DOI: 10.1002/hon.3275

WILEY

INVITED COMMENTARY

Discovery Science highlights from the 17th International Conference on Malignant Lymphoma

Gabriela Forestieri | Joyce Marques de Almeida | Sara Napoli | Deborah Piffaretti | Chiara Tarantelli | Fangwen Zhang | Afua Adjeiwaa Mensah

Faculty of Biomedical Sciences, Institute of Oncology Research, Università della Svizzera italiana, Bellinzona, Switzerland

Correspondence
Afua Adjeiwaa Mensah, Institute of Oncology Research, Via F. Chiesa 5, Bellinzona 6500, Switzerland.
Email: afua.mensah@ior.usi.ch

KEYWORDS
17-ICML, biology, collaboration, epigenetics, lymphoma, lymphomagenesis, single cell omics

Since its inception over 4 decades ago, the International Conference on Malignant Lymphoma (ICML) has steadily grown to become the leading international forum for lymphoma experts. Now with a biennial occurrence and more than 3000 participants, the ICML provides a unique opportunity for lymphoma clinicians, healthcare workers and scientists to come together and discuss novel data gleaned from discovery science, translational research, and clinical research efforts. Many pivotal findings in the lymphoma research community were first reported at ICML meetings and some of these have driven practice-changing approaches in lymphoma patient care.

As lymphoma scientists working at the Institute of Oncology Research in Bellinzona, Switzerland, we are proud to be involved in the ICML. In collaboration with Women in Lymphoma, we are excited to present to you our selected *Discovery Science highlights* from the 17th ICML meeting. Our aim is for these highlights to complement the clinical take-home messages presented at the *17-ICML highlights* session and as such champion the importance of collaborative research which combines the expertise of clinical and non-clinical investigators, for the effective prevention, diagnosis and treatment of lymphomas.

Over the years, the work of a multitude of lymphoma researchers together with the emergence of new technologies, have resulted in a richer understanding of the molecular features that initiate and support lymphomagenesis. Laura Pasqualucci (New York, USA), is aptly recognised as having made seminal contributions to our understanding of lymphoma biology and as such, a number of her team's findings featured in her *Meet the Professor* session,[1] which focused on the role of the germinal center (GC) in the genesis of lymphomas. After giving an authoritative overview of the genetic, epigenetic and microenvironmental perturbations involved in the pathogenesis of lymphomas originating from the GC, she explained how she and Riccardo Dalla-Favera (New York, USA), embarked on opening the "big black box" that is the non-coding human genome to better study GC-derived lymphomas. Their investigations revealed that superenhancers (SE) were frequently hypermutated in diffuse large B cell lymphomas (DLBCLs). Over 90% of DLBCLs were found to harbor at least two mutations within SE regions thus indicating a selective pressure to acquire mutations in these regulatory domains. The activation induced cytidine deaminase, AID, was identified as a central player in the introduction of these mutations and the SE of key genes involved in lymphomagenesis were among those targeted. Working in the lab of Riccardo Dalla-Favera and Laura Pasqualucci, Elodie Bal, whose investigations uncovered this frequent targeting of SE in DLBCL, described her findings in more detail in two different sessions, *Epigenetic mechanisms and targeted therapies in B- and T-cell lymphomas* and *Lymphoma Biology*.[2,3] In the latter session, she focused on defining the pathogenic role of mutations targeting the intragenic SE of the transcriptional coactivator gene, *BTG2*.[4] The rescue of hotspot mutations via CRISPR/Cas9 editing in mutated DLBCL cell lines resulted in reduced fitness and lower *BTG2*

A Women in Lymphoma (WiL) initiative in collaboration with lymphoma scientists from the Institute of Oncology Research (IOR).

expression, confirming a direct link between SE mutations, deregulated *BTG2* expression and DLBCL cell fitness. Since SE are sites of dense binding by transcription regulatory factors, a logical next step was to determine if the *BTG2* SE hotspot mutation affected the recruitment of DNA binding proteins. Using gel shift assays and chromatin immunoprecipitation, Bal and colleagues showed that binding of the transcription factor TFAP4 to the *BTG2* SE was inhibited in the presence of the mutation but correction of the mutation to the wildtype sequence restored TFAP4 binding to the *BTG2* SE. To close the circle on these findings they introduced the SE hotspot mutation in wildtype DLBCL cell lines and observed increased *BTG2* expression. This work demonstrated how a change in a single nucleotide within the non-coding part of the genome can affect the binding of a transcription factor that regulates a coding gene and strikingly shed some new light on the "big black box."

Seeking to understand another aspect of the role of SE in lymphomas, Sara Napoli (Bellinzona, Switzerland), presented her work on enhancer RNAs (eRNAs), noncoding RNAs transcribed from active SE,[5] in the session, *Mechanisms of treatment resistance*. Napoli and colleagues used marginal zone lymphoma (MZL) cells with acquired resistance to the BTK inhibitor ibrutinib, to try to uncover the role of eRNAs in mediating therapeutic escape. By applying de novo reconstruction to RNA-Seq profiles of these resistant models, they were able to identify hundreds of expressed eRNAs, some of which were novel and previously unannotated. Via chromatin immunoprecipitation with antibodies that specifically bind to active enhancer regions, followed by DNA sequencing of regions pulled down by these antibodies (Chip-Seq), Napoli and colleagues first showed that almost ten times more active enhancers were lost than gained in the MZL model of ibrutinib resistance. Importantly, for both lost and gained active enhancers, only a minor fraction was associated with altered expression of proximal coding genes indicating the possibility that some, or even most, of these enhancers might themselves function directly in acquired drug resistance. As a proof of concept to demonstrate that an enhancer switch could mediate acquired resistance in MZL, Napoli performed a CRISPR experiment using a CRISPR interference (CRISPRi) library to transcriptionally silence the transcriptional start sites of around 700 eRNAs expressed in the MZL ibrutinib resistance model and its parental counterpart (while under treatment with drug or vehicle alone). This experiment provided important indications of the relevance of eRNAs in therapeutic response because it showed that eRNA expression could robustly distinguish resistant cells from parental cells and also separate resistant cells treated with ibrutinib from those treated with vehicle alone. From this screen individual eRNAs favoring ibrutinib activity and candidate eRNAs that could be targeted in combination with ibrutinib were also identified. Further CRISPRi screens were then performed on parental cells treated with other small molecules inhibiting PI3K (copanlisib, umbralisib), BCL2 (venetoclax) or CD20 (rituximab), with the goal of identifying eRNAs that are essential under treatment. This vast exploration of eRNAs functions in drug responses has so far led to some promising findings that Napoli and colleagues are investigating in more depth.

In the session *Epigenetic mechanisms and targeted therapies in B- and T-cell lymphomas*, Ari Melnick (New York, USA), co-chair of the session with Margaret Shipp (Boston, USA), gave a scholarly overview on the epigenome,[6] defining it as "…the set of instructions that describe how the cell behaves," and emphasizing that the interdependent, multi-layer modifications that define the epigenome are only beginning to be understood. Among the new discoveries being made by Melnick's team, a recurrent finding is the involvement of epigenetic mechanisms in immune synapse signaling between B cells and T cells. Indeed many somatic mutations occurring in DLBCL and follicular lymphoma (FL) disrupt the immune synapse or alter the flow of information across it. Recently, Melnick's team elegantly demonstrated that *BTG1* missense mutations, which are frequent in the MCD/C5 subtype of DLBCL, accelerate the efficiency with which immune synapse help is given to B cells resulting in enhanced GC kinetics and increased fitness.[7] It is notable that two independent studies[3,7] have shown how deregulation of two different BTG family members, *BTG1* and *BTG2*, either through mutations in the coding sequence or within an intragenic non-coding SE, increase lymphoma cell fitness. Questions remaining to be answered are if *BTG1* and *BTG2* aberrations are mutually exclusive, which would suggest that they have overlapping functions in DLBCL, and whether *BTG2* SE mutations also affect the immune synapse and GC kinetics.

Later on in the *Epigenetic mechanisms and targeted therapies in B- and T-cell lymphomas* session, François Lemonnier (Paris, France) discussed the importance of epigenetic alterations and the microenvironment in follicular helper T-cell lymphoma (TFHL).[8] He expounded on experiments by his team, describing how adoptive transfer murine models were used to transfer T cells with wildtype *IDH2* or *TET2*, mutated *IDH2* or *TET2* or both *IDH2* and *TET2* mutated, to recipient mice. The recipient mice developed either a myeloid-like disease or lymphomas. The double IDH2; TET2 murine model readily developed an angioimmunoblastic T cell lymphoma (AITL)-like disease with concomitant remodeling of the microenvironment, while the wildtype and single mutant models did not, indicating that the co-occurrence of IDH2 and TET2 mutations was specific for AITL development. Interestingly, the models with TET2 or dual mutated T cells also frequently showed clonal expansion of GC B cells, thus alluding to the key role of aberrant immune crosstalk in B cell lymphoproliferation. Lemonnier concluded by showing some studies that corroborate targeted treatment approaches for TFHL patients since they often respond better to these agents (e.g., histone deacetylase inhibitors and demethylating agents), than other peripheral T cell lymphomas.

The effectiveness of epigenetic agents in modulating the B cell lymphoma tumor microenvironment (TME) and response to chimeric antigen receptor (CAR) T therapy was meticulously explored by Wendy Beguelin and team (New York, USA).[4] DLBCL and FL depend on the histone methyltransferase EZH2 for their proliferation and survival. Somatic gain-of-function mutations of EZH2, found in 20%–30% of FL and GCB-DLBCL, drive lymphomagenesis at least in part through the generation of immune evasive phenotypes. Taking advantage of a genetically engineered mouse model (GEMM)

designed for conditional expression of mutated EZH2 and overexpression of BCL2 ("EZB") in GC B-cells, they showed that in vivo tazemetostat treatment of EZB GEMM significantly reduced EZB lymphoma B-cells and increased CD4+ and CD8+ cells, while reducing Tregs. They found that EZH2 inhibition not only directly affected T-cells, but also increased the immunogenicity of EZB lymphoma cells. Strikingly, exposure of murine CAR T-cells to EZH2i enhanced in vivo CAR T tumor killing by increasing memory CAR T and inhibiting T cell exhaustion. Through these coherent approaches, they persuasively showed that EZH2i enhances CAR T antitumor effects on different levels by inhibiting lymphoma cell growth, inducing lymphoma immunogenicity and synapse with T-cells, modulating the TME and enhancing T-cell function. Based on these promising laboratory findings, a Phase Ib clinical trial has been approved by the United States Food and Drug Administration for the treatment of relapsed/refractory DLBCL, FL and mantle cell lymphoma patients with combined tazemetostat and CD19 CAR T.

For the keynote Henry Kaplan memorial lecture,[9] Margaret Shipp recounted the establishment of an international prognostic index (IPI) for aggressive B cell lymphomas at the fourth edition of the ICML in 1990. While useful for predicting survival, an important limitation of the IPI was that it provided no biological insight into better treatment approaches. Just a decade later, microarray transcriptome profiling of DLBCL determined two distinct molecular subtypes of DLBCL with gene expression signatures that largely overlapped those of normal germinal center B (GCB) cells and activated (ABC) B cells. This molecular cell of origin (COO) classification provided early rationale for including targeted agents such as ibrutinib and lenalidomide in the standard R-CHOP regimen. Disappointingly, these treatment approaches did not achieve a therapeutic endpoint suggesting the presence of additional heterogeneity not captured by the COO molecular classification. To better define DLBCL genetic subsets, Shipp and colleagues comprehensively analyzed 304 newly diagnosed DLBCLs for mutations, structural variants and somatic copy number alterations, identifying five genetic clusters, C1–C5, with a significant impact on prognosis. The concomitant identification of overlapping genetic subtypes by Louis Staudt's team and by the Haematological Malignancy Research Network a couple of years later further strengthened these observations. As emphasized by Shipp, these genetic analyses provided several important lessons: multiple mechanisms can perturb the same target or pathway, multiple pathways are perturbed within the same genetic cluster and targeting complementary pathways is crucial for impeding treatment escape. Ongoing studies using a combined Shipp/Staudt cohort of 699 newly diagnosed DLBCL aim to determine a prospective molecular classifier that can be used for patient incorporation into new targeted therapy trials and are also helping to define genetic bases of immune evasion using spatially defined tumor-immune microenvironments in DLBCL.

Central nervous system (CNS)–DLBCL is a highly aggressive lymphoma with genetic features that largely overlap with the MCD/C5 subtypes of DLBCL. The CNS localisation of this lymphoma limits the choice of therapeutic agents to those able to cross the blood brain barrier, further complicating its treatment. Laboratory models aiming to recapitulate the human disease are frequently in vivo murine models and although these have been useful, recent studies demonstrate that there are important differences between human and murine brains with respect to their development, architecture and complexity. Arianna Baggiolini (Bellinzona, Switzerland), a stem cell and cancer biologist, shared her expertise in the field of human brain organoids during the AACR-ICML joint session, *Technology that will change lymphoma understanding and care*.[10] Using human pluripotent stem cells, Baggiolini's team has fine-tuned procedures for the generation of complex brain organoids which closely recapitulate human brain structure and function. Compared to murine models, these in vitro brain organoids better reproduce characteristics of the human brain including tropism, cellular adaptation, cellular crosstalk and niche remodeling, making them ideal for the study of CNS-DLBCL. In a recently initiated collaboration with Francesco Bertoni's team (Bellinzona, Switzerland), this exciting technology is being used to study CNS lymphomas with the goal of gaining new insights in the understanding and treatment of these diseases.

A new technology that featured heavily in the 17-ICML was single cell omics. The inter- and intra-patient heterogeneity of lymphomas makes them well-suited for analyses at the single cell level since these approaches provide a snapshot of the type, frequency, distribution and spatial organisation of individual cells within a tumor. Katia Basso (New York, USA) provided an instructive overview of single cell omics techniques, showing the results of a collaborative effort with the group of Lara Mussolin (Padova, Italy). This collaboraton, initiated in Padova, utilized single cell transcriptomics and immunoglobulin repertoire analysis to investigate pediatric sporadic Burkitt lymphoma (BL), with the aim of determining molecular features specific to relapsed/refractory cases.[11] Basso, Mussolin and colleagues observed inter- and intra-patient heterogeneity of tumor cells but noted that non-tumor cells were very similar across patients, with cell phenotype rather than molecular features driving different clusters. Relapsed BL were depleted for a specific subtype of GC light zone cells compared to non-relapsed BL. Further, they identified the transcript *TPM2* as preferentially enriched in relapsed/refractory BL, suggesting it my serve as a prognostic marker for progression-free survival in BL patients. Thus, although treatment responsive and relapsed/refractory BL overlap in clinical and phenotypic features, single cell transcriptomics revealed clinically relevant differences in cellular sub-populations and in the expression of specific transcripts.

Single cell omics techniques also featured prominently in work presented by Christian Steidl (Vancouver, Canada), where they were used to decipher the Hodgkin lymphoma (HL) microenvironment.[12] In HL, the characteristic predominance of the TME and low abundance of tumor cells precludes analysis of the latter by single cell RNA-Seq (which requires dissociation of tumor tissue into live single cells), but the opposite is true for the more prevalent cells of the surrounding microenvironment. From their HL "Atlas of immune cells" Steidl's team confirmed the predominance of Tregs within the HL TME, identifying *LAG3* and the previously reported *CTLA4* as

enriched within this subpopulation. Using imaging mass cytometry (IMC), Steidl and colleagues elegantly validated the presence of LAG3+, CD4+ T cells and determined their spatial arrangement in non-dissociated HL tumors. The IMC revealed the proximity of LAG3+ Tregs to MHC-II negative tumor cells while MHC-II positive tumor cells were typically surrounded by numerous FOXP3+ Tregs. Using multicolor immunofluorescence (IF), another spatial single cell analysis approach, they then demonstrated the presence of CXCR5 positive Hodgkin Reed Sternberg (HRS) cells, which were associated with an adverse disease specific survival at primary diagnosis. Based on their observations from spatial analyses at the single cell level, Steidl and colleagues devised a spatial scoring system to capture the interaction ranges of cells within the vicinity of HRS cells, postulating that longer versus shorter distances between these cell types could determine diverse tumor biologies and patient outcomes. Starting with a panel of 35 HL-specific markers, they identified six spatial biomarkers that could predict patient outcome, thus proving their hypothesis and providing a novel prognostic tool for the stratification of HL patients.

In his Gianni Bonadonna memorial lecture,[13] Ralf Küppers (Essen, Germany), systematically described key findings made in *"Elucidating the enigmatic pathobiology of Hodgkin lymphoma."* In the first of seven topics covered during his talk, he described how his team determined the cellular origin of HRS cells via molecular analysis of immunoglobulin V gene sequences from single HRS cells laser microdissected from frozen tissues. This early single cell analysis approach enabled them to demonstrate that HRS cells derived from GC B cells with deleterious mutations in immunoglobulin genes. These "crippled" cells did not undergo apoptosis but were able to exit the GC and undergo additional transforming events that produced HRS cells. Gene expression profiling of HRS cells showed an almost complete loss of B cell-specific gene expression. This loss of the B cell program likely enables HRS precursor cells to evade apoptosis in the GC since re-expression of B cell genes is toxic for HRS cells. A characteristic hallmark of HRS cells—their bi- or multi-nucleated structure—was postulated as likely not deriving from the fusion of two cells some time before a team comprising Küppers showed, using time-lapse microscopy of HL cell lines with fluorescently labeled tubulin, that they are the result of incomplete cytokinesis during mitosis. Although the precise mechanisms governing incomplete cytokinesis of HRS cells are unknown, the strong downregulation of various mitosis and cytokinesis factors observed in these cells likely plays a role. Expression of the cell surface receptor CD30 was identified as a characteristic feature of HL more than 4 decades ago. To study the role of CD30 in HL, Küppers' team knocked it out using CRISPR/Cas9 gene editing. They observed a growth disadvantage of CD30-depleted cells, increased death of HRS cells, altered chemokine & cytokine expression and downregulation of MYC and its targets in HL thus demonstrating that CD30 contributes to pathogenic signaling in HL. Concluding his presentation, Küppers described novel findings of transcriptional reprogramming by mutated IRF4 in HRS cells. A transcription factor required for plasma cell differentiation, IRF4 is highly expressed in HRS cells, which paradoxically do not have a plasma cell program. Mutated IRF4 detected in microdissected HRS cells from a subset of HLs showed strongly altered binding behavior with loss of binding to canonical sites and neomorphic binding to non-canonical composite sites. This switch in the genomic sites targeted by mutated IRF4 compared to wildtype IRF4 causes downregulation of plasma cell genes in cells with mutated IRF4 and upregulation of HRS cell genes. Mutated IRF4 is therefore another feature of HL that can contribute to maintaining the HRS cell transcriptional program.

The abstracts we have highlighted here represent new and recurrent themes of lymphoma research that intrigued, informed and enthused us during the 17-ICML. We conclude with some closing remarks from Peter Johnson's (Southampton, UK) expansive overview covering *"25 years of antibody treatments for lymphomas,"* given during his John Ultmann memorial lecture[14]: "We have learned a lot over the last 25 years: not to make too many predictions about mechanisms, to look for the mechanisms when things work, and to understand why they don't in order to build better medicines; that by understanding the biological effects of molecules we find better treatments, and finally, that it begins and ends with the relationship between clinical researchers and discovery scientists and how they work together."

ACKNOWLEDGEMENT

Open access funding provided by Universita della Svizzera italiana.

CONFLICT OF INTEREST STATEMENT

We have no conflicts of interest to disclose.

ORCID

Afua Adjeiwaa Mensah https://orcid.org/0000-0001-8113-3689

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/hon.3275.

REFERENCES

1. Pasqualucci L. The germinal center in the pathogenesis of B cell lymphomas. *Hematol Oncol*. 2023;41(S1):62-69. https://doi.org/10.1002/hon.3141
2. Bal E, Corinaldesi C, Holmes AB, Pasqualucci L, Basso K, Dalla-Favera R. Super-enhancer hypermutation in DLBCL: role of alterations in the glucocorticoid receptor pathway. *Hematol Oncol*. 2023;41(S2):89-90. https://doi.org/10.1002/hon.3163_53
3. Bal E, De Simone P, Holmes AB, et al. BTG2 super-enhancer mutations disrupt TFAP4 binding and dysregulate BTG2 expression in diffuse large B-cell lymphoma. *Hematol Oncol*. 2023;41(S2):108.
4. Isshiki Y, Porazzi P, Melnick A, Ruella M, Béguelin W. EZH2 inhibition enhances CAR T antitumor effect by inducing lymphoma immunogenicity and enhancing T cell function. *Hematol Oncol*. 2023;41(S2):228.
5. Napoli S, Cascione L, Ramnarayanan S, et al. Enhancer RNAs (eRNAs) play a role in the response to small molecules and in the

development of acquired resistance in marginal zone lymphoma (MZL). *Hematol Oncol*. 2023;41(S2):189.
6. Melnick AM. Epigenetic basis and therapy of DLBCL. *Hematol Oncol*. 2023;41(S2):88-89. https://doi.org/10.1002/hon.3163_50
7. Mlynarczyk C, Teater M, Pae J, et al. BTG1 mutation yields super-competitive B cells primed for malignant transformation. *Science*. 2023;379(6629):eabj7412. https://doi.org/10.1126/science.abj7412
8. Lemonnier F. Epigenetic basis and therapy of follicular helper T-cell lymphomas. *Hematol Oncol*. 2023;41(S2):89.
9. Shipp MA. Lessons learned from the genetic heterogeneity of lymphoid malignancies. *Hematol Oncol*. 2023;41(S2):30.
10. Baggiolini A. The human brain microenvironment of primary CNS lymphoma: human pluripotent stem cells-derived brain models. *Hematol Oncol*. 2023;41(S2):882.
11. Basso K. Single cell omics in the study of B cell lymphoma. *Hematol Oncol*. 2023;41(S2):37.
12. Steidl CG. The tumor microenvironment of Hodgkin lymphoma at single cell resolution. *Hematol Oncol*. 2023;41(S2):38.
13. Küppers R. Elucidating the enigmatic pathobiology of Hodgkin lymphoma. *Hematol Oncol*. 2023;41(S2):30.
14. Johnson PMW. 25 years of antibody treatments for lymphoma. *Hematol Oncol*. 2023;41(S2):30-31. https://doi.org/10.1002/hon.3163_3

How to cite this article: Forestieri G, de Almeida JM, Napoli S, et al. Discovery Science highlights from the 17th International Conference on Malignant Lymphoma. *Hematol Oncol*. 2024; e3275. https://doi.org/10.1002/hon.3275