genetic mechanisms. These subtypes map onto, and complement, the newly minted genetics-based classifications of DLBCL. In order to arrive at a tractable unified molecular classification for aggressive B-cell lymphoma, ongoing efforts are needed to integrate GE and genetic aberrations across the disease spectrum. Such a classification framework holds the promise of

improved diagnostic accuracy and reliability while providing the foundation for improving patient outcomes through precision medicine.

IA09 Genomics of common AYA lymphomas: Hodgkin lymphoma and primary mediastinal B-cell lymphoma. Lisa G. Giulino-Roth. Weill Cornell Medical College, New York, NY.

Hodgkin lymphoma (HL) and primary mediastinal B-cell lymphoma (PMBCL) are both B-cell malignancies that commonly arise in the mediastinum and have a peak incidence in the adolescent and young adult (AYA) age range of 15-39y. Advances in sequencing technology with low-input samples have allowed for recent advances in our knowledge of the key molecular alterations in these lymphoma subtypes. In this session, we will discuss the unique genomic features of HL and PMBCL and highlight what is known about differences across the age spectrum. We will also discuss how genomics can inform the use of novel therapies for HL and PMBCL.

Hodgkin lymphoma has traditionally been challenging to study by standard genomic platforms given the rarity of the Hodgkin Reed Sternberg (HRS) cell among the extensive inflammatory infiltrate. Recently, our group and others have utilized flow cytometry to sort HRS cells and intratumoral T and B cells for genomic analyses. Optimization of library preparation for ultralow (<5ng) input now allows for whole-exome characterization of classical HL. Sequencing of the first 10 HL exomes revealed inactivating mutations in *β*-2-microglobulin (*B2M*) in 7 of 10 cases. B2M alterations resulted in loss of MHC Class I expression, providing a potential mechanism of HRS cell immune escape (Reichel et al., Blood 2015). Other common alterations observed in this cohort were *TNFAIP3* (in 6/10 cases) and *HISTH1E* (5/10 cases). More recently, a larger cohort of 23 cases of cHL was evaluated by whole-exome sequencing (Wienand et al., Blood Advances 2019). Here the most frequent alterations occurred in *B2M* (39%), *NFKNIE* (26%), and *TNFAIP3* (26%). Further analysis of this cohort integrating genomic mutations, somatic copy number alterations, and structural variants identified key pathways that are altered in cHL including genomic mechanisms of immune evasion, enhanced JAK/STAT signaling, and alterations in NF-κB signaling. It is not known if differences in genomic alterations exist across the age spectrum in cHL and how this might guide therapy. For example, loss of *B2M* is associated with younger/AYA age range (median age of B2M negative (altered) vs. positive = 30y vs. 47y, $p<0.001$). Further studies are needed to fully characterize the HL genome and understand the implications of alterations for therapy in both pediatric and adult cohorts.

Primary mediastinal B-cell lymphoma is a rare subtype of non-Hodgkin lymphoma, accounting for 2% to 3% of NHL. The disease typically presents as a large mediastinal mass in patients in the AYA age range, with an increased incidence in females. Although previously considered a subtype of diffuse large B-cell lymphoma, PMBCL is

now recognized as its own clinical and molecular/pathologic entity that shares many similarities with Hodgkin lymphoma. Recent genomic characterization of PMBCL has been performed using whole-exome sequencing (Mottok et al., Blood 2019; Chapuy et al., Blood 2019). These studies reported recurrent alterations in components of JAK/STAT and NF-κB signaling as well as genetic mechanisms of immune evasion similar to that observed in cHL including alterations in *B2M, CD274*, and *CIITA*. In addition, cases were observed to have high tumor mutational burden and an apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) mutation signature, which may confer sensitivity to PD-1 blockade.

In summary, recent genomic findings in HL and PMBCL demonstrate molecular similarities between these lymphoma subtypes and define the genetic basis for immune evasion sensitivity to PD-1 blockade. This has translated to national clinical trials for AYAs with HL and PMBCL. A randomized phase III trial of nivolumab + AVD vs. brentuximab + AVD for children and adults with advanced stage HL is currently ongoing (NCT03907488). In PMBCL, a randomized phase III trial evaluating nivolumab in combination with standard therapy for upfront disease is in development and will include pediatric and adult patients across NCTN sites.

IA11 AYA lymphomas: Bridging the divide. Kara Kelly. Roswell Park Comprehensive Cancer Center, Buffalo, NY.

Lymphomas account for approximately 20-25% of annual cancer diagnoses in the adolescent and young adult (AYA) population, defined as ages 15–39 years. Despite continued improvements in lymphoma outcomes, AYAs have not exhibited survival gains to the same extent as other age groups. At present, this survival gap is a manifestation of many compounding factors including a lack of sufficient understanding of the tumor biology or the optimal therapeutic approaches, as well as delays in diagnosis, the supportive care needs, long-term toxicity risk, developmental stage of the AYA, and socioeconomic factors. There is no established standard of care for AYA lymphomas, and there is significant divergence in treatment approaches between medical and pediatric oncology.

Data on the heterogeneity of lymphoid malignancies in the AYA population as compared with younger pediatric and older adult groups will be reviewed. The highest proportion (35-70%) of lymphoid malignancies are due to Hodgkin lymphoma and lymphoblastic leukemia/lymphoma, followed by diffuse large B-cell lymphoma (10%-20%). Sex, race, and ethnic differences in the AYA lymphomas are also observed. These findings suggest that AYA lymphomas are biologically distinct from those present in the pediatric and adult populations. Factors that may contribute to the observed age-based variation in disease include evolution of the immune system with age, changes in the impact of genetic susceptibility, and viral and/or environmental exposures.

There is a marked difference between the pediatric and adult approaches for AYA lymphomas. The example of AYA Hodgkin lymphoma will be reviewed. The pediatric backbone chemotherapy regimen has been doxorubicin, bleomycin, vincristine, etoposide, prednisone, and cyclophosphamide (ABVE-PC), whereas the adult regimen has been doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD). Radiation therapy is incorporated more often in pediatric high-risk regimens and adult low-risk approaches. Because disease biology does not conform to an 18-year-old cutoff, which of these approaches is most appropriate for AYAs has not been established. Treatment approach is not standard and is often driven by the experience of the physician. Recent progress in developing collaborative clinical trials through the North American National Clinical Trials Network will be reviewed.

IA13 Oncogenic Rag GTPase signaling links cellular nutrients with the FL microenvironment. Ana Ortega-Molina, Cristina Lebrero-Fernández, Nerea Deleyto-Seldas, Alba Sanz, Alejo Efeyan. Spanish National Cancer Research Center (CNIO), Madrid, Spain.

During the humoral response, B cells undergo a sudden anabolic shift that requires high cellular nutrient levels to sustain the subsequent proliferative burst. Follicular lymphoma (FL) originates from B cells that have participated in the humoral response, and 15% of FL samples have selected for point, activating mutations in *RRAGC*, a member of the Rag GTPase family that controls the activation of the mechanistic target of rapamycin complex 1 downstream of the sensing of cellular nutrients. S74C and T89N, two of the most frequent activating single-amino acid changes in RRAGC, when targeted to the endogenous *Rragc* locus in mice, confer only a partial insensitivity to nutrient deprivation but strongly exacerbate B-cell responses and accelerate lymphomagenesis. Surprisingly, this moderate increase in nutrient signaling affected the interaction of B cells with the cellular microenvironment, synergizing their effects on mTORC1 activation with paracrine cues from the supportive T-cell microenvironment that activate B cells via the PI3K–Akt–mTORC1 axis. Hence, *Rragc* mutations sustain induced germinal centers and murine and human FL in the presence of decreased T-cell help. From a therapeutic standpoint, *Rragc* mutations impose a selective vulnerability to pharmacologic inhibition of mTORC1. Our results support a model in which activating mutations in the nutrient signaling pathway foster lymphomagenesis by corrupting a nutrient-dependent control over paracrine signals from the T-cell microenvironment.

While pharmacologic inhibition of mTORC1 with rapamycin yielded exciting preclinical responses in murine lymphomas with activating mutations in *Rragc*, targeting the nutrient signaling cascade itself, instead of using such allosteric, partial inhibition of mTOR, may constitute a more efficacious intervention. Because nutrient signaling inhibitors are still in development phase, their efficacy and safety remain unproven. Previous genetic approaches to investigate the consequences of inhibition of Rag GTPase signaling relied on deletion of the Rags in mice and led to severe phenotypes and death. Incomplete inhibition of nutrient signaling, an approach that would mirror more closely the effect of small molecules, has not been pursued to support both their efficacy and safety. We have generated knock-in mice endogenously expressing a point-mutant form of RagC (Q119L) that partially suppresses nutrient signaling. $RagC^{Q119L/Q119L}$ mice are not viable, but $RagC^{Q119L/+}$ mice show minimal phenotypic alterations with partially decreased nutrient signaling. While B-cell development was unaffected, B-cell activation and the humoral response were impaired in $RagC^{Q119L/+}$ in a B cell-intrinsic manner. When bred to the

FL- and autoimmunity-prone strain VavP-Bcl2, $RagC^{Q119L/+}$ mice were protected against development of both FL and autoimmunity. No obvious systemic tradeoff for the suppression of nutrient signaling seems to occur, because $RagC^{Q119L/+}$ mice show normal physiology and longevity with a similar age-dependent health decline. Altogether, our work supports the oncogenicity of activating mutations in components of the nutrient signaling pathway, such as RagC, and both the efficacy and safety of a moderate inhibition of nutrient signaling against pathologic B cells without detrimental systemic effects.

IA15 CARs and armored CARs: Improving CAR T-cell therapy for cancer. Renier J. Brentjens. Memorial Sloan Kettering Cancer Center, New York, NY.

Significant progress has been made in the field of cancer cell therapy over the last 2 decades. Specifically, a patient's own T cells may be genetically engineered to express an artificial T-cell receptor, termed a chimeric antigen receptor (CAR), which, when expressed in a T cell, can alter the specificity of the T cell. A CAR consists of a binding domain, typically derived from a monoclonal antibody, fused to T-cell signaling domains including a co-stimulatory domain, CD28 or 4-1BB, fused to a distal CD3 zeta chain cytoplasmic signaling domain. CAR T cells targeted to the CD19 antigen, expressed on most B-cell malignancies, have demonstrated the greatest clinical efficacy, and commercial CAR T-cell products have recently been FDA approved in the treatment of relapsed and refractory B-cell lymphomas as well as B-cell acute lymphoblastic leukemias. Similarly, CAR T cells targeting the BCMA antigen, expressed on most multiple myeloma tumor cells, have demonstrated impressive antitumor responses in relapsed and refractory disease in both early-phase clinical trials as well as ongoing registration trials. While response rates in the context of CAR T cells targeted to CD19 and BCMA have been impressive, unfortunately the durability of response in some patients is limited and others fail to respond to treatment entirely. The complete list of etiologies responsible for treatment failures remains to be fully defined. Likely causes for treatment failures include the loss of target antigen expression by the tumor, limited persistence of CAR T cells in vivo, and suppression of CAR T cells in the context of an immune-suppressive tumor microenvironment. While this list is likely incomplete, investigators in the CAR T cell field are working on designing next-generation CAR T cells that could address these current limitations. Armored CAR T cells are CAR T cells further modified to enhance persistence and cytotoxicity. Specifically, CAR T cells may be modified to express or secrete biologically active cytokines or ligands designed to modulate the tumor microenvironment and recruit endogenous antitumor immune effectors to enhance the antitumor immune response. Examples of armored CAR T cells include cytokine-secreting CAR T cells (IL-12, IL-18), co-stimulatory ligand-expressing CAR T cells (4-1BBL, CD40L), CAR T cells that secrete single-fragment-length antibodies (scFvs) targeted to immune checkpoint receptors (PD-1), and CAR T cells that express dominant negative chimeric receptors (Fas, TGF-B). Preclinical studies in the context of these armored CAR T cells have demonstrated markedly enhanced antitumor activity in mice when compared to T cells modified to express the CAR alone. Outcomes of planned and ongoing early-stage clinical trials are pending but may markedly change the field in the foreseeable future.

IA17 CAR T cells for T-cell lymphoma. Maksim Mamonkin. Baylor College of Medicine, Houston, TX.

Development of CAR T-cell therapies for T-cell lymphoma has been hampered by a limited range of targetable antigens. Shared expression of most T-lineage antigens between malignant and normal T cells promotes fratricide of CAR T cells and increases the risk of T-cell aplasia. We developed a CAR targeting CD5, a pan-T cell antigen commonly expressed in T-cell lymphoma. CD5 CAR T cells evade fratricide by rapidly downregulating CD5 protein expression and retain high cytotoxicity against T-cell lymphoma. An ongoing phase I clinical trial demonstrates curative potential of autologous CD5 CAR T cells in patients with peripheral T-cell lymphoma. CD5 CAR T-cell infusions were well tolerated and did not produce prolonged ablation of endogenous T cells. Other CAR T cell-based approaches for T-cell lymphoma will also be discussed.

IA21 Biomarker-informed studies in peripheral T-cell lymphoma. Steven M. Horwitz. Memorial Sloan Kettering Cancer Center, New York, NY.

Peripheral T-cell lymphomas (PTCL) are a heterogeneous group of non-Hodgkin's lymphomas (NHL) with 29 distinct subtypes (counting provisional entities) in the latest WHO classification (1). Historically, therapeutic approaches for PTCL were derived from aggressive B-cell lymphomas. More recent therapies have been studied specifically in PTCL with four drugs approved by the FDA in the last decade, including pralatrexate, romidepsin, brentuximab vedotin (ALCL only), and belinostat (2-5). The most frequent approach to studying new agents has been empiric with some continued success in identifying additional tools, with "all-comer" overall response rates between 25-30% and only moderate durability (median PFS 2.5-4 months). The one standardly used exception is the CD30 targeted antibody drug conjugate brentuximab vedotin. When the CD30 target is strongly and consistently expressed as in anaplastic large-cell lymphoma, brentuximab vedotin is remarkably active with an overall response rate of 86% (57% CR) and a median PFS >12 months (4). Brentuximab vedotin has activity in other variably CD30-expressing PTCL including AITL, PTCL-NOS, and MF, although the consistency, depth, and duration of responses are less than those seen in ALCL (6-7). Whether those differences are explained primarily by the density and intensity of target as opposed to the underlying biology of those diseases is unclear. Nonetheless, when BV was added to upfront chemotherapy for patients with untreated PTCL, and enriched for those with CD30 expression, significant improvements in PFS and OS were seen with the greatest benefit for those with ALCL (8).

In an attempt to expedite our understanding and application of new therapies in T-cell lymphoma, we expanded and formalized a clinical research partnership in part supported by an LLS SCOR grant (Translational Discovery in Peripheral T-cell lymphoma; PI David Weinstock) to prospectively incorporate correlative science plans and institute standards of sample collection into new clinical trials of targeted agents.

Several of our initial trials have shown efficacy at times in subtype-specific ways. The SYK/JAK inhibitor cerdulatinib (NCT01994382) showed particular activity in AITL and intriguing but transient activity in several subjects with strong syk-expressing g/d TCLs but an absence of activity in those with PTCL-NOS (9). For the Pi3K d/g inhibitor duvelisib as part of combination therapy (NCT02783625), the responses and complete responses were consistent across subtypes (10). In that trial over 80% of subjects had samples collected, and RNAseq, whole-exome sequencing, and multicolor immunofluorescence are being conducted. For the trial of ruxolitinib, we

set out to both test a prespecified hypothesis and collect tissues pretreatment, on treatment, and at relapse to assess additional predicators of response and understand mechanisms of resistance. In this study (NCT02974647, PI Alison Moskowitz). We divided patients into cohorts based on presence of JAK/STAT mutation (Cohort 1), absence of mutation but presence of phosphorylated (p)STAT3 or pSTAT5 by IHC/phosphoflow (Cohort 2), or neither (Cohort 3). As hypothesized, absence of either pathway activation or mutation was associated with low rates of response, and preliminary assessment of pretreatment biopsies by multicolor immunofluorescence identified pS6 as a predictive biomarker to inform subsequent studies of ruxolitinib in PTCL (11).

These early attempts have demonstrated a proof of principle that biomarker-driven hypotheses can be embedded into prospective clinical trials of PTCL and adequate sample collection is feasible. These samples allow us to best match therapies to patients and, critically, by obtaining samples on treatment and at progression, provide the best opportunity to understand mechanisms of resistance to facilitate the design of combination therapies.

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IA23 The role of EZH2 gain-of-function mutations in lymphoma. Wendy Béguelin. Weill Cornell Medicine, New York, NY.

Follicular lymphomas (FLs) are slow-growing, indolent tumors containing extensive follicular dendritic cell (FDC) networks and recurrent EZH2 gain-of-function mutations. Paradoxically, FLs originate from highly proliferative germinal center (GC) B cells with proliferation strictly dependent on interactions with T follicular helper cells. Herein, we show that EZH2 mutations initiate FL by attenuating GC B-cell requirement for T-cell help and driving slow expansion of GC centrocytes that become enmeshed with and dependent on FDCs. By impairing T-cell help, mutant EZH2 prevents induction of proliferative MYC programs. Thus, EZH2 mutation fosters malignant transformation by epigenetically reprograming B cells to form an aberrant immunologic niche that reflects characteristic features of human FLs, explaining how indolent tumors arise from GC B cells.

IA25 CREBBP: Not all mutations are created equal. Michael R. Green. The University of Texas MD Anderson Cancer Center, Houston, TX.

Follicular lymphoma (FL) and the EZB/C3 subtype of diffuse large B-cell lymphoma (DLBCL) are characterized by frequent mutations of chromatin-modifying genes, including KMT2D, CREBBP, and EZH2. Genomic analysis of serial biopsies from FL patients has allowed the reconstruction of evolutionary phylogenies and highlighted CREBBP mutations as occurring predominantly as early events in disease evolution and residing within common progenitor cells that seed relapse. Patient tumor cells with CREBBP mutations have significantly reduced MHC class II expression and perturbed T-cell responses to tumor, suggesting a role for CREBBP mutations in driving immune evasion. Knock-out and knock-down studies of Crebbp confirmed its tumor-suppressor function in animal models, but uncovered some differences compared to the phenotypes observed in association with CREBBP mutations in primary tumors. Mutations of CREBBP most often encode missense amino acid substitutions within the catalytic lysine acetyltransferase (KAT) domain rather than nonsense/frameshift variants that result in a loss of protein expression. Here I will discuss recently published and unpublished data regarding the function of CREBBP mutations in B-cell lymphoma, functional differences between classes of mutations (KAT domain missense vs. nonsense/frameshift) and between different mutational hotspots within the KAT domain. Furthermore, I will discuss how these functional insights provide a rationale for targeted therapeutic strategies in B-cell lymphoma.

IA26 What do the results from the trials tell us? Peter W. M. Johnson. University of Southampton, Southampton, United Kingdom.

The relatively high prevalence of somatic mutations in several epigenetic modifying genes in germinal center lymphomas suggests a role in pathogenesis, with a variety of potential mechanisms for this. The first of these somatic changes to be successfully targeted by small-molecule inhibitors was the activating mutation of the enhancer of Zeste Homolog-2 (EZH2), which is found in up to a quarter of follicular lymphomas and germinal center-type diffuse large B-cell lymphomas, where it drives the methylation of histone H3 on lysine 27, inhibiting exit from the germinal center and accelerating lymphoma development in preclinical models. Two inhibitors of EZH2 have entered clinical trials, GSK2816126 and tazemetostat. GSK2816126 is a highly selective and potent inhibitor of wild-type (WT) and mutant (Y641N, A677G, and A687V) EZH2, with IC50 of 28–861 nM for proliferation in EZH2-mutant lymphoma cell lines. It is not orally bioavailable, but pharmacokinetic/pharmacodynamic studies suggested that therapeutic levels could be achieved with intravenous doses of between 900mg and 2,700mg twice a week. Tazemetostat is a similarly selective and orally bioavailable small-molecule inhibitor of EZH2 with an IC50 of 0.49-5800 nM for proliferation in EZH2-mutant lymphoma cell lines. Both of these molecules have been tested in phase I dose escalation studies. GSK2816126 was given by twice-weekly intravenous infusion at doses of up to 3,000mg, with dose-limiting liver enzyme rises at the highest dose, achieving trough levels of 410 ng/mL. No changes in H3K27me3 could be seen in peripheral blood mononuclear cells, and only one short-lived partial response was seen among 20 patients with B-cell lymphoma. Tazemetostat was given at doses of up to 1,600mg twice daily without reaching dose-limiting toxicity, although significant thrombocytopenia was seen at this dose level. The mean pre-dose trough level at the recommended phase II dose of 800mg bd was 75 ng/mL, and skin biopsies showed a reduction of H3K27me3 by approximately 40% from baseline. Tazemetostat has shown moderate efficacy as a single agent in relapsed/refractory follicular lymphoma (FL), with overall response rate (ORR) 33/43 (77%) and median duration of response (mDOR) 8.3 months in EZH2-mutant cases and ORR 18/53 (34%), mDOR 13 months in those without mutations. In relapsed/refractory diffuse large B-cell lymphoma (DLBL) the response rate was lower, ORR 6/36 (17%), mDOR 11+ months in mutant cases and 20/121 (17%), mDOR 7+ months in wild-type. In both studies the treatment was well tolerated, with the most frequently reported adverse events being thrombocytopenia, anemia, asthenia, vomiting, and fatigue, all in less than 5% of patients with FL and less than 20% in DLBL. Combination therapy of tazemetostat with atezolizumab did not increase the response rate in DLBL, but giving it with R-CHOP chemotherapy was well tolerated in a phase Ib study, and a dose of 800mg BD

could be given without apparently increased toxicity over R-CHOP in patients between 60 and 80 years old, and without adverse effects on the pharmacology of the chemotherapy or tazemetostat. In keeping with many agents targeting a single molecular abnormality, inhibitors of EZH2 have demonstrated clear but transient efficacy, with the most promising results in follicular lymphoma. The higher response rate among cases with an activating mutation is notable, but wild-type lymphomas also show responsiveness in a minority of cases, indicating other potential mechanisms of action. The combination of tazemetostat with chemoimmunotherapy appears promising, although the relatively good prognosis for DLBL bearing an EZH2 mutation may make it difficult to demonstrate clear clinical benefit with this approach.

IA28 Disparities in late effects incidence among adolescent and young survivors of lymphoma. Qian W. Li, Ann Brunson, Ted Wun, Renata Abrahao, Theresa H. M. Keegan. Center for Oncology Hematology Outcomes Research and Training (COHORT), Division of Hematology and Oncology, University of California Davis School of Medicine, Sacramento, CA.

Background: Survival after lymphomas, the most common malignancies in adolescents and young adults (AYAs), has improved significantly over time as a result of advances in diagnostic procedures and therapeutic management. However, studies have shown that survival varies substantially by race/ethnicity, socioeconomic status (SES), health insurance coverage, and clinical factors. Additionally, therapies that lead to cure, such as chemotherapy and radiation, can result in an elevated lifetime risk of chronic medical conditions ("late effects") that can considerably impair the quality of life and increase mortality of survivors. We aimed to estimate the burden of late effects in AYA lymphomas survivors and identify sociodemographic and clinical factors associated with late effects incidence.
Methods: We used data from the California Cancer Registry linked to hospitalization data from the California Office Statewide Health Planning and Development. Eligible patients were those diagnosed with a first primary non-Hodgkin (NHL) or Hodgkin lymphoma (HL) from 1996 to 2012 who survived at least 2 years after diagnosis. Patients were followed through 2014. The late effects included cardiovascular, respiratory, kidney, liver, endocrine, and neurologic diseases, as well as avascular necrosis and second cancers. We estimated the cumulative incidence of each condition, accounting for death as a competing risk. Cox proportional hazards regression models were used to examine the relation of sociodemographic and clinical factors to late effects, providing an estimate of the hazard ratio (HR) and associated 95% confidence intervals (CI). NHL and HL models were adjusted for age at diagnosis, year of diagnosis, stage at diagnosis, sex, race/ethnicity, cancer subtype (NHL), B-symptoms (HL), initial treatment (chemotherapy and radiation), receipt of a hematopoietic stem cell transplant (HSCT), health insurance, and neighborhood SES. Patients with human immunodeficiency virus (HIV) infection were considered separately in the NHL analyses and excluded from the HL analyses.
Results: We identified 5,085 HL and 4,817 NHL survivors. Of those with NHL, 425 (9%) were HIV infected. In both HL and NHL cohorts, the highest cumulative incidence of late effects was observed for endocrine, cardiovascular, and respiratory diseases. Among NHL survivors, those with HIV infection had a higher incidence of all late effects, including an over 3-fold higher risk of second cancers compared with HIV-uninfected survivors (8.1% vs. 2.6%, respectively). We observed that, except for hypothyroidism, HL patients diagnosed with late (vs. early) stage disease had a greater incidence of all late effects. In multivariable adjusted models, HL survivors

who underwent an HSCT experienced 1.7 to 3.4-fold higher risk of all late effects than non-HSCT recipients. Similarly, among HIV-uninfected NHL survivors, those who underwent an HSCT had a higher risk of most late effects, particularly avascular necrosis (HR=4.6, 95% CI 2.4–8.8). Except for second cancers, uninsured/publicly insured HL survivors experienced a 1.3–1.8 greater risk of all late effects compared with those with private insurance. For NHL, AYAs with public/no insurance had 1.6 to 2.4 higher risk of several late effects, particularly neurologic diseases (HR=2.4, 95% CI 1.5–3.8). Additionally, we observed that HL and NHL patients who lived in lower SES neighborhoods had a higher risk of respiratory and endocrine diseases and NHL survivors living in lower SES neighborhoods also had a higher risk of cardiovascular diseases. Compared to non-Hispanic whites, Black HL survivors were more likely to have cardiovascular diseases, and Black and Hispanic survivors were more likely to have endocrine diseases. Furthermore, among NHL survivors, Hispanics experienced a 73% and Blacks a 91% greater risk of renal diseases. Differences by race/ethnicity were more pronounced among NHL survivors with HIV infection, with those of non-white race/ethnicity experiencing a nearly 6-fold higher risk of renal diseases. Importantly, HL AYA survivors who experienced any late effect had an over 2-fold increased risk of death (HR=2.1–6.2).

Conclusion: To our knowledge, this is the first US population-based study to examine the influence of sociodemographic factors on the incidence of late effects, as well as to compare the risk between NHL HIV-infected vs. HIV-uninfected survivors. We found substantial incidence of late effects in AYA survivors of both NHL and HL in California and identified populations with a higher incidence of these conditions. Specifically, we found that AYA survivors of Black or Hispanic race/ethnicity, those who had public or no health insurance, who underwent a HSCT, or resided in lower SES neighborhoods were consistently at higher risk of developing late effects. In addition, NHL survivors with HIV infection had a strikingly greater risk of all conditions. These sociodemographic and clinical factors have been associated with worse survival among AYAs. In order to improve outcomes and reduce health disparities in these young cancer survivors, our findings highlight the critical need to address these late effects by increasing education of AYAs on their risks and the importance of survivorship care and reducing health insurance and financial barriers to receiving quality care.

IA29 Genome-estimated African ancestry is associated with distinct tumor mutations and poorer survival in patients with diffuse large B-cell lymphoma. Christopher R. Flowers. The University of Texas MD Anderson Cancer Center, Houston, TX.

Significant differences have been observed in the incidence, age of onset, and clinical outcomes of diffuse large B-cell lymphoma (DLBCL) by race in the United States. However, it remains unclear what factors underlie these differences and whether genomic differences contribute to these disparities. In this talk Dr. Flowers will address the identified differences in baseline clinical characteristics for black and white patients with DLBCL and the relationships between race, sociodemographic factors, and outcomes. To understand the influences of genetic ancestry on tumor genomic alterations, in a recent paper (Lee et al., Cancer 2020), we estimated the genetic ancestry of 1,001 previously described patients with DLBCL (Reddy et al., Cell 2017) using unsupervised model-based Admixture global ancestry analysis applied to exome sequencing data and examined the mutational profile of 150 DLBCL driver genes in tumors obtained from this cohort. Global ancestry prediction identified 619 patients with >90% European ancestry, 81 patients with >90% African ancestry, and 50 patients with >90% Asian ancestry. Compared with patients with DLBCL with European ancestry, patients with African ancestry were aged >10 years younger at the time of diagnosis and were more likely to present with B symptoms, elevated serum lactate dehydrogenase, extranodal disease, and advanced stage disease. Patients with African ancestry demonstrated worse overall survival compared with patients with European ancestry (median, 4.9 years vs. 8.8 years; P = .04). Recurrent mutations of MLL2 (KMT2D), HIST1H1E, MYD88, BCL2, and PIM1 were found across all ancestry groups, suggesting shared mechanisms underlying tumor biology. The authors also identified 6 DLBCL driver genes that were more commonly mutated in patients with African ancestry compared with patients with European ancestry: ATM (21.0% vs. 7.75%; P < .001), MGA (19.7% vs. 5.33%; P < .001), SETD2 (17.3% vs. 5.17%; P < .001), TET2 (12.3% vs. 5.82%; P = .029), MLL3 (KMT2C) (11.1% vs. 4.36%; P = .013), and DNMT3A (11.1% vs. 4.52%; P = .016). Distinct prevalence and patterns of mutation highlight an important difference in the mutational landscapes of DLBCL arising in different ancestry groups. This characterization of genetic alterations among patients with African descent who are diagnosed with DLBCL provides a roadmap for future studies in other lymphoid malignancies and a pathway for drug development in areas where these genetic alterations are found to be associated with worse clinical outcomes.

IA30 Targeting MYC deregulation in cancer. Gregor A. Lueg[1], Monica Faronato[1], Andrii Gorelik[1], Andrea G. Grocin[1], Miriam Llorian-Sopena[2], Probir Chakravarty[2], Bernadette Brzezicha[3], Martin Janz[4], Mathew J. Garnett[5], Dinis P. Calado[6], Edward W. Tate[1]. [1]Imperial College and The Francis Crick Institute, London, United Kingdom, [2]The Francis Crick Institute, London, United Kingdom, [3]Experimental Pathology & Oncology Berlin-Buch, Berlin, Germany, [4]Max Delbrück Center for Molecular Medicine and Charité, Berlin, Germany, [5]Wellcome Sanger Institute, Cambridge , United Kingdom, [6]The Francis Crick Institute and King's College London, London, United Kingdom.

Human N-myristoyltransferase (NMT) 1 and 2 catalyze N-terminal protein myristoylation, a modification that regulates membrane trafficking and interactions of >100 proteins. NMT has been proposed as a target in cancer, but a rationale for selectivity is lacking due to the complex impact of NMT inhibition (NMTi) on multiple cellular pathways. Here, large-scale screens of hundreds of cancer cell lines against a panel of potent and selective NMTi were combined with systems-level analyses to reveal that NMTi is synthetically lethal with deregulated MYC or MYCN. Synthetic lethality is mediated by post-transcriptional failure in (NADH) Complex I protein synthesis followed by mitochondrial dysfunction in MYC-deregulated cancer cells. We observed that NMT inhibitors blocked the growth of patient-derived tumors with deregulated MYC, without overt toxicity. This mechanistic framework supports NMTi as a novel targeted therapeutic approach and provides a new paradigm in which targeting of a constitutive protein modification is synthetically lethal in MYC-deregulated cancers.

IA31 Modeling and targeting double-hit lymphoma. Bruno Amati. European Institute of Oncology (IEO), Milan, Italy.

High-grade B-cell lymphoma with concurrent activation of MYC and BCL2, also known as double-hit lymphoma (DHL), shows dismal prognosis with current front-line therapies (e.g., R-CHOP), calling for the development of new therapeutic regimens. The synergy between MYC and BCL2 in lymphomagenesis is explained by the ability of BCL2 to block the proapoptotic activity of MYC, while leaving intact its proliferative potential; on this basis, we reasoned that compounds that exacerbate MYC-induced apoptosis might cooperate with BCL2 inhibitors in killing DHL cells. We previously observed that genes encoding components of the mitochondrial ribosome were coordinately activated in MYC-driven lymphoma (1) and were critical for tumor maintenance in transgenic mice (2). In line with the genetic data, inhibition of mitochondrial translation with the antibiotic tigecycline was synthetic-lethal with MYC activation and extended lifespan in lymphoma-bearing mice (2). Combining tigecycline with the BCL2 inhibitor venetoclax revealed synergy in the induction of apoptosis in human DHL cell lines, as well as marked antitumoral activity in xenografted mice (3). These preclinical data warrant the repurposing of venetoclax and tigecycline for the treatment of refractory and/or relapsed DHL. By blocking the synthesis of mitochondrially encoded proteins, tigecycline impairs the assembly of the electron-transport chain (ETC) complexes and oxidative phosphorylation. We thus hypothesized that direct ETC inhibitors might also preferentially kill MYC-overexpressing cells and synergize with venetoclax against DHL. We will present data that verify these predictions for IACS-010759, a small-molecule inhibitor of mitochondrial complex I. A current limitation for further preclinical development and mechanistic analysis lies in the lack of a bona fide mouse model of DHL. While the cooperation between MYC and BCL2 in lymphomagenesis was amply documented over almost three decades, the models described so far led to tumor onset at early stages of B-cell ontogeny; as a consequence, no model is available that reliably reproduces the development of DHL from germinal center (GC) B-cells, as observed in the clinic. In order to overcome this limitation, we combined CRE-activated alleles of MYC and BCL2 with Cgamma1-CRE, a CRE-expressing transgene that is specifically expressed in GC B cells. Concerted activation of both oncogenes led to increased expansion of GC B cells upon immunization, followed within a few months by the onset of aggressive, invasive B-cell lymphomas. Our progress in characterizing this disease model will be presented at the meeting.

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IA35 Follicular lymphoma dynamics through single-cell analysis. Bertrand Nadel.
Centre d'Immunologie de Marseille-Luminy, Marseille, France.
Follicular lymphoma (FL) is the second most common non-Hodgkin lymphoma in the Western world, generally characterized by a disseminated disease at diagnosis, an indolent clinical course, and recurrent, increasingly chemoresistant relapses. Overt FL is preceded by an insidious phase of asymptomatic growth and might emerge from common precursor clones, evolving over decades, and which might participate in subsequent relapses. Consequently, the cell of origin remains ambiguous. Although FL results from the malignant transformation of germinal center B cells, FL precursors emerge much earlier in B-cell ontogeny with the hallmark t(14;18) translocation and ensuing constitutive expression of BCL2 in bone marrow pre-B cells. While t(14;18) is considered the necessary early first hit to transformation, BCL2 as such is a very weak driver, as evidenced by the detection of t(14;18) in peripheral blood from a large fraction of the adult healthy population and the long latency and low penetrance observed in various BCL2 mouse models. Recent data of clonal dynamics in human and mouse models strongly suggest that additional illegitimate events likely accumulate over iterative GC passages, generating a highly mutagenic dynamics linked with slow oncogenic progression. Combined with the power of next-generation sequencing, molecular interrogations of t(14;18)+ clones in healthy individuals and FL patients recently provided the extent of FL genetic diversity in space (distinct tissues) and time (diagnosis vs. relapse). Thus, the path to transformation appears as a complex multi-hit process occurring along B-cell ontogeny, escalating along successive derailments of B-cell receptor diversification mechanisms, and subversion of specific immunologic properties of B cells. This protracted clonal evolution generates a massive accumulation of subclones and an associated complex overall genetic heterogeneity. This raises several questions. While FL originate from germinal center B cells, it is unclear to what extent overt lymphoma B cells retain GC B-cell functional dynamics or are blocked in a particular stage of the GC reaction. Furthermore, although one might intuitively assume that distinct subclones likely have a different gene expression profile associated to specific functions (and potentially driving distinct capacity to respond to therapy), it is still unclear to which extent subclonal genetic heterogeneity imprints functional heterogeneity. Because subclonal heterogeneity is hidden in bulk, where all distinct subclones are mixed and averaged, we recently used integrative single-cell analysis of phenotype, gene expression, and IGH sequence to track the characteristic human GC B-cell program in FL B cells. We used pseudo-time approaches to model the cyclic continuum of GC B-cell transitional states and identified characteristic patterns of synchronously expressed gene clusters in GC B cells. Strikingly this GC-specific gene expression synchrony was lost in single lymphoma B cells. Yet, distinct and conserved

FL-specific cell states co-existed within single patient biopsies. Our data show that lymphoma B cells are not blocked in a GC B-cell state but may adopt new dynamic modes of functional diversity, opening novel definitions of lymphoma identity.

IA38 Defining lymphoma reservoirs: Clues from the genomics. Jessica Okosun. Barts Cancer Institute, Queen Mary University of London, London, United Kingdom.
Large-scale technology and advanced computational analyses have revolutionized our understanding of the genomic underpinnings of lymphoma subtypes across the board, providing near-complete genomic encyclopedias. A characteristic feature of indolent lymphomas is their propensity to initially respond to therapeutic intervention, only to subsequently relapse or transform to a more aggressive lymphoma at a later stage. As a consequence, the population disease burden remains significant as, for the majority, their lymphomas are incurable. There is growing evidence that persisting cancer-repopulating cells act as lymphoma reservoirs and are the harbingers for lymphoma recurrence and therapy resistance. Follicular lymphoma (FL) is the best studied of the indolent B-cell lymphomas and represents a tractable model system given its protracted clinical course. The evidence for the existence of a tumor-propagating reservoir in FL (referred to as the common precursor cell, CPC) has primarily arisen from genetic studies in two key areas. The first are unique cases of donor-derived lymphomas, where both donor and recipient of stem cell transplants develop clonally related FL several years later, suggesting precursor cells were transferred at the time of the transplantation. The second include studies of temporal genetic profiling of sequential diagnostic, relapsed, and transformed FL (tFL) tumors from the same individual, undertaken by us and others more recently using higher-resolution next-generation sequencing. This has allowed us to infer the genetic composition of these putative ancestral or reservoir populations and indeed determine that epigenetic alterations such as mutations in the histone-modifying enzymes, CREBBP and KMT2D, represent early lymphoma-initiating events. Recent genomic studies in histologically related entities such as pediatric-type FL and in situ follicular neoplasia (ISFN) have provided further clues to the relevance of the genetic events. A number of crucial questions remain: What is the exact origin, phenotype, and niche of these reservoir populations, and do particular patient-specific niches promote a degree of dormancy and continued molecular evolution of these lymphoma reservoirs? Capturing the characteristics and behavior of these reservoir populations in FL has the potential to reveal novel biomarkers and facilitate new approaches to measuring, monitoring, and tracking this population. Furthermore, defining the characteristics of these populations would allow the generation of murine models that more suitably recapitulate this disease state, providing a resource to guide and test rational novel therapies. In conclusion, we now have early insights into the genetic fingerprints of these persisting tumor-propagating reservoir populations. Targeting these populations, perceived as the “root’’ of the cancer, may provide our best chance of preventing relapse and realizing a cure for FL.

IA39 Investigating malignant transformation in Waldenstrom's macroglobulinemia. Zachary R. Hunter[1], Maria Luisa Guerrera[2], Guang Yang[1], Nicholas Tsakmaklis[2], Xia Lu[2], Steven P. Treon[1]. [1]Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, [2]Dana-Farber Cancer Institute, Boston, MA.
Waldenström's macroglobulinemia (WM) is an uncommon B-cell lymphoma that corresponds to the histopathologic classification of IgM-secreting lymphomplasmacytic lymphoma. It is also a disease characterized by a series of highly recurrent mutations. Whole-genome sequencing of CD19+ bone marrow cells from patients with WM led to the discovery of a somatic heterozygous c.978T>C mutation in Toll-like receptor adaptor protein *MYD88*, resulting in a leucine-to-proline substitution p.Leu265Pro (L265P) in over 90% of WM patients. Somatic activating mutations in MYD88 induce constitutive homodimerization and downstream signaling through the nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB) pathway independent of receptor activation. Other somatic events characteristic of WM include deletions in chromosome 6q21-23 and activating nonsense and/or frameshift mutations in the carboxyl-terminal tail of *CXCR4*, found in 40-60% and over 30% of WM patients, respectively. Both of these events are found predominantly in MYD88 mutant WM and are frequently restricted to a subclonal population.

Similar to related lymphomas, WM is thought to evolve from IgM-secreting monoclonal gammopathy of undermined significance (MGUS). The MYD88 mutations are easily detectible IgM MGUS stage, suggesting that it is a very early event in clonal development but may predate the malignant transformation into WM. This theory is support by several transgenic mouse models of mutant *MYD88* that have demonstrated that p.Leu265Pro MYD88 mutation in B cells alone is not sufficient for lymphomagenesis (Knittel et al., Blood 2016; Sewastianik et al., Blood Adv 2019). Mutations in *CXCR4* have been detected in IgM MGUS, though at lower rates than are typical of WM. Given that this and the transcriptional profile of *CXCR4* activating mutations in WM are consistent with a pattern of suppression of tumor suppressors upregulated by mutant *MYD88*, it has been proposed as a possible transformation event from IgM MGUS to asymptomatic WM. Likewise, a number of careful genetic studies of IgM MGUS have suggested the emergence of somatic copy number alterations, particularly the deletions in 6q, to be related to the malignant transformation of WM (Schop et al., Cancer Genet Cytogenet 2006; Paiva et al., Blood 2015).

We therefore reanalyzed our whole-genome sequencing data of WM patients and performed allele-specific PCR and 6q copy number assays to study the rates and

relationship of *CXCR4* mutations and chromosome 6q deletions in untreated *MYD88* mutated WM. Both our 30-patient whole-genome and our 25-patient PCR cohort found no relationship between symptomatic and asymptomatic WM and the somatic events, indicating that they both were occurring at an earlier stage of WM development. Both studies also found that the large clonal deletions in chromosome 6q were not observed in the presence of *CXCR4* mutations ($p<0.01$ for both). Using our previous RNASeq data of 55 WM patients, we compared the transcriptional signatures for *CXCR4*-mutated and 6q-deleted WM relative to WM patients with the *MYD88* mutation alone, revealing an overlapping signature of 19 genes. Notably, the magnitude and relative direction of the changes were similar for *CXCR4*-mutated and 6q-deleted WM. Impacted genes included biologically relevant targets such as *FAM110C*, *WNK2*, *CDK14*, *FOXO3*, *IGF2R*, and *HRK*. Preliminary validation studies using qPCR and immunohistochemistry have confirmed these findings. We therefore propose that *CXCR4* mutations and 6q deletions are not only appearing during the transformation from MGUS to WM but target a related set of genes that may play a critical role in the pathogenesis of *MYD88* mutant WM. Functional studies to further characterize these genes and evaluate their potential as therapeutic targets for WM are ongoing.

IA40 Defining precursors in low-grade lymphomas that seed second lymphomas.

Abner Louissaint[1], David Weinstock[2]. [1]Massachusetts General Hospital, Harvard Medical School, Boston, MA, [2]Harvard Medical School, Dana-Farber Cancer Institute, Boston, MA.

Most patients with low-grade lymphoma will achieve a clinical remission (CR) with standard chemoimmunotherapy. Yet, relapse from minimal residual disease (MRD), either as low-grade or high-grade disease, remains the leading cause of death. Several studies have provided consistent evidence showing the strong prognostic value of measurable MRD using flow cytometry, PCR, next-generation sequencing, or other approaches. While MRD includes the malignant cells that remain in a patient who achieves CR, not all MRD cells may have the functional capability to proliferate into relapse. We recently introduced a nomenclature to more precisely distinguish cells included within MRD. In this nomenclature, M-REC (Minimal-RElapsable Cancer) denotes fully transformed cells capable of driving a relapse; MN-REC (Minimal Non-RElapsable Cancer) denotes fully-transformed cells incapable of driving a relapse; and MRP (Minimal Residual Precursors) denotes residual cells that harbor somatic and/or phenotypic alterations but are not fully malignant, such as those causing a dysplastic “field-effect” in cancers like those of the bladder, head and neck, or esophagus. Studies that defined the genetic phylogeny of lymphoma using diagnostic, relapsed, and transformed B-cell lymphomas from the same patients have identified examples where MRPs repeatedly evolve into clinical lymphomas. This conflicts with the traditional model of cancer evolution, in which “relapses” result from M-RECs that persist after therapy. There are two immediate implications to this paradigm-shifting concept: 1) Many “relapses” are actually second lymphomas, and 2) defining and targeting the vulnerabilities of MRPs may be essential for improving lymphoma-free survival among patients who achieve a CR. It is important to note that the vulnerabilities of MRD may differ within the bone marrow, blood, lymph node, and other sites, so sampling of each is essential. To make this possible, we have advanced two strategies. First, we have established xenografts of human follicular lymphoma explants within immunocompromised mice that capture both malignant and nonmalignant cellular components, as well as predictable transformation to large-cell lymphoma in vivo. Second, we have initiated rapid autopsies of patients with disseminated lymphoma to obtain specimens from multiple organs and thereby define the heterogeneity of environmental niches that support the persistence of MRD.

IA41 Approaches for personalized medicine in lymphoma through liquid biopsies. David M. Kurtz. Stanford University, Stanford, CA.

Over the last decade, circulating tumor DNA (ctDNA) has emerged as an important biomarker across a range of cancers, including lymphomas. The potential clinical applications of ctDNA technologies are broad, including tumor mutational genotyping, detection of molecular responses and minimal residual disease, as well as early detection of relapsing disease with specific attention to mechanisms of resistance. Numerous opportunities therefore exist to apply ctDNA methodologies to tailor therapy for individual patients. By applying targeted next-generation sequencing of ctDNA via Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) in patients with lymphoma, these opportunities for precision oncology largely fall into two groups—identification of specific targetable molecular features and quantitative assessment of disease burden and risk. Here we explore these approaches in patients with diffuse large B-cell lymphoma (DLBCL) undergoing first-line or salvage therapy. Utilizing CAPP-Seq from pretreatment plasma, mutational genotyping of diverse somatic alterations is possible, including single-nucleotide variants (SNVs), translocations, and copy number alterations. By combining these mutations through an integrated Bayesian framework, identification of specific disease subtypes is possible, including activated and germinal center B-cell molecular cell-of-origin subtypes. In addition, detection of emergent mutations during and after treatment can provide insights into mechanisms of therapeutic resistance, many of which are potentially targetable. In addition to these insights into disease biology, ctDNA can serve as a quantitative handle on disease burden. By measuring ctDNA disease burden in DLBCL patients from six centers throughout North America and Europe, we demonstrate the prognostic value of ctDNA levels prior to treatment. Additionally, we found that a molecular response to treatment, as detected by the change in ctDNA after one or two cycles of therapy, is highly prognostic for patient outcomes in both front-line and later lines of therapy. Moreover, these molecular response features can be combined with additional outcome predictors, such as the IPI and interim PET/CT scans, to build a dynamic risk model termed the Continuous Individualized Risk Index (CIRI). CIRI provides a personalized probability of likely patient outcomes that updates over time as more information becomes available. Outcome prediction with CIRI significantly improved on predictions using ctDNA molecular response, interim PET/CT scans, or the IPI alone. In summary, liquid biopsies through ctDNA afford numerous opportunities for personalized medicine, ranging from mutational profiling and molecular subtyping to disease quantification and response prediction. Further studies of novel clinical approaches are likely to change treatment paradigms in the near future.

IA42 Detecting and quantifying mutations associated with treatment resistance in aggressive lymphomas using ctDNA. Nicole Thomas[1], Laura K. Hilton[1], Neil Michaud[2], Kevin Bushell[1], Ryan Rys[3], Michael Jain[4], Lois Shepherd[5], Marco A. Marra[6], John Kuruvilla[7], Michael Crump[7], Koren Mann[3], Sarit Assouline[3], Christian Steidl[6], Mark S. Cragg[8], David W. Scott[6], Nathalie Johnson[3], Ryan D. Morin[1], Christopher K. Rushton[1], Sarah E. Arthur[1], Miguel Alcaide[1], Matthew Cheung[1], Aixiang Jiang[1], Krysta M. Coyle[1], Kirstie L. S. Cleary[8]. [1]Simon Fraser University, Burnaby, BC, Canada, [2]Epizyme, Boston, MA, [3]McGill University, Montreal, QC, Canada, [4]Moffitt Cancer Centre, Tampa, FL, [5]Queens University, Kingston, ON, Canada, [6]BC Cancer, Vancouver, BC, Canada, [7]Princess Margaret Cancer Centre, Toronto, ON, Canada, [8]University of Southampton, Southampton, United Kingdom.

A significant proportion of diffuse large B-cell lymphoma (DLBCL) patients treated with immunochemotherapy containing rituximab (R-CHOP) exhibit either primary or acquired treatment resistance. The advancement of therapeutics in the relapse setting has likely been encumbered by our limited understanding of the molecular features that underlie resistance to R-CHOP. Unfortunately, our knowledge of DLBCL genetics is mostly limited to analyses conducted on diagnostic tissue biopsies, which have not been exposed to the selective pressures imposed by therapy. Identifying genetic alterations that contribute to treatment resistance may reveal additional treatment options and lead to biomarkers allowing patients to be paired with appropriate treatments. Genetic subgroups are gaining popularity as a new strategy to implement precision medicine in DLBCL (1). The relevance of these and other biomarkers in the relapse setting remains unclear due to limited genetic exploration of relapsed and refractory DLBCL (rrDLBCL). Progress has been limited, in part, by the requirement of tissue biopsies collected after relapse. It is well established that quantitative genomic techniques such as digital PCR and targeted sequencing can be used to determine the proportion of tumor DNA in plasma from lymphoma patients (2). With a sufficiently broad panel, sequencing affords additional opportunities including the ability to identify subclonal structure and population dynamics over time. This presentation will discuss our recent analysis of a large collection of ctDNA primarily comprising DLBCL patients on various clinical trials (3). Targeted sequencing of these samples and comparison to exome data from a meta-cohort of previously characterized untreated DLBCL biopsies revealed six genes significantly enriched for mutations upon relapse. We found both TP53 and KMT2D were mutated in the majority of rrDLBCLs, and these mutations persisted in the dominant clone following relapse, suggesting a role in primary treatment resistance. By inferring subclonal dynamics, we observed recurrent patterns of clonal expansion and contraction following rituximab-based therapy, with MS4A1 mutations representing the only example of consistent clonal expansion. MS4A1 missense mutations within the

transmembrane domains led to loss of CD20 expression in vitro, and patient tumors harboring these mutations lacked CD20 protein expression. Our analysis nominates TP53 and KMT2D mutation status as novel prognostic factors that may facilitate the identification of high-risk patients prior to therapy. Moreover, we have demonstrated the potential to identify tumors with loss of CD20 surface expression stemming from MS4A1 mutations. Implementation of noninvasive assays to detect such features of acquired treatment resistance may allow timely transition to more effective treatment regimens. In certain scenarios whole-exome sequencing (WES) or whole-genome sequencing (WGS) can be successfully applied to ctDNA, thereby allowing the identification of mutations, structural variation, and copy number changes. Low-pass sequencing of shotgun libraries can also be used to ascertain course estimates of ctDNA levels as well as the copy number landscape (4). Given the importance of copy number and structural alterations in the inference of genetic subgroups, these methods may allow the exploration of these groups and their stability over time. Through a series of illustrative examples, this presentation will explore the benefits of each of these techniques in the study of tumor evolution and acquired treatment resistance in DLBCL.

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IA44 ctDNA in indolent lymphoid cancers treated with targeted therapies. Constantine S. Tam, Piers Blombery. Peter MacCallum Cancer Centre, Royal Melbourne Hospital and University of Melbourne, Melbourne, VIC, Australia. The use of circulating tumor DNA (ctDNA) in diagnosing and monitoring treatment responses in hematologic malignancies has multiple advantages: (1) ctDNA is noninvasive, allowing for frequent, serial, real-time tracking of multiple tumor clones; (2) ctDNA is highly sensitive for clone-specific genomic aberrations, and may rival traditional minimal residual disease (MRD) methodologies in detecting low-volume disease; and (3) ctDNA "integrates" the tumor genome across multiple body compartments, allowing the detection of emerging resistant clones that would otherwise be missed by single-site biopsies. Since 2014, our group has evaluated the use of ctDNA in patients with hematologic cancers initially as an investigational tool, and more recently as an accredited (ISO15189) hybridization-based NGS panel that can perform sequence variant detection, genome-wide copy number calling, and structural variant detection from ctDNA. In this talk, we will discuss different clinical scenarios and the evolution of ctDNA at our center. Our initial proof-of-concept study was conducted in patients receiving BTK inhibitor (BTKi) and/or BCL2 inhibitor (BCL2i) therapy for chronic lymphocytic leukemia (CLL) or mantle cell lymphoma (MCL). Clinical challenges in this population include response assessment being confounded by BTKi redistribution lymphocytosis, differential compartmental responses to different classes of novel agents, and emergence of resistant clones in disparate disease sites. In our study of CLL, serial samples from 32 patients treated with either ibrutinib (BTKi, n=22) or venetoclax (BCL2i, n=10) were analyzed, including 3 patients who developed RS. Using a bespoke TS panel of 7 commonly mutated genes in CLL (*SF3B1*, *NOTCH1, ATM*, *TP53, MYD88*, *KRAS*, *and BIRC3*), somatic mutations comprising 0.1% to 90% of total ctDNA were detected in 25 of 32 (78%) patients. Serial monitoring of MAF was concordant with disease response as determined by CT scans and, importantly, ctDNA was able to "look beyond" the artificial rise in circulating tumor cells caused by BTKi, showing an early and consistent fall in MAF. ctDNA was sensitive to the detection of disease clones in occult body compartments—of 88 serial timepoints assessed by both ctDNA and MNL DNA after the initiation of novel therapy, mutations were found to be detectable only in ctDNA in 18 (20%) timepoints. In 30 timepoints where MRD testing was performed by both ctDNA and high-resolution flow cytometry, there was 100% concordance in results. In a second study, serial ctDNA analyses were performed in a cohort of 24 patients with MCL receiving combination ibrutinib and venetoclax therapy. Using TS and a panel of 42 genes known to be recurrently mutated in MCL, trackable ctDNA mutations were detected in 71% of patients, with baseline MAF ranging from 0.4-60%. Similar to our experience with CLL, dynamic

changes in ctDNA closely mirrored tumor responses as determined using traditional staging methods of CT, PET, endoscopy, and bone marrow examination. In one patient, ctDNA started rising several weeks before relapse was evident by traditional MRD methods. A further patient had progression in an occult site (pleura), with acquisition of new CNAs on chromosome 6, including 6p loss. In this patient, these new CNAs were readily identified by LC-WGS of plasma. Finally, in a patient with primary refractory disease, an acquired loss of chromosome 9p was identified in plasma 8 weeks before disease progression was evident on imaging. We have since optimized and validated ctDNA testing for routine use into our diagnostic laboratory NGS workflow and we will discuss recent cases of BTK and BCL2 mutations being readily detectable in patients in early stages of novel agent resistance, facilitating early transition to next-line therapy. In aggressive lymphomas, we will demonstrate the value of ctDNA in detecting CD274(PD-L1)/PDCD1LG2 (PD-L2) copy number abnormalities and structural variants in patients with lymphomas in inaccessible tissue sites, guiding the rational use of checkpoint inhibitors. Other important structural variants that are able to be detected in ctDNA through this methodology are IGH-MYC and IGH-BCL2 translocations, thus allowing rapid categorization of patients with diffuse large B-cell lymphoma as “double-hit” lymphoma. Taken together, these studies underscore the value of ctDNA as a noninvasive, highly sensitive tool for assessment of genomic landscape and tracking clonal dynamics across multiple body compartments in patients with hematologic malignancies.

IA46 Molecular and genetic profiling in canine lymphoma unravels targets for the human counterpart. Luca Aresu. Department of Veterinary Sciences, Turin, Italy. Canine lymphoma represents an excellent cancer model for the human counterpart and is the most widely investigated tumor both in veterinary and comparative oncology. Knowledge of the diagnosis, molecular biology, genetic, and epigenetic has progressively grown in recent years, but prognosis in both B- and T-cell lymphoma remains poor. Several lymphoma subtypes are recognized in canine species and diffuse large B-cell lymphoma (DLBCL) is the most frequent, followed by marginal zone lymphoma (MZL) and a peculiar T-cell malignancy named T-zone lymphoma. DLBCL resembles in many elements the human counterpart, including frequency, molecular abnormalities, and similar therapeutic responses to chemotherapy. However, many other aspects are still scarcely known, and a complete characterization becomes fundamental when including dogs with this lymphoma in clinical trials for new molecules. Indeed, the inability to accurately predict outcome poses several limitations when testing efficacy of new drugs. With NGS advent and the dramatic reduction of costs, researchers in veterinary medicine have started to describe canine lymphoma in a similar fashion to rodent models. The aims of this presentation are to provide an overview of the most significant results in canine lymphoma that have been published in the last decade, focusing on molecular and therapeutic aspects of this tumor. About B-cell lymphoma, transcriptomic studies in canine DLBCL, MZL, and follicular lymphoma (FL) have revealed similarities with the human histotypes. Genes involved in B-cell receptor signaling were found to be enriched both in canine DLBCL and MZL, and several Toll-like receptors were overexpressed, suggesting mechanisms associated with human activated B-cell (ABC)-like subtype DLBCL pathogenesis. The aberrant expression of these genes seems to contribute to enhancing proliferation and protection of canine B cells from apoptosis by stimulating NF-κB activity. The biologic role of NF-kB was tested in dogs with DLBCL using NEMO-binding domain peptide in two clinical trials and the compound resulted in safe blocking of NF-kB activity. Previously, treatment with Ibrutinib was found effective in dogs with B-cell lymphoma. Also, genes coding for proteins involved in MYC signaling and PI3K/AKT/mTOR pathway were found to be overexpressed in both DLBCL and MZL. A peculiar MYC deregulation controlled by LIN28B/Let-7 axis was identified only in DLBCL and LIN28B resulted the most upregulated gene both in vivo and in vitro, revealing an important functional relevance and opening new scenarios for treatment of this tumor in dogs. Within this context, the pan-BET inhibitor OTX015, targeting BRD4, was able to reduce proliferation and expression of MYC-associated genes in vitro. When investigating the cell of origin of canine DLBCL, a study from our group showed a segregation of this lymphoma in two transcriptomic subgroups that were further characterized by

distinct methylation profiles and associated with survival. The dogs with inferior survival showed a higher expression of transcripts involved in T-cell and macrophage regulation (CD163, CD96, PD-1, PDL-1, CTLA4, CD8a, CD4). Recently, a study explored the mutation profile of canine B- and T-cell lymphoma in three pure breeds. B-cell lymphomas were categorized based on their mutation profile, and TRAF3, BIRC3, and MAP3K14 were found to be frequently mutated. For TRAF3, a combination of somatic frameshift mutations and truncating SNVs in causing down-expression was identified and germ-line mutations were retrieved in some dogs, possibly indicating that inherited mutations in this gene contribute to genetic predisposition to the tumor. In future, we expect an increasing number of preclinical trials including data from dogs with different lymphoma histotypes, but a detailed pathologic and molecular classification of the tumors is definitively needed to avoid biases related to the heterogeneity of this disease. Inclusion of dogs with lymphoma as part of preclinical testing or in parallel with human trials will be a serious advantage both for human and veterinary medicine. However, limitations, including that dogs are from private owners and toxicities, should always be considered.

IA49 MALT1 targeting for B-cell lymphomas. Lorena Fontan. Weill Cornell Medicine, New York, NY.

MALT1 biology and function MALT1 is an oncogene frequently translocated to API2 t(11;18)(q21;q21) or IgH t(14;18)(q32;q21) in MALT lymphomas. MALT1 forms part of the CBM complex along with CARD11 and BCL10. This is a high-order complex where MALT1 acts as a scaffolding protein to recruit TRAF6 and the IKK complex and leads to NF-kappaB activation. MALT1 is also a cysteine protease. We now know 10 substrates of MALT1 (including itself) and 2 neosubstrates for the API2-MALT1 fusion protein. MALT1 substrates include several regulators of NF-kappaB and MAPK activation (A20, CYLD, RELB, and HOIL1), and their cleavage amplifies and/or prolongs activation of these pathways. HOIL1 cleavage by MALT1 might also contribute to negative feedback at late stages of signal transduction. Moreover, MALT1 cleaves at least three RNases that modulate the expression of proinflammatory genes and play an important role in T-cell differentiation. Less is known about their role in B-cell biology. Interestingly, CARD11 and MALT1 have been implicated in glutamine transport and its metabolism and, although their role has not been fully elucidated, they may contribute to the metabolic switch from oxidative phosphorylation to aerobic glycolysis that accompanies T- and B-cell activation. MALT1 knockout mice are immunosuppressed and fail to activate T-cell and B-cell receptors but are generally healthy and fertile. However, older mice tend to develop atopic dermatitis. On the other hand, in MALT1 C472A mice where protease activity is lost while scaffolding activity is intact, T and B cells can activate NF-kappaB signaling downstream of TCR/BCR. However, signaling is reduced, likely due to increased activity of MALT1 targets A20, CYLD, RELB, and HOIL1. These mice develop severe autoimmunity that leads to death and that has been attributed to loss of T-regulatory cells, which are very dependent on MALT1 protease activity for differentiation and maintenance. Loss of T-regulatory cells allows unrestricted T-effector cell function leading to the autoimmune syndrome, which is likely antigen driven because independently generated models showed symptoms in different organ systems. Therapeutic targeting of MALT1 A genome-wide shRNA screen uncovered MALT1 as an essential gene for ABC-DLBCL, and subsequent studies using a substrate mimetic inhibitor of MALT1 determined that ABC-DLBCLs were dependent on its protease activity. These findings arouse great interest in targeting MALT1's protease activity and, in the last 8 years, there have been numerous small molecules developed that target MALT1 through different mechanisms. Active site and allosteric inhibitors have been developed that show activity and efficacy in vivo in ABC-DLBCL xenograft models, serving as proof of concept of the therapeutic value of MALT1 inhibition. The first clinical trial for a MALT1 inhibitor was launched in April 2019 (NCT03900598) and is now open in 32 centers. All efficacy studies published to

date are based on xenograft studies in immunodeficient mice. Studies in genetically engineered ABC-DLBCL models will shed light on what will be the effects of MALT1 inhibition in the antitumoral immune response. Recent studies using syngeneic solid tumor models in immunocompetent mice showed decreased engraftment of tumors in mice lacking MALT1 protease activity. Moreover, use of a MALT1 inhibitor in combination with PD1 blockage greatly enhanced each other's activity, particularly in highly immunogenic tumors. This effect was attributed to the effects of MALT1 on T-regulatory cells, but deeper mechanistic studies are needed to fully understand the mechanisms at play. Nonetheless, these results suggest that MALT1 inhibition could have both tumor-intrinsic and -extrinsic effects that could cooperate to kill ABC-DLBCL. If general effects over the antitumoral immune response are confirmed, MALT1 inhibition could have a broader application as adjuvant for other immune-oncology approaches in cancer. Little is known to date on what mechanisms of resistance could be deployed by tumor cells to escape MALT1 inhibition. However, based on what is known for BTK and other signaling mediators, resistance will likely arise and combinatorial regimens will be needed to attain durable responses and maximal effectiveness. Our recent work on combinations anchored in MALT1 inhibition showed that BCR, PI3K, and TLR directed inhibitors were either additive or synergistic with MALT1 inhibition. MALT1/PI3Kdelta-i combinations showed enhanced but not durable responses in vivo. Tumors in mice treated with this combinatorial regime resumed growth while in treatment and displayed active NF-kappaB and MTORC1 signaling. This study revealed that MTORC1 activation could constitute a critical feedback mechanism limiting the effect of MALT1 inhibitors. In concordance, a MALT1/MTORC1 combinatorial regime suppressed MTORC1 activation and was highly effective in vivo, leading to tumor regression and significant enhanced survival after only one cycle of treatment. Future studies using clinical candidates and patient samples will further our understanding of this mechanism and elucidate other mechanisms that can be deployed by cells to overcome MALT1 inhibition and will help shape the future of MALT1 inhibition-centered oncologic regimens.