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Follicular Lymphoma: State of the art ICML Workshop in Lugano 2015

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State-of-the-art workshop report from a meeting held in Lugano, Switzerland on June 16, 2015
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Abstract:

The 13th international Conference on Malignant Lymphoma held in Lugano in June 2015 was preceded by a closed workshop (organized in collaboration with the American Association for Cancer Research and the European School of Oncology) with the aim of developing an up-to-date understanding of the biology of follicular lymphoma and the clinical implications of new findings in the field. Discussed topics included the mutational spectrum at diagnosis, the clinical correlates of genetic and epigenetic alterations, the mechanisms of clonal evolution and histological transformation, the crosstalk between tumor cells and microenvironment and the development of novel treatments. This report represents a summary of the workshop.

Introduction:

A closed workshop was held in Lugano on June 16, 2015, Switzerland prior to the opening of the International Congress on Malignant Lymphomas (ICML). The purpose of the workshop was to bring together experts in the field of follicular lymphoma (FL) spanning basic biology, clinical translational research and clinical medicine and included a number of researchers active in the area of clinical trial design and implementation. The meeting was co-chaired by professors Emanuele Zucca and Randy Gascoyne. The meeting first addressed key topics involved in FL biology and pathogenesis, including molecular ontogeny, clonal heterogeneity and mechanisms underlying histological transformation, altered regulatory circuits in FL gene expression and signaling, epigenetic modifications, the role of the tumoral microenvironment and the clinical implications of driver genetic alterations impacting FL treatment outcomes. The afternoon session focused on state-of-the-art therapies for FL, opportunities to target the microenvironment as a novel treatment paradigm, the role of chemotherapy-free regimens and the promise of novel targeted therapies in the future of FL therapeutic strategies with emphasis on clinical trial design and international collaboration. What follows is an overview of the presentations, much of which introduced unpublished work, and discussions by experts in the field.

Our understanding of the biology and pathogenesis of FL is rapidly evolving, in part following the application of next-generation sequencing strategies to this cancer [1-14] (see Table 1, recurrent

genetic events in FL). Several sequencing studies published over the last few years have served to highlight the genetic landscape of FL including potentially targetable recurrent mutations involving oncogenic pathways characterized by predominantly loss-of-function mutations. More work is still needed to determine the functional consequences of many of the mutations and related genetic alterations. From a clinical perspective, a clear realization has emerged that the majority of patients with FL (approximately 80%) have a relatively indolent clinical course and manageable disease with currently available therapies, while ~20% of cases will show early progression of disease (POD) or transformation within 24 months following initial diagnosis [15,16]. The 80% of indolent FL patients might benefit from reduced dose intensity and subsequent toxicities. The early POD group requires a focus of intensive research efforts, as it identifies the subset of patients with FL where the disease substantially compromises life expectancy and those with the greatest need for novel treatment approaches designed to achieve the goal of precision medicine.

Follicular lymphoma ontogeny:

The Nadel laboratory in Marseilles has focused on studying the early molecular events involved in FL pathogenesis. The vast majority of cases of advanced stage FL harbor a t(14;18)(q32;q21.3), placing the *BCL2* oncogene under control of the *IGH* enhancer element on chromosome 14 [17]. This leads to the deregulated and constitutive expression of BCL2, an anti-apoptotic protein. The normal default program for germinal center B cells is apoptosis, so over expression of BCL2 maintains the viability of these cells that would otherwise die. Early work by Nadel and colleagues established that rare cells in the peripheral blood of otherwise normal individuals harbor a t(14;18) [18-21]. The translocation , which occurs early on in B cell development, as a by-product of the VDJ recombination process in the bone marrow, is found predominantly in the memory B cell compartment and these cells account for ~1 in 10^6 cells in ~ 70% of healthy adults and increases with age [22-24]. Despite the presence of these cells, the majority of healthy adults never develop clinical FL. Subsequent work established that those apparently normal individuals with an increased frequency of t(14;18)-positive

cells were more likely to develop FL later in life [25-27]. Moreover, rare reports of clonally related FL following transplant in donor and recipient pairs further established the long latency period and highlight the complexity of additional (shared) alterations years before the development of FL [28]. These findings were insightful and helped establish the concept that the t(14;18) was necessary, but insufficient on its own, for the development of clinically overt FL.

Important work from this group established that the B cells harboring the t(14;18) were not naïve B cells. They clearly established that circulating *BCL2*-rearranged cells had undergone the germinal center reaction, revealing evidence of immunoglobulin gene somatic hypermutation [19]. These cells were termed FL-like cells (FLLC) because they showed some specific features of "frozen FL" cells such as imprints of genomic instability and an allelic paradox (whereby a surface IgM is expressed despite active class-switch recombination on the translocated allele). In aggregate, these findings indicate that t(14;18)-positive cells in healthy individuals are mainly post-germinal center memory B cells. Further molecular characterization of FLLCs established an iterative process whereby these cells undergo multiple germinal center re-entries likely driven by antigen exposure or possibly lectin interactions [29]. As a result, they are prone to acquire additional mutations in the highly mutagenic germinal center milieu, characterized by the action of activation-induced cytidine deaminase (AID) introducing multiple mutations into *IG* and non-*IG* loci, some of which possibly represent further early oncogenic hits. Surface IgM may favor multiple germinal center re-entries, while BCL2 over-expression allows these mainly non-proliferating, centrocyte-like FLLCs to survive [30,31].

In 2002, Cong *et al* described FL *in-situ* (FLIS) where small clusters of t(14;18)-positive cells colonize germinal centers of otherwise reactive appearing benign lymph nodes [32]. FLIS is to be distinguished from partial involvement by FL[33]. When lymph nodes are removed for reasons other than establishing a diagnosis of lymphoma; ~2% are found to show FLIS on careful histologic examination [34]. Although by standard morphology appearing unremarkable, these nodes reveal a characteristic appearance with immunohistochemical staining, as scattered germinal centers show strong staining of cells with both BCL2 and CD10, distinguishing them from surrounding reactive germinal centers [33].

Fewer than 5% of people with FLIS have been observed to subsequently develop clinical FL [33]. FLLCs refer to the circulating t(14;18)-positive cells found in most healthy adults and Tellier *et al* using innovative techniques was able to establish that these cells colonize the germinal centers of benign nodes, exposing these cells to repeated rounds of physiologic DNA damage where subsequent genetic hits alter specific pathways that eventually lead to overt FL in only a small subset of people [35]. These findings established a link between FLLCs and FLIS, helping to clarify early steps in FL ontogeny. Finally, Nadel and colleagues established a $BCL2^{tracer}$ mouse model that recapitulates circulating t(14;18)-positive FLLCs in humans. This technological innovation has proven valuable as a model of early FL development [29,36] (see Figure 1).

In aggregate, these data have been instrumental to our improved understanding of the early events in FL pathogenesis, but equally raise a number of unanswered questions [37]. Is the process of germinal center re-entries driven by a specific antigen or chronic antigen exposure in general; does the nature of the antigen exposure evolve over the course of FL development; does the nature of the antigen exposure or host genetics explain the relatively low prevalence of FL in the Japanese population; how does overexpression of BCL2 contribute; if FLLCs are reminiscent of non-proliferative centrocytes in the light zone, how are mutations introduced into cells with a diminished replicative capacity; what is the role of *IGH* mutations introducing N-glycosylation sites and what lectins and/or cell types in the germinal center help support survival of FLLCs in FLIS; and finally what critical mutations or combination of hits on a background of the t(14;18) lead to overt FL in a subset of affected patients?

Follicular lymphoma biology:

A fundamental understanding of the biology of the normal germinal center (GC) has been critical to improving our knowledge regarding the underlying pathogenesis of FL. Under normal conditions, reactive GCs combine two potentially genetically reckless properties, genomic damage in the form of double stranded DNA breaks and rapid cellular proliferation, in the absence of proper DNA

damage responses due to the suppression of p53 and ATR transcription by BCL6 [38,39]. Both are necessary for the generation of antibody diversity. A number of seminal papers have been recently published that provide insight into GC biology, including light zone-dark zone transitions, the role of MYC and regulatory pathways that bestow properties of "stem-ness" on GC B cells [40-46]. FL is a GC-derived neoplasm and thus neoplastic B cells reveal a gene expression program characteristic of the normal GC, and resembling that of light zone B cells, altered by a background of constitutive expression of BCL2 resulting from the t(14;18) and subsequently by acquired, somatic mutations [47].

Recently published work from Payton and Oltz provides further insight into FL pathogenesis [48,49]. A combination of genetic and epigenetic perturbations conspires to alter gene expression programs in FL B cells. Some of these changes are mediated by point mutations in gene regulatory elements that disrupt transcriptional factor (TF) binding. More global changes to chromatin structure may be secondary to acquired alterations in histone modifying genes including *EZH2*, *KMT2D*, *CREBBP, EP300* and others [1,2,50]. Using an experimental approach comparing purified neoplastic B cells from FL to normal control GC B cells, Payton and Oltz used genome-wide strategies to highlight the distinguishing features of GC B cell transformation [48]. In non-transformed FL, gene expression more closely resembles normal centrocytes and can be distinguished from centroblasts [47]. Detailed comparisons of the "regulome" (the landscape of regulatory elements in the genome that control gene expression in concert with TF binding) further defined two subtypes of FL; one resembling centrocytes and the other reminiscent of activated peripheral blood B cells. Their work further defined the abnormal circuitry of gene expression patterns in FL, revealing that some regulatory elements characteristically active in GC B cells are decommissioned, while others are co-opted as part of oncogenesis. The latter include general cancer related features such as proliferation and survival pathways. They also determined that lineage-inappropriate gene expression results from commandeered TF binding to enhancers not normally active in GC B cells. Moreover, they identified both acquired and inherited single nucleotide variants (SNVs) in regulatory elements that perturb TF binding and thus downstream gene expression in FL [48]. These findings suggest that more in-depth

analysis of the non-coding genomic space in FL might be of significant value. Such an effort will be resource intensive, given the need to produce whole genomes at reasonable depth and quality. Moreover, it will likely be technically complicated owing to reduced tumor cell content in whole FL biopsies and lower DNA quality metrics. Importantly, Payton and Oltz demonstrated that these disruptive non-coding SNVs can be identified by epigenome profiling (e.g. ChIP-sequencing) [48].

Ari Melnick and colleagues have performed seminal work exploring the role of genetic alterations and epigenetic switches and their contribution to FL pathogenesis [39,51-53]. The genes implicated in these pathways include *EZH2*, *BCL6* and *KMT2D* (also known as *MLL2*). Recurrent somatic mutations in *EZH2* occur in ~20-25% of FL and target a hotspot at Y641 [50,54]. EZH2, the enzymatic component of the polycomb repressor complex 2, is a H3K27 methyltransferase that adds methyl groups to lysine 27 (H3K27) to promote transcriptional silencing [55,56]. Balancing gene expression in cells is another histone mark (H3K4me3) that promotes gene expression. *EZH2* mutations in FL are characterized as gain-of-function alterations, are always heterozygous, and show enhanced methylation of the dimethylated lysine tails (H3K27me2) [55,56]. Thus mutated EZH2 cooperates with wild type EZH2 to shift the steady state in cells to trimethylated H3K27me3 and the resultant gene silencing of polycomb targets. EZH2 function is required for germinal center formation [52,57]. It enables B cell proliferation by silencing DNA checkpoint genes and also transiently silences GC exit programs. EZH2 helps establish bivalent chromatin marks in GC B cells (genes with both H3K27me3 and H3K4me3 marks) that render target genes poised (i.e. genes that can be rapidly toggled on or off by removal of one or the other histone mark). Conditional expression of the *EZH2* activating mutants in mice causes GC hyperplasia and accelerates lymphomagenesis in cooperation with BCL2 [52].

BCL6 is a transcriptional repressor and is also required for GC formation [58,59]. Forced expression of BCL6 promotes lymphomagenesis [60]. For BCL6 to drive proliferation and survival of lymphoma cells it must recruit corepressors (SMRT, NCOR, and BCOR) to form complexes that target gene promoters and enhancer regions [61,62]. Down regulation of BCL6 and post-translational modification of corepressors promotes GC exit by restoring expression of BCL6 target genes [59,63]. Small molecule inhibitors to both

EZH2 and BCL6 have been developed and together might represent lead compounds for rational combinatorial therapies for GC derived lymphomas [51,64-67].

KMT2D or MLL2 is a histone methyltransferase responsible for introducing H3K4 mono- and dimethylation marks associated with gene enhancers [68]. It is frequently mutated in FL and appears to be a driver mutation occurring early during follicular lymphomagenesis [1]. The pattern of mutations is consistent with a loss-of-function tumor suppressor gene and homozygous loss resulting from either deletion or mutation of the 2nd allele is not uncommon. Two elegant studies published recently in *Nature Medicine* establish *KMT2D* as a *bona fide* tumor suppressor gene [69,70]. Mutations in *KMT2D* impair enzymatic activity leading to loss of H3K4 methylation marks at gene enhancers and essentially remodel the epigenetic landscape in FL. Conditional loss of *KMT2D* early during B cell development leads to increased GC formation, and in a B cell context, cooperates with BCL2 deregulation to promote lymphoma development. This may be achieved in part, by silencing other tumor suppressor genes, and genes involved in cell cycle regulation, BCR signaling and termination of the GC reaction.

Tumor microenvironment in FL:

The role of the tumor microenvironment (TME) in FL is an intense area of research focused on interactions between non-neoplastic immune-related cells, stromal cells and extracellular matrix components that are involved in crosstalk with malignant B cells. These cells provide a number of proliferation and survival signals necessary for FL growth and also help to foster immune escape mechanisms. Tarte and colleagues have contributed greatly to our understanding of this important biological phenomenon by studying the two main niches that contribute to FL pathogenesis; the bone marrow (BM) and the lymph node (LN) [71,72]. BM involvement in FL is very common and typically shows lower grade cytology in comparison to the LN [73]. Mesenchymal stromal cells (MSCs) in the BM directly support the growth of neoplastic FL B cells and foster a tumor-supportive TME through monocyte recruitment and M2 polarization mediated by their increased secretion of CCL2 triggered by direct contact with malignant B cells [74]. In the LN, FL B cells derive growth and survival signals from follicular

dendritic cells (FDCs), fibroblastic reticular cells (FRCs) and follicular T-helper cells (TFH) through receptor-ligand interactions and cytokines [75]. A specific TFH subset, defined by expression of CD10, is significantly expanded in FL and provides both survival drug resistance signals to FL B cells within the GC niche [76]. Cytotoxic T cells are rendered dysfunctional through a number of mechanisms that produce an "exhausted" and unresponsive phenotype [77-80]. Moreover, these cells have defective immune synapse formation and altered motility, rendering them less able to kill malignant B cells [81,82]. Purified CD4 and CD8 expressing cellular populations from LNs involved by FL in comparison to reactive lymph nodes from healthy individuals show altered gene expression patterns induced by the FL B cells [82]. FL cells also produce, as a result of crosstalk with TFH cells, chemokines recruiting Treg cells that further dampen the immune response to the tumor [83,84]. The tumor cells express CD70 and the FL tumor-associated macrophages express PD-1 ligands, both leading to an exhausted phenotype for tumor infiltrating lymphocytes [85]. Specific mutations in the neoplastic cells also foster immune privilege. *CREBBP* mutations lead to reduced MHC class II expression by FL B cells that decreases the number and activation state of infiltrating CD4+ and CD8+ T cells [13]. Other somatically acquired mutations in FL may also contribute to direct genetic mechanisms of immune escape and indirect polarization of a tumor-supportive microenvironment.

Most cases of FL have acquired N-glycosylation sites in their *IG* variable region resulting from somatic hypermutation [86,87]. These are not found in normal B cells and only occasionally in other B cell lymphomas. Lectin binding to sIG stimulates the B cell receptor (BCR) and induces downstream intracellular calcium fluxes [88,89]. In FL, M2 macrophages can induce a dendritic cell-specific intracellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) dependent adhesion of highly mannosylated IgM+ FL B cells and similarly trigger BCR activation that can be blocked by either SYK or BTK inhibitors [90]. Other lectins within the FL niche can also function similarly. These somatically acquired genetic alterations introduced into the *IG* variable regions appear to be selected for and thus likely contribute to a growth advantage. Moreover, these collective data suggest that lectins in the FL TME contribute

growth and survival signals through BCR activation by antigen-independent means and might represent the critical 2nd hit needed to manifest clinically overt FL [89].

Cells of the innate immune system, including neutrophils, have also been implicated as active elements in the TME in FL through the activation of supportive stromal cells [91]. A number of additional cell types and pathway perturbations within the TME have also been implicated as contributors to FL transformation [82,92,93]. The TME in FL is biologically complex, with many of the functional interactions and cell involved yet to be fully defined. None the less, crosstalk between the malignant B cells and immune cells and stromal elements within the TME suggests a number of potential therapies in FL that are further discussed below.

Transformation of follicular lymphoma:

Histologic transformation of FL (TFL) is a prognostically dominant clinical event in the course of FL, often heralded by new onset systemic or local symptoms and an increased pace of disease progression. It occurs at a rate of 2-3%/year over the first 12 - 15 years and is usually associated with significantly reduced survival [16,94-98]. The histology most often encountered at the time of re-biopsy is diffuse large B cell lymphoma (DLBCL), but other histologies can also be seen [99]. Previous studies of paired diagnostic and transformed disease had implicated a number of dominant oncogenic alterations, including acquired *MYC* translocations, *TP53* mutations and/or copy number loss and alterations of chromosome 9p21 involving *CDKN2A* [100-103]. More recently, the application of next-generation sequencing combined with high-density copy-number analysis has given us an unbiased insight into the genetic landscape of events that underpin transformation of FL. Two elegant studies, published in 2014 by Okosun and Pasqualucci, respectively, provided a base-pair resolution analysis of the genetic perturbations associated with TFL [5,9]. Transformation in most cases arises from a common ancestral precursor cell harboring founder mutations of key genes including chromatin modifying genes *CREBBP*, *EZH2* and *KMT2D* and anti-apoptotic genes such as *BCL2* translocations, while later events implicate cell cycle deregulation, DNA damage response, JAK-STAT pathway hits and NF-κB

signaling. The evolutionary pathway from disease initiation to transformed clone is in most cases is divergent, with phylogenetic histories revealing either a sparse (few mutations) or rich (more numerous mutations) clonal progenitor [5]. Immune evasion as a transformation event was also seen, implicating genes such as *CD58* and *B2M* [9]. Mutations involving linker histone core genes and *STAT6* identify chromatin remodeling and JAK-STAT pathway perturbations in TFL. The mutation profile of TFL is most frequently analogous to germinal center (GCB)-type DLBCL, but also unique with respect to hits in *TNFAIP3*, *CD79B*, *MYD88* and *PRDM1* that are more characteristic of the *de novo* activated B cell-like (ABC) DLBCL [9]. Accompanying gene expression studies indicate that most TFL are GCB-like, but approximately 16% of cases are ABC-like [104]. These data have possible therapeutic implications. Recently, mutations involving the coding regions of *BCL2* have been implicated as contributing to both diminished survival in FL as well as an increased risk of transformation [8]. In the supplemental data from the m7FLIPI study and unpublished data from analysis of the PRIMA trial (G. Salles, unpublished), these findings could not be confirmed; thus additional studies of the role of *BCL2* mutations in FL are required. Further studies to define the evolutionary trajectories of FL ancestral precursors under the influence of specific treatments may also provide leads that inform both overall prognosis and risk of transformation.

Clinical translation:

The mutational landscape of FL is largely characterized as a result of a number of next-generation sequencing studies. Recurrent mutations include *KMT2D* (*MLL2*), *EZH2*, *CREBBP*, *EP300*, *STAT6*, *TNFRSF14*, *HIST1H1E, RRAGC* and other less common mutations including *GNA13*, *SGK1*, *MEF2B* and *FOXO1* (see Table 1). Importantly, the clinical implications of any of these recurrent mutations were essentially unknown. In an international collaborative effort spanning three countries and two continents recently published by Weigert and colleagues, a prognostic model that incorporated the mutational status of seven genes together with the FL International Prognostic Index (FLIPI) and the ECOG performance status (m7FLIPI) was constructed using failure-free survival (FFS) as the primary

outcome measure from data derived from a randomized, phase III clinical trial of R-CHOP in FL [105,106]. In total, 74 genes were analyzed across 151 patients in the training set with seven genes contributing to FFS in the final model. The seven mutated genes included four associated with treatment outcome (*EP300, FOXO1, CREBBP* and *CARD11*) and three associated with favorable outcome (*MEF2B, ARID1A* and *EZH2*). Importantly, the final model reproducibly identified a subset of ~ 20% of FL patients with a markedly inferior survival. The FLIPI alone had far inferior specificity in identifying these truly poor risk patients. The enhanced performance of the m7FLIPI was accomplished because of migration of high-risk profile based on the FLIPI into the low-risk category by m7FLIPI, most frequently due to the presence of an *EZH2* mutation. The model was carried forward into an independent validation cohort of 107 FL patients treated with a different rituximab-containing regimen (R-CVP) with similar performance, the majority also receiving rituximab maintenance.

These findings demonstrate that mutational data combined with clinical findings can contribute to refined prognostication and might represent a useful clinical tool to identify high-risk FL patients and hold promise to be useful for risk-adapted treatment strategies. However, before it can be routinely adopted, the model needs to be thoroughly tested in additional cohorts including asymptomatic FL patients or those with low-volume disease and those treated with different chemoimmunotherapy regimens such as bendamustine/rituximab. In addition, further refinements to improve its performance seem warranted. If successful, this might be a useful tool in clinical practice, as it was designed using routinely available formalin fixed paraffin embedded biopsy material and thus could represent an important step forward towards risk-adapted treatment strategies for many patients with FL. In time, together with incremental improvements in our knowledge of fundamental FL biology, transition to biology-adapted treatment strategies may be possible.

Clinical questions and novel therapies in follicular lymphoma:

In the current era, no universally accepted standard-of-care therapy for symptomatic, advanced-stage FL has been identified [107]. Asymptomatic patients with low volume disease not-

fulfilling GELF criteria can still be reasonably approached with a watch & wait strategy [108-110]. The available treatment options for symptomatic patients or those with substantial disease volume warranting treatment are broad and span a spectrum from single agent rituximab through to chemo-immunotherapy employing a number of different chemotherapy regimens. Recent studies clearly show that median overall survival for patients with FL is now beyond 15 years, with a real possibility that some patients may currently be over-treated with more aggressive regimens [15]. Given the median age of diagnosis for FL and the more routine use of biologic agents, a growing number of patients appear to be dying of causes other than FL. Moreover, progression-free survival as an endpoint may not be an ideal surrogate of clinical benefit given the very long time required to accumulate sufficient events. Some have suggested that sustained complete response (CR) rates at 30 months could represent a better and more efficient surrogate measure of treatment efficacy to allow more rapid evaluation of new therapies. Others advocate for a composite endpoint that incorporates quality of life and toxicity evaluations, given the often asymptomatic nature of this indolent disease.

A recent analysis from the National LymphoCare Study (NLCS) cohort in the United States led by Friedberg and colleagues enrolled over 2,700 patients with follicular lymphoma between the years 2004-2007 and followed them for 10 years, the most common induction chemo-immunotherapy treatment used was R-CHOP [15]. Twenty percent of patients treated with R-CHOP had disease which either did not respond or progressed within two years; these patients had a markedly increased hazard ratio for death, and the median overall survival for this subgroup was only 5 years. With almost 9 years of follow-up, two thirds of the deaths in R-CHOP treated NLCS patients occurred in these twenty percent of patients with early disease progression. These results have been validated in an independent cohort from the Iowa-Mayo SPORE, who has also preliminarily demonstrated in collaboration with the LySA lymphoma group that patients with follicular lymphoma without early PFS events have similar overall survival to age-matched controls [15]. Taken together, these datasets indicate that the current largest unmet clinical need in FL is the “high-risk” subset of patients who progress early (i.e. early POD), where it remains imperative to change the natural history of FL.

A clear path forward for the research agenda in FL was described by Dr. Friedberg and supported by most other clinicians in attendance. The roughly 20% of FL patients dying early are the unmet clinical group in need of better therapeutic approaches and also represent the patients who should be identified and subsequently prioritized for enrollment on clinical trials. We require concerted effort brought to bear on developing predictive tests that can be deployed at the time of diagnosis to reproducibly identify these high-risk patients. It is not yet known whether these patients with early progression of disease are those identified as high risk by the m7FLIPI. Ideally, a strategy to identify those patients at-risk for transformation would also have considerable traction in the clinical trial setting. Some leads, such as the m7FLIPI show promise, but more work is required to identify the majority of early POD patients [106]. Low density gene expression that integrates pathway perturbations downstream of recurrent mutations and/or approaches to identify patients with increased immune response-2 expression profiles might also be useful, but the latter requires validation in the current era of therapy. Clearly, we need to develop robust biomarkers to be used in conjunction with cutting-edge clinical trials testing promising new agents alone or in combination for high-risk patients with FL. To accomplish this, diagnostic specimens from patients enrolled in clinical trials worldwide must be stored for future interrogation when response and remission duration are determined.

Dr. Seymour reviewed the large number of new agents being evaluated for the treatment of FL (see Table 2) Recent data were discussed including new CD20 monoclonal antibodies ofatumumab and obinutuzumab as well as novel antibody drug conjugates; IMIDs such as lenalidomide; BTK inhibitors including Ibrutinib and other drugs in this class, PI3K inhibitors (isotype selective and broadly acting), BCL2 inhibitors including both the more broadly acting navitoclax and the BCL2-specific venetoclax; immune checkpoint inhibitors involving the PD1/PD-L1 axis and novel small molecule inhibitors of specific targets such as EZH2. Despite convincing data regarding the importance of the BCR in FL, ibrutinib single-agent activity in relapsed/refractory or untreated FL is somewhat disappointing [111]. Chronic active BCR signaling is thought to be an important pathway in FL, likely through IGH-mannose lectin interactions [112]. Responses to PI3Kδ inhibition following single agent or combination therapies

with idelalisib suggest that FL cells may be more dependent upon this pathway [113-115]. Previous discussions highlighted how new knowledge concerning the underlying biology of FL informs epigenetic therapy considerations including HDAC inhibitors and small molecule BCL6 and EZH2 inhibitors. Moreover, the rationale behind efforts to target the crosstalk between FL and the TME was a talk unto itself presented by Professor John Gribben from Bart's in London. Both Dr.'s Gribben and Tarte discussed overcoming the T cell exhaustion phenotype in FL through the use of lenalidomide, including evidence that the defect in immune synapse formation can be overcome [81]. The rationale behind the use of anti-PD-L1 antibodies (nivolumab) or antibodies to PD-1 (pidilizumab) were discussed including promising data in the relapsed/refractory setting in FL [116]. A number of other approaches targeting aspects of the TME were briefly highlighted, including anti-CD47 and CD137 antibodies, CSF1-R antagonists and CAR T cells.

Dr. Seymour reviewed mostly phase I and II data regarding the aforementioned agents. He also discussed the need for caution regarding new, seemingly rational combination therapies, using the toxicity profile that resulted from the combination of lenalidomide, idelalisib and rituximab that produced unacceptable hepatic toxicity and death [117]. To move some of these strategies into the clinical setting will require further testing in the context of carefully monitored clinical trials.

Conclusions and future directions:

The meeting ended with an overview of the conclusions drawn from both presentations and discussions, but also a list of priorities for future studies. There was uniform agreement that we need robust biomarkers to identify the roughly 20% of patients with advanced-stage FL that could be considered high-risk (early POD) in order to identify these patients for inclusion in clinical trials testing new agents and novel strategies. In addition, it was recognized that to deliver on the promise of improvements for this subset of patients we would need to establish international collaborations using

standardized lab-based biomarker studies combined with uniform treatment approaches required to enroll and more rapidly analyze outcome correlates in FL. As improvements in our understanding of the fundamental biology unfold together with additional genome-wide genomic landscape studies and biomarker development, carefully constructed clinical trials can be designed that will allow us to finally deliver on the promise of precision medicine for patients with FL.

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Table 1: Recurrent genetic alterations in FL

Genetic alteration	Frequency	Function	References
Gene regulatory element mutations	100%	Transcriptional control	41
t(14;18)(q32;q21.3)	85-90%	Anti-apoptosis	17
KMT2D mutations	80-90%	Histone modification	1, 3, 5 , 9, 15, 106
Mutations of *IGH* epitopes that promote N-glycosylation	85-95%	BCR signaling	86
CREBBP mutations	40-65%	Histone modification and immune escape	1, 2, 3, 4, 5, 106
BCL2 mutations*	40-65%	Anti-apoptosis?	1, 8, 106
Deletion 6q including *EPH7A* deletion/methylation, *TNFAIP3* mutation/deletion and *PRDM1* deletions[118,119]	60-70%	Tumor suppressor	12, 118, 119
TNFRSF14 mutations/deletions, LOH	50%	Direct proliferative signal? Immune escape?	4, 10, 11, 14, 106
EZH2 mutations	20-30%	Histone modification	1, 4, 47, 48, 106
Core histone genes e.g. *HIST1H1E*	20-30%	Histone modification	1, 3, 5, 106
RRAGC mutations	~17%	mTORC1 signaling	6
MEF2B mutations	10-15%	Transcription factor	1, 106
STAT6 mutations	10-15%	JAK-STAT signaling	7, 106
EP300 mutations	~ 10%	Histone acetylation	1, 2, 5, 106
ARID1A mutations	~10%	Chromatin remodelling	1, 3, 5, 106
OCT2 mutations	5-10%	Transcription factor	3, 106
CARD11 mutations	5-10%	BCR signaling	1, 5, 106
FOXO1 mutations	5-10%	BCR signaling	1, 5, 14, 106
GNA13 mutations	5-10%	Focal adhesion/mobility	1, 5, 9, 14, 106
B2M mutations	5-10%	Immune escape	1, 5, 9, 14
SGK1 mutations	5-10%	Protein kinase	1, 5, 7, 106

*Includes *BCL2* coding region and other mutation types. LOH = loss of heterozygosity.

Table 2: Response rates to novel agents in FL

Agent	# of patients	Prior therapies Median (range)	ORR/CR (%)	Median PFS	References
Obinutuzumab:					120-123
Phase 1	13		69 / 38	Not reported	
Phase 1 with maintenance	10		40 / 10	Not reported	
Phase 2 dose finding	34	3 (1–11)	50 / 31	11.9 months in high-dose cohort	
Randomised phase 2	74	2 (1–7)	45 / 5	17.6 months	
Polatuzumab vedotin	25		28 / 12	5.7 months	124-126
Polatuzumab & rituximab	20		70 / 40	11.5 months	
Polatuzumab 1.8 mg and rituximab (RP2D)	20		70 / 20	61% at 12 months	
BTK Inhibitors:					127-129
Ibrutinib – phase 1	16	3 (1–5)	38 / 19	13.4 months at ≥2.5 mg/kg	
Ibrutinib – phase 2	40	3 (1–11)	30 / 3	9.9 months	
ONO-4059 [111,120-122]	5		0	N/A	
PI3K inhibitors:					113, 115, 130
Idelalisib - phase 1	38		45 / 0	7.6 months	
Idelalisib - phase 2	72		54 / 14	11 months	
Duvelisib[113,115,123]	32*		65 / 16		
BCL2 inhibitors:					131-133
Navtioclax	16	5 (1–11)	6 / 0	~ 3 months	
Navitoclax & rituximab	12		75 / 42		
Venetoclax	29	3 (1–10)	38 / 14	11 months	
PD1 axis inhibitors					134
Nivolumab	10		40 / 10	68% at ~ 6 months	
BiTE antibodies					135
Blinatumomab	28	3 (1–10)	43 / 21	N/A	
EZH2 inhibitors	4		1 PR	N/A	136

ORR = overall response rate, CR = complete remission, PFS = progression-free survival, N/A = not available

* mixed indolent histologies