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To cite this article: Duncan M. Gascoyne & Alison H. Banham (2017) The significance of FOXP1 in diffuse large B-cell lymphoma, *Leukemia & Lymphoma*, 58:5, 1037-1051, DOI: [10.1080/10428194.2016.1228932](https://doi.org/10.1080/10428194.2016.1228932)

To link to this article: <http://dx.doi.org/10.1080/10428194.2016.1228932>



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REVIEW



## The significance of FOXP1 in diffuse large B-cell lymphoma

Duncan M. Gascoyne and Alison H. Banham

Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

### ABSTRACT

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of mature B-cell lymphoma. While the majority of patients are cured with immunochemotherapy incorporating the anti-CD20 monoclonal antibody rituximab (R-CHOP), relapsed and refractory patients still have a dismal prognosis. DLBCL subtypes including an aggressive activated B-cell-like (ABC) and a more favorable prognosis germinal center-like (GCB) DLBCL have been identified by gene expression profiling and are characterized by distinct genetic abnormalities and oncogenic pathways. This identification of novel molecular targets is now enabling clinical trials to evaluate more effective personalized approaches to DLBCL therapy. The forkhead transcription factor FOXP1 is highly expressed in the ABC-DLBCL gene signature and has been extensively studied within the context of DLBCL for more than a decade. Here, we review the significance of FOXP1 in the pathogenesis of DLBCL, summarizing data supporting its utility as a prognostic and subtyping marker, its targeting by genetic aberrations, the importance of specific isoforms, and emerging data demonstrating a functional role in lymphoma biology. FOXP1 is one of the critical transcription factors whose deregulated expression makes important contributions to DLBCL pathogenesis. Thus, FOXP1 warrants further study as a potential theranostic in ABC-DLBCL.

### ARTICLE HISTORY

Received 17 July 2016  
Revised 17 August 2016  
Accepted 21 August 2016

### KEYWORDS

FOXP1; diffuse large B-cell lymphoma; ABC-DLBCL

### FOXP1 and the FOXP family

Murine Foxp1 was identified by the Tucker laboratory as a DNA-binding glutamine-rich transcription factor having similarity to HNF-3/forkhead proteins and being expressed at the terminal stage of B-cell differentiation.[1] Subsequently, we cloned the human FOXP1 gene, which encoded a novel antigen recognized by the JC12 murine monoclonal antibody. The human FOXP1 protein was preferentially expressed in mantle zone rather than germinal center B cells and was particularly abundant in a subset of diffuse large B-cell lymphomas (DLBCL).[2]

There are four members comprising the FOXP subfamily of forkhead transcription factors, FOXP1-4, which share significant homology. All have been shown to participate in normal development and to have roles in cancer. Their interactions are complex and regulated by the existence of multiple isoforms and the ability of FOXP proteins to form both homo- and heterodimers that regulate distinct patterns of gene expression.[3,4] FOXP1 has been most extensively studied in cancer, and much of the non-DLBCL data are summarized in a previous review.[5]

### Diffuse large B-cell lymphoma

Mature B-cell lymphomas are collectively the 10th most common cancer worldwide, with nearly 386,000 new diagnoses annually (2012 Cancer Research UK statistics), and of these ~40% are DLBCL. Although DLBCL display both clinical and molecular heterogeneity, [6] patients receive a standard CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy regimen, that in latter years has been improved by addition of the anti-CD20 monoclonal antibody rituximab (R-CHOP).[7] While most patients are curable with R-CHOP, outcome for those that fail to respond or relapse remains dismal.[8]

Distinct DLBCL subtypes including a relatively good prognosis germinal center B-cell-like (GCB-DLBCL) subtype and a poor prognosis activated B-cell-like (ABC-DLBCL) subtype have been revealed by gene expression profiling (GEP).[9,10] The former is likely of germinal center (GC) origin, and the latter potentially derived from a transient plasmablast stage prior to terminal plasma cell differentiation.[9,11] Further biological and next-generation sequencing studies have provided a wealth of information regarding the

**CONTACT** Alison H. Banham [alison.banham@ndcls.ox.ac.uk](mailto:alison.banham@ndcls.ox.ac.uk) University of Oxford Medical Sciences Division, NDCLS-Radcliffe Department of Medicine, Level 4 Academic Block, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, United Kingdom of Great Britain and Northern Ireland

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oncogenic pathways active in DLBCL and an array of molecular targets for therapeutic intervention. Critical events in ABC-DLBCL include, among others, constitutive NF- $\kappa$ B activation,[12] chronic B-cell receptor (BCR) signaling (potentially stimulated via self-antigens),[13–15] and inactivation of terminal plasma cell differentiation via aberrations in the *PRDM1/BLIMP1* transcription factor.[16] These and other studies have led to new clinical trials in DLBCL, including those to evaluate Bruton's tyrosine kinase (BTK) inhibitors (reviewed in Young *et al.* [15]) as strategies to inhibit BCR-dependent NF- $\kappa$ B signaling in DLBCL.

Here, we review the role of FOXP1 in the pathogenesis of DLBCL. We have summarized its utility as a prognostic and DLBCL subtyping marker, presented the evidence for its targeting by genetic aberrations, addressed the importance of specific FOXP1 isoforms and discussed the functional role of FOXP1 in lymphoma pathogenesis.

### Identification of FOXP1 as an ABC-DLBCL biomarker and prognostic indicator

We initially reported increased FOXP1 protein expression in DLBCL with a post-GC phenotype (CD10<sup>−</sup>, BCL6 < 50%), [17,18] while *FOXP1* transcript expression was identified within the ABC-DLBCL GEP signature.[11]

Early studies investigated whether small gene expression profiling panels could routinely distinguish GCB- versus ABC-DLBCL, although FOXP1 was not always included. For example, *FOXP1* was included in a 27-gene DLBCL subtype predictor but not in the final 14 gene signature used to validate the predictor in an independent dataset.[19] Another study refining 18 key regulatory genes from a larger 36-gene panel incorporated *FOXP1* as an ABC-DLBCL marker.[20] More recently in the rituximab era, expression of 16 cell-of-origin and immune response genes, including *FOXP1*, predicted the outcome of elderly DLBCL patients treated with R-CHOP in the Groupe d'Etude des Lymphomes de l'Adulte (GELA) centers. A multivariate model comprising cell-of-origin genes (*FOXP1*, *MME*, *LMO2*, and *LPP*) and two immune response genes (*APOBEC3G* and *RAB33A*) was highly predictive of patient outcome.[21]

The Hans algorithm (CD10, BCL-6, and MUM1/IRF4) was among the first approaches to routinely identify GEP-defined DLBCL subtypes using immunohistochemistry.[22] FOXP1 was expressed in 48% of GCB and 71% of non-GCB tumors, but its expression did not correlate with survival and FOXP1 was not incorporated into the algorithm. However, as the significance

of FOXP1 protein expression in DLBCL became more firmly established, it was incorporated into multiple immunohistochemistry DLBCL-subtyping algorithms (Figure 1).[23–29]

### FOXP1 as a prognostic marker in DLBCL in the prerituximab era

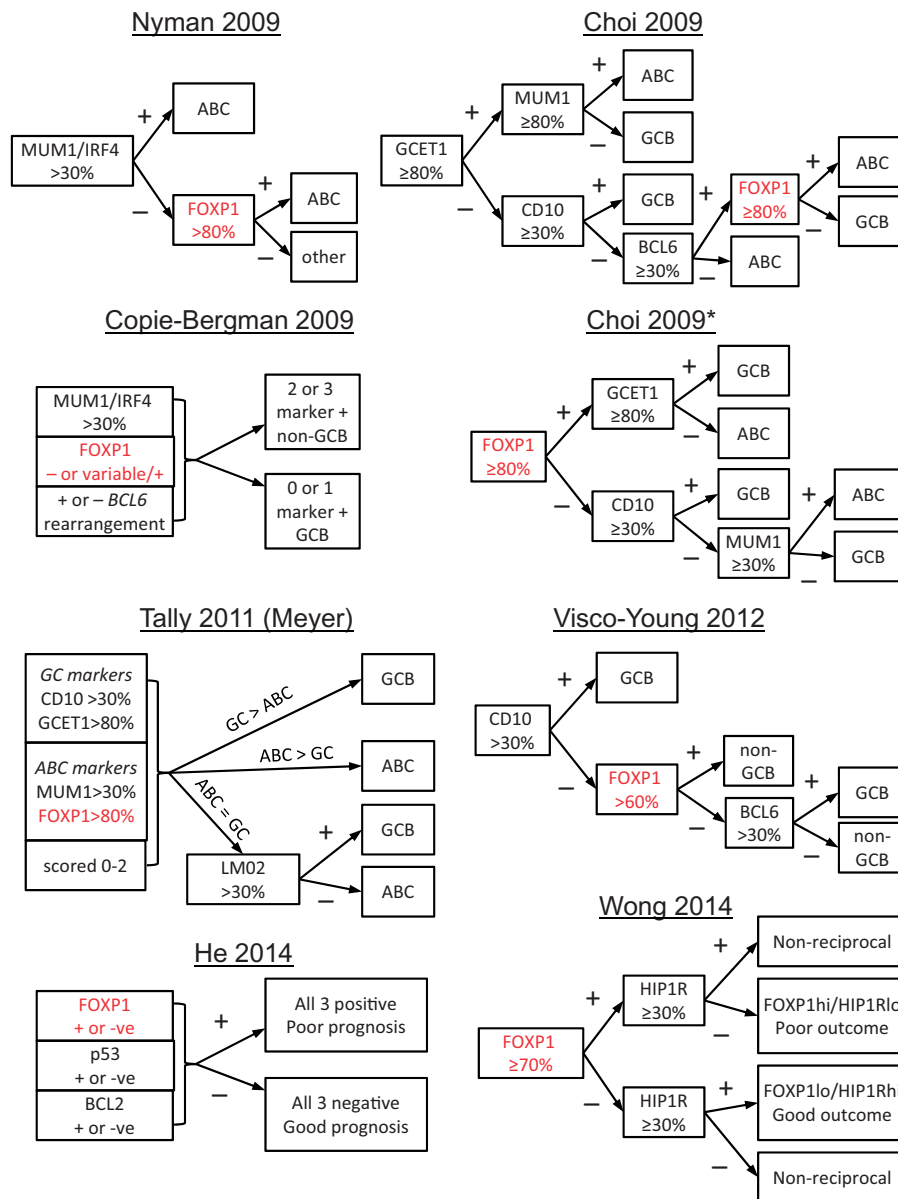
A relationship between high FOXP1 protein expression and inferior outcome was first identified in previously untreated *de novo* DLBCL ( $n=101$ ) from the British Columbia Cancer Center.[30] FOXP1-positive patients had median overall survival (OS) of only 1.6 years, compared to 12.2 years for FOXP1-negative patients ( $p=.0001$ ).[31] In an independent DLBCL series, tumors with uniform strong FOXP1 expression were predominantly of non-GCB phenotype (CD10<sup>−</sup>, BCL6 < 50%, MUM1<sup>+</sup>), BCL2 positive in the absence of the  $t(14;18)$  translocation, and exhibited poor clinical outcome.[32] In contrast, neither of two DLBCL studies by Hans *et al.* identified FOXP1 protein expression as clinically relevant.[22,33] These and the results of additional immunohistochemistry studies analyzing FOXP1 as an individual marker are summarized in Table 1.

### FOXP1 as a prognostic marker in DLBCL in the rituximab era

The addition of rituximab to DLBCL CHOP chemotherapy necessitated reevaluation of existing DLBCL prognostic markers. A phase-I dose-escalation study of intraventricular rituximab monotherapy in patients with recurrent CNS and intraocular lymphoma provided the first evidence for elevated *FOXP1* gene expression being associated with poor rituximab response.[34]

Evaluation of both the Hans algorithm and FOXP1 protein in *de novo* R-CHOP/R-CHOEP-treated DLBCL ( $n=117$ ) initially reported that neither had an impact on two-year survival.[26] However, while the Hans algorithm remained nonpredictive, a modification using MUM1/IRF4 ( $\geq 30\%$  positivity) and then FOXP1 (100% positivity) to define an ABC-DLBCL category did distinguish R-CHOP-treated DLBCL patients with inferior three-year failure-free survival and a trend toward inferior OS.[35]

Although the Hans algorithm exhibited approximately 80% concordance with GEP-defined DLBCL subtyping,[22] Choi *et al.* used new antibodies recognizing GCB cell markers to improve the predictive power of an immunohistochemistry DLBCL-subtyping algorithm and study both CHOP ( $n=84$ ) and R-CHOP ( $n=63$ )-treated patients. This new algorithm,



**Figure 1.** IHC algorithms incorporating FOXP1. Choi 2009\* is a modified Choi algorithm proposed by Meyer et al. [25] which performed equivalently to the original algorithm [29] but without dependency on BCL6. ABC: activated-B like; GCB: germinal center B.

using GCET1, CD10, BCL6, MUM1 alongside FOXP1, reduced the dependence upon BCL-6 staining and was 93% concordant with GEP classification. Both GEP-defined subtyping and the Choi immunohistochemical algorithm exhibited clinical relevance in CHOP and R-CHOP-treated DLBCL.[29]

The French GELA group also associated FOXP1 expression with a non-GCB DLBCL phenotype using two different scoring cut offs.[23] In R-CHOP-treated patients ( $n = 113$ ), uniform moderate-to-high-level FOXP1 protein expression in all tumor cells was associated with a trend toward inferior survival ( $p = .06$ ). An immunoFISH index, whereby positivity was conferred by at least two-thirds of either FOXP1 or MUM1/IRF4 protein expression or *BCL-6* gene rearrangement,

identified high-risk patients with shorter five-year OS independently of the age-adjusted IPI. High-level FOXP1 expression also conferred poor OS and progression-free survival (PFS) in relapsed/refractory DLBCL patients treated with R-ICE or R-DHAP regimens in the CORAL trial.[36]

### Factors affecting reproducibility of DLBCL prognosticators

The Lunenburg Lymphoma Biomarker Consortium identified variable detection of several DLBCL subtyping markers (FOXP1 not studied) across multiple laboratories, due to the use of different reagents, staining methods and variation in scoring.[37] Consequently,

**Table 1.** IHC Studies of FOXP1 protein expression as an individual biomarker in DLBCL.

Antibody clone	IHC cutoff	Treatment	Patients (n)	Correlation high FOXP1 to outcome	Citation
JC12	30%	CHOP	147	ns	Hans et al. [22]
JC12	uniform high	CHOP	126	inferior OS $p = .015$ , independent of IPI	Barrans et al. [32]
JC12	>30%	CHOP	99	inferior OS $p = .0001$ , independent of IPI, PFS ns $p = .073$	Banham et al. [31]
JC12	$\geq 30\%$	Chemo/no R	196	ns for OS or EFS	Hans et al. [33]
JC12	Barrans	R not included	76	Inferior DSS $p = .03$	Kodama et al. [96]
na	60%	CHOP	208	Inferior survival $p = .019$ , independent of stage not independent of IPI	Adams et al. [97]
na	na	na	47	ns (CNS DLBCL)	Cheng et al. [98]
JC12	mod/strong >80%	CHOP $\pm$ R EPOCH $\pm$ R	73	ns (HIV + DLBCL)	Chadburn et al. [99]
JC12	100% strong	R-CHO(E)P	117	ns	Nyman et al. [35]
JC12	30%/Barrans	R-CHOP	113	ns (30% cutoff), trend poor OS (Barrans score)	Copie-Bergman et al. [23]
JC12	$\geq 47.5\%$	Chemo-no R	217	inferior DSS $p = .044$ , independent of IPI and COO Inferior DSS $p = .014$ (nodal), ns (extranodal) Inferior DSS ( $p = .047$ (non-GCB), ns (GCB-DLBCL))	Hoeller et al. [40]
JC12	Pos/neg	CHOP	141	ns (all or nodal cases), inferior OS $p = .024$ (extranodal)	Yu et al. [100]
na	Barrans	R-ICE, R-DHAP	217	poor OS $p = .036$ and PFS $p = .024$ , multivariate PFS $p = .047$ relapsed/refractory patients	Theiblemont et al. [36]
na	$\geq 60\%$	R-CHOP	475	poor PFS $p = .006$ , poor OS $p = .0009$	Visco et al. [27]
JC12	$\geq 30\%$	R-CHOP	92	poor PFS $p = .002$ , Poor OS $p = .017$ , Poor PFS $p = .014$ (GCB), poor OS $p = .009$ (GCB), ns (non-GCB)	Hu et al. [101]
na	na	na	71	inferior OS $p = .0015$ , ns in multivariate $p = .065$ (PCNSL)	Shen et al. [102]
sc-6283	>40% strong	na	51	ns (PCNSL)	Chen et al. [103]
JC12	multiple	R-CHOP	168	ns	Culpin et al. [104]
JC12	30, 60, 80%	R-CHOP	141	ns	Coutinho et al. [38]
JC12	$\geq 70\%$	R-CHOP	157	inferior OS $p = .0013$ , inferior PFS $p = .0012$ , independent of IPI and non-GCB	Wong et al. [28]
JC12	Allred >2	CHOP $\pm$ R	58	inferior PFS $p = .027$ , inferior OS $p = .008$ (Gastric DLBCL)	He et al. [24]
Ab16645	Barrans	R-CHOP, R-ESHAP	100	poor OS $p = .011$	Bellas et al. [41]
Abcam	$\geq 80\%$	R-CHOEP	87	ns (young DLBCL patients)	Horn et al. [105]
SP133	>50%	R-CHOP-14	123	inferior OS $p = .00018$ , independent of stage and IPI	Tzankov et al. [42]
1-21	50%	R-CHOP	60	ns, OS $p = .062$ (PT-DLBCL)	Deng et al. [106]
JC12	$\geq 70\%$	R-CHOP	73	ns (series 2)	Wong et al. [68]

Where possible the number of patients refers to those with FOXP1 expression data, not the number in the entire series. With regard to therapy the designation refers to primarily whether or not samples were from the rituximab era, treatment information was not always available for all patients and in some studies patients were also treated with other regimens such as radiotherapy and stem cell transplantation.

COO: cell-of-origin subtype; chemo: chemotherapy; DSS: disease-specific survival; DLBCL: diffuse large B-cell lymphoma; EFS: event-free survival; GCB: germinal center B-cell; IPI: international prognostic index; LBCL: large B-cell lymphoma; mod: moderate; na: information not available; Neg: negative; ns: not significant; OS: overall survival; PCNSL: primary nervous system diffuse large B-cell lymphoma; PFS: progression-free survival; Pos: positive; PT-DLBCL: primary testicular DLBCL; R: rituximab; wk: weak.

multiple studies report different findings, and even comparisons of multiple DLBCL-subtyping algorithms within the same group of patients have demonstrated variability in their predictive accuracy.[25,38] FOXP1 has been reported to be a reproducibly assessable marker in DLBCL by an international inter- and

intrainstitutional and inter- and intraobserver study, [39] and most but not all immunohistochemistry studies have used the JC12 antibody. However, variable cutoffs for FOXP1 frequency and/or intensity (Table 1) have prevented direct comparisons across studies. While not all studies report FOXP1 expression as



clinically significant, those that do reproducibly link its expression to poor outcome and never favorable outcome (Table 1). Intriguingly, across multiple studies including a prospective trial (NCT00544219), FOXP1 alone exhibited greater predictive value than the cell-of-origin algorithms tested.[28,40–42]

### **FOXP1 and MYC-positive, double-hit and triple-hit lymphomas**

Many studies have demonstrated that the ~10% of DLBCL-exhibiting MYC translocation have a poor outcome. FOXP1 expression correlates with that of MYC, [41,42] and both FOXP1 activation of MYC [43] and indirect MYC-mediated upregulation of FOXP1 [44] have been reported. When combined with BCL2 and BCL6 translocations/rearrangements, these double- and triple-hit B-cell lymphomas are particularly aggressive and novel therapies are actively being sought.[45] A clinicopathological study of 11 triple-hit cases, of which five were DLBCL, demonstrated that while four immunohistochemistry algorithms classified them as GCB-like, all cases were positive for FOXP1.[46] Such cases provide one explanation for why FOXP1 expression may predict survival in some DLBCL series more effectively than cell-of-origin algorithms.

### **Genetic aberrations targeting FOXP1 in DLBCL**

FOXP1/IGH translocations were first identified in MALT lymphoma (10%), which commonly exhibit trisomy FOXP1 and elevated FOXP1 transcript expression, and were mutually exclusive of those targeting MALT1 and BCL10.[47] FOXP1 expression correlated with unfavorable relapse rate and disease-free survival in MALT lymphoma, while strong FOXP1 positivity with trisomy 3 and 18 defined a polymorphic subset at risk of transformation to DLBCL.[48]

Wlodarska et al. first identified recurrent chromosome translocations targeting FOXP1 in DLBCL.[49] In a non-GCB DLBCL, a gastric MALT lymphoma, and a B-NHL otherwise nonspecified, FOXP1 (3p13) was juxtaposed to the immunoglobulin heavy chain (IGH) locus by a t(3;14)(p13;q32) translocation. Another B-NHL displayed a translocation between FOXP1 and 2q36. Strong FOXP1 protein expression accompanied both translocations and amplification (one case, 4–16 copies) and importantly was present also in nontranslocated tumors, thus evidencing additional mechanisms upregulating FOXP1 protein expression.[49] A gastric DLBCL with a chromosome translocation placing FOXP1 under the control of IGH locus also exhibited high-level expression.[50] A further study identified 30/

102 DLBCL with FOXP1 copy number gains and five cases with a FOXP1 translocation. Only two of the translocations involved the IGH locus, none had any low-grade component or history of a previous low-grade lymphoma, and there was a strong association with extranodal presentation.[51] However, Barrans et al. detected common extra FOXP1 copies (39/58, 67%) but not FOXP1 translocation in 58 (from 499) DLBCL with high uniform FOXP1 protein expression.[52] These early studies demonstrated that FOXP1 translocation occurs only in a minority of DLBCL, more commonly in extranodal DLBCL, and that high-level protein expression can be exhibited in the absence of genetic FOXP1 abnormalities.

The identification of recurrent FOXP1 translocations provided genetic evidence that FOXP1 might be a lymphoma oncogene. Furthermore, since other MALT lymphoma translocations activate the NF-κB pathway [47], these findings provided insight into potential FOXP1 functions in DLBCL, as ABC-DLBCL require constitutive NF-κB activity for survival.[12]

Genomewide studies of genetic aberrations in lymphoma have also highlighted frequent alterations at the FOXP1 locus. Comparative genomic hybridization (CGH) analysis of 224 untreated DLBCL identified frequent trisomy 3 in ABC-DLBCL (15%) but not GCB-DLBCL.[53] Lenz et al. combined high-density array-CGH with gene expression profiling in an investigation of 203 DLBCL, showing that trisomy 3 was detected in 26% ABC-DLBCL and only 1% GC-DLBCL, with FOXP1 being the third most highly expressed of ~1092 annotated chromosome 3 genes in DLBCL with trisomy 3. FOXP1 copy number increases were detected in 38% ABC-DLBCL versus only 4% GCB-DLBCL.[54] The authors concluded FOXP1 was a candidate oncogene associated with trisomy 3 in ABC-DLBCL. A subsequent association of FOXP1 gains with non-GCB-DLBCL by FISH reported that these were associated with nodal, rather than extranodal presentation. However, these gains had no correlation with survival, or with FOXP1 protein expression, which did correlate with poor outcome.[40] Thus, similar results regarding FOXP1 gains have been identified using different methods to analyze comparable size patient cohorts.

### **Smaller FOXP1 isoforms are expressed in ABC-DLBCL**

While FOXP1 represented a potential oncogene in DLBCL, it appeared to be a tumor suppressor in carcinomas.[2,55] We initially considered that this might reflect context dependent differences in full-length protein activity. However, an additional explanation

was provided by our identification of abundant smaller (~65 kDa) FOXP1 proteins (FOXP1<sub>s</sub>) in ABC-DLBCL cell lines and in primary NGC (non-germinal center)-DLBCL.[56] These were detectable also in activated nonmalignant B cells but were not abundant in tonsillar lymphoid tissue. Thus, B-cell activation represents one mechanism for upregulating FOXP1 expression independently of genetic aberrations, and strong FOXP1<sub>s</sub> expression may be a characteristic of the ABC-DLBCL cell-of-origin.

Initial analyses suggested that smaller human FOXP1 isoforms lacked N-terminal regions and/or were derived from alternatively spliced transcripts (isoforms 3 and 9).[56] Viral integration sites giving rise to avian nephroblastoma were clustered around the equivalent of human *FOXP1* exon 7 and have also been predicted to generate N-terminally truncated oncogenic foxP1 proteins.[57] N-terminal FOXP1 truncation in ABC-DLBCL was subsequently validated by mass spectrometry and the use of a FOXP1 antibody whose epitope was encoded by exon 7.[58] Smaller FOXP1 proteins were suggested to have oncogenic activity in DLBCL, either through intrinsic activity or by regulating function of the full-length ~75 kDa protein. Furthermore, we proposed that *FOXP1* translocations might regulate relative levels of FOXP1 isoforms.[56]

### Relationships between translocations and FOXP1 isoform expression

Mapping *FOXP1* breakpoints in translocation-positive MALT and DLBCL cases (16/595) demonstrated that these generally occurred within the 5' end of the gene.[59] Furthermore, in several instances, the breakpoints were found within *FOXP1*-coding sequence, including two cases having breakpoints upstream of what we now know to be the first FOXP1s coding exon (exon 8).[58,59] Thus, *FOXP1* translocations did appear to potentially promote expression of variant transcripts.

Further support for this hypothesis was provided by the Wlodarska laboratory's comprehensive cytogenetic and molecular characterization of four cases of non-*IGH* *FOXP1* rearrangements (compared with *IGH-FOXP1* lymphomas and FOXP1+ lymphomas lacking detectable *FOXP1* rearrangements).[60] *FOXP1* translocations with partner genes such as *PAX5* [61] or *ABL1* [62] in acute lymphoblastic leukemia and *PDGFRA* in a myeloproliferative disease [63] have been reported to generate fusion proteins containing domains from each partner. However, in contrast, the *FOXP1* non-*IGH* rearrangements in B-cell lymphomas were found to

disrupt the full-length *FOXP1* transcript and promote expression of N-terminally truncated isoforms.[60] A similar translocation was also identified in primary testicular lymphoma, where *FOXP1* rearrangements were detected in 7% of cases using a break-apart FISH probe.[64] Analysis of sequential samples revealed that non-*IGH* *FOXP1* rearrangements became detectable during the course of the disease.[60] The authors therefore hypothesized that *IGH*-translocations might be an early oncogenic event activating full-length FOXP1, with two cases demonstrating full-length FOXP1 protein expression, while non-*IGH* translocations may N-terminally truncate FOXP1 to promote disease progression.[60] Differing *FOXP1* breakpoints of *IGH* and some non-*IGH* *FOXP1* translocations may simply reflect differential alternative FOXP1 internal promoter activity (6b/7b/7c) in the initiating tumor cell.

### FOXP1<sub>s</sub> protein expression and function

Wlodarska's group proposed that the 65 kDa smaller FOXP1 isoforms might be single species with altered post-transcriptional modifications [60] and our subsequent exon targeted siRNA and transcript expression analyses in DLBCL are consistent with translational start for FOXP1<sub>s</sub> in exon 8. *FOXP1* transcripts encoding FOXP1<sub>s</sub> proteins have variable 5' noncoding exons (both groups identifying abundant exon 6b expression) and are not encoded by splice variants lacking exons 8, 9 and/or 10 (isoforms 3, 5, and 9).[58] Truncated FOXP1 isoforms are not exclusive to DLBCL and have been associated with an active NF-κB signature in follicular lymphoma, [65] and have been observed in prostate and breast cancer cell lines.[66,67]

While studying whether FOXP1<sub>s</sub> proteins in ABC-DLBCL might functionally differ from the abundant full-length protein in some GCB-DLBCL, we identified HIP1R (an endocytic protein) as a GCB-DLBCL marker directly repressed by FOXP1 in ABC- but not GCB-DLBCL cell lines.[28] This could reflect differential FOXP1 isoform activity, FOXP1<sub>s</sub> being a stronger transcriptional repressor (as reported for the murine protein in 293T cells) [3] and/or context-dependent differences in these distinct DLBCL molecular subtypes. In R-CHOP-treated DLBCL ( $n = 157$ ), HIP1R expression (>10%) identified patients with a favorable outcome, while FOXP1 (≥70%) identified those with a poor prognosis. Interestingly, FOXP1<sup>hi</sup>HIP1R<sup>lo</sup> status identified an R-CHOP-resistant subgroup in the whole series and in low-risk IPI (0–2) and GCB-DLBCL subgroups. Conversely, patients with FOXP1<sup>lo</sup>HIP1R<sup>hi</sup> tumors exhibited ≥80% 5-year overall survival in both GCB-

and ABC-DLBCL subgroups and in the low-risk IPI subgroup. The *FOXP1<sup>hi</sup>HIP1R<sup>lo</sup>* group scored by transcript abundance was also predictive of poor outcome in an independent series.[28] Furthermore, in a third small series ( $n=73$ ) where FOXP1 protein alone was not predictive of outcome, the *FOXP1<sup>hi</sup>HIP1R<sup>lo</sup>* group again identified DLBCL patients with poor OS and PFS.[68] We hypothesize that *FOXP1<sup>hi</sup>HIP1R<sup>lo</sup>* DLBCL have transcriptionally active FOXP1<sub>s</sub> protein and that HIP1R may represent a DLBCL tumor suppressor potentially increasing the efficiency of CD20-targeted therapy.

## FOXP1 regulation by microRNAs in B cells and DLBCL

### miR-150

Reductions in FOXP1, MYB, and survivin expression in the Burkitt lymphoma cell-line DG75 were achieved by experimental induction of miR-150, after profiling of microRNA expression in B-cell subsets showed downregulation of miR-150 in GC B cells compared to naïve and memory B cells.[69] This miR-150-dependent FOXP1 regulation occurred in mature, rather than pro- or pre-B cells, and in chronic lymphocytic leukemia miR-150-mediated repression of FOXP1 and GAB1 impaired BCR signaling.[70] Thus, strong downregulation of miR-150 expression in DLBCL versus lymph nodes may facilitate FOXP1 upregulation.[71]

### miR-34a

Genetic *Foxp1* deletion blocks murine B-cell development at the pro- to pre-B-cell transition,[72] a defect also produced by constitutive expression of a p53-regulated microRNA, miR-34a.[73] *Foxp1* (lacking its 3' UTR) rescued B-cell maturation in the presence of miR-34a and was validated as a direct miR-34a target gene. This finding connects *Foxp1* suppression to the p53 network,[73] and DLBCL with *TP53* deletion exhibited significantly lower miR-34a.[74] A later study also demonstrated that coexpression of FOXP1, p53 and BCL2 (another miR-34a target) identified DLBCL and MALT lymphomas with poor outcome.[24]

MALT lymphoma can transform into gastric DLBCL, and a comparison of their microRNA expression profiles identified a 27-microRNA signature (largely shared with nodal DLBCL) transcriptionally repressed by MYC.[44] Silencing of MYC blocked DLBCL proliferation, and miR-34a overexpression in DLBCL cells most effectively recapitulated this phenotype. FOXP1 was identified as a direct miR-34a target that was overexpressed in gastric DLBCL and its knockdown was

reported to inhibit DLBCL proliferation in U-2932, SU-DHL-4, SU-DHL-6, and SU-DHL-7.[44] Later studies have reported a significant reduction of miR-34a expression in the serum [75] and tumors [24] of DLBCL patients.

### miR-181a

A study of microRNA expression in 176 R-CHOP-treated DLBCL identified three microRNAs (miR-18a, miR-181a and miR-222) individually associated with DLBCL patients' survival, of which miR-18a and miR-181a also predicted outcome in multivariate analysis.[76] FOXP1 was identified as a potential miR-181a target, and transfecting the miR-181a precursor into HBL-1 DLBCL cells reduced endogenous FOXP1 protein levels and activity of 3' *FOXP1* UTR luciferase reporters.[76]

## Oncogenic processes and pathways regulated by FOXP1 in DLBCL

As described previously, early studies in DLBCL were focused primarily on the utility of FOXP1 as a biomarker and its regulation by genetic aberrations and microRNAs. More recently, there have been a number of studies seeking to understand its function (Table 2).

### FOXP1 regulates germinal center differentiation and function

*Foxp1* deletion in the mouse identified its requirement for the pro-B to pre-B-cell transition, control of *Rag* gene expression and the control of *IGH* VDJ recombination.[72,77] Subsequently, Sagardoy et al. generated transgenic mice ( $E\mu$ SR $\alpha$ -hFOXP1) overexpressing human FOXP1 (5–10 gene copies) in B and T cells.[77] In young mice, both B- and T-cell populations were largely normal, and although older mice showed a modest expansion of IgM<sup>hi</sup>IgD<sup>hi/lo</sup> mature B cells and of CD21<sup>+</sup>CD23<sup>+</sup> marginal zone cells, there was no evidence of tumors or other disease. Immunization studies to investigate B-cell phenotypes during an acute immune response identified smaller GCs and a role for FOXP1 in negatively regulating the expansion phase of GC development and impairing GC function, via a decrease in IgM to IgG1 switching. These data suggest that although overexpression of full-length FOXP1 may indeed be an early oncogenic event during lymphomagenesis,[60] additional factors are also probably necessary.

FOXP1 is expressed in only a minority of normal GC cells, [2] and while some of these coexpressed the BCL6 transcription factor, FOXP1/BCL6 expression



**Table 2.** FOXP1 functionality in DLBCL.

FOXP1 manipulation	Phenotype on FOXP1 manipulation	Notes	Citation
DLBCL cell line siRNA (U-2932, SU-DHL-4,6,7)	inhibited proliferation (~50% reduced <sup>3</sup> H-Thymidine incorporation)	Identified indirect Myc regulation of FOXP1 via miR-34a	Craig et al. [44]
cell line ChIP and siRNA (OCI-Ly1), transgenic Eμ-driven hFOXP1 in immunized mouse	reduced GC formation, inhibited IgG1 class switching	<i>PIM1</i> and <i>POU2AF</i> repressed, <i>PRDM1</i> activated in OCI-Ly1	Sagardoy et al. [77]
non-DLBCL B-cell cell line siRNA (Raji)	reduced BCR signaling (calcium flux), no effect on viability, downregulation pAKT	modest FOXP1 depletion single siRNA, CLL sensitive to BCR ligation had elevated <i>FOXP1</i> transcripts	Mraz et al. [70]
overexpression in primary human B cells	expansion by the inhibition of apoptosis, no increase in proliferation, IKK-CA treatment identified NF-κB cooperation	GEP shows apoptotic targets, top GO term immune response	van Keimpema et al. [78]
overexpression, siRNA, ChIP-seq in DLBCL cell lines OCI-Ly10, Ly1, Ly7, additionally Ly3 for ChIP-seq	no effect of siRNA on apoptosis or proliferation	50–70% FOXP1 knockdown, genes <i>HRK</i> , <i>BIK</i> , <i>RASSF6</i>	van Keimpema et al. [78]
DLBCL cell line siRNA (HBL-1, DB, K-422, OCI-Ly3)	increased expression of GCB marker HIP1R in ABC- not GCB-DLBCL	effective FOXP1 knockdown, marker of transcriptionally active FOXP1	Wong et al. [28]
overexpression in human primary B cells	repression of CD38+ plasma cell IgG secretion	<i>PRDM1</i> , <i>CD44</i> , <i>CD69</i> , <i>p21</i> , <i>p27</i> repressed	Tsai et al. [83]
overexpression in primary human memory B cells and DLBCL cell lines OCI-Ly7, SKW6.4, ChIP-seq	inhibition of plasma cell differentiation, IgG not IgM	<i>PRDM1</i> , <i>IRF4</i> , <i>XBP1</i> are directly FOXP1 repressed targets	van Keimpema et al. [82]
DLBCL cell line shRNA + <i>in vivo</i> (DB), overexpression and siRNA (DB, K-422)	WNT signaling regulated, FOXP1 shRNA slowed tumor growth, overexpression in DB and K422 increased Wnt activity	FOXP1 complexed with β-catenin, TCF7L2, CBP. FOXP1 levels may indicate sensitivity to Wnt inhibitors	Walker et al. [79]
DLBCL cell line siRNA (HBL-1, DB, K-422, OCI-Ly3)	MHC class II signature increased in ABC-DLBCL, CD74 and MHC class II upregulated on cell surface	GC signature genes <i>LPP</i> , <i>VNN2</i> , <i>NEIL1</i> FOXP1 repressed in ABC-DLBCL cell lines	Brown et al. [85]
DLBCL cell line siRNA (U-2932, SU-DHL-4)	reduced surface CD19 with long FOXP1 isoform-biased depletion	total FOXP1 depletion has no effect on surface CD19. FOXP1 isoforms differ in U-2932 subpopulations	Brown et al. [58]
DLBCL cell line dox-inducible shRNA and GEP (HBL-1 TMD-8), ChIP-seq (HBL-1, TMD-8, OCI-Ly8,10,19, HT, BJAB)	apoptotic cell death after siRNA, no effect in OCI-Ly10, HT, Ly19. MHC class II activation	regulator of multiple signatures including GC, PC, MHC, MYD88, JAK-STAT, NF-κB (pIKK), c-MYC	Dekker et al. [43]
DLBCL cell line siRNA (SU-DHL-6, U-2932, RIVA), RNA-seq, ChIP-seq (SU-DHL-6, U-2932)	induced apoptotic cell death	<i>S1PR2</i> critical target. FOXP1 total silencing more effective than long-isoform biased siRNA	Flori et al. [86]

BCR: B-cell receptor; ChIP(seq): chromatin immunoprecipitation (sequencing); dox: doxycycline; GC: germinal center; GEP: gene expression profiling; IgG and IgM: immunoglobulin G and M; IKK-CA: I kappa B kinase constitutively active; MHC: multiple histocompatibility complex; PC: plasma cell; RNA-seq: ribonucleic acid sequencing.

patterns are generally reciprocal.[77] Chip-on-chip analysis to identify FOXP1 target genes in the OCI-Ly1 DLBCL cell line (predominantly full-length FOXP1 protein) identified approximately a 50% overlap with BCL6 target genes and enrichment in biological pathways corresponding to antigen presentation, immune response and cancer. Transiently silencing FOXP1 in OCI-Ly1 (GCB-DLBCL) cells led to *PIM1* and *POU2AF* upregulation and repression of *PRDM1*, demonstrating the potential of FOXP1 to both activate and repress gene expression in malignant B cells.[77]

### FOXP1 cooperates with NF-κB and promotes B-cell survival

Correlation between FOXP1 expression and the ABC-DLBCL subtype [11] and identification of FOXP1 translocations in MALT lymphoma [47] both pointed to an association with NF-κB dependency. In this regard, van Keimpema et al. identified FOXP1 target genes by

gene expression profiling of primary human B cells retrovirally transduced with FOXP1 and cultured on CD40L-L cells, and upon FOXP1 manipulation (overexpression or silencing) in GCB-DLBCL (OCI-Ly1 and OCI-Ly7) and ABC-DLBCL (OCI-Ly10) cell lines.[78] This uncovered FOXP1 repression of proapoptotic genes and a seven-gene apoptotic signature (*EAF2*, *BIK*, *TP63*, *HRK*, *TP13INP1*, *RASSF6*, and *AIM2*) sufficient to identify DLBCL patients with poor R-CHOP response, irrespective of their cell-of-origin subtype. ChIP-sequencing analysis of OCI-Ly1, OCI-Ly3, OCI-Ly7, and OCI-Ly10 identified widespread FOXP1 binding in each cell line (≥20,000 regions), with approximately half the FOXP1-regulated genes having a FOXP1 ChIP peak within 20 kb of the transcriptional start site. ABC-DLBCL had enrichment of IRF4- and SpiB-bound regions and NF-κB consensus motifs, suggesting that FOXP1 might cooperate or interact with such factors. Gene ontology analysis identified enrichment of genes involved in cell death and apoptosis across all lines. ABC-DLBCL were

characterized specifically by the regulation of chemotaxis, immune response, and cell/lymphocyte activation, while GCB-DLBCL showed regulation of cell cycle among others.

FOXP1 overexpression *ex vivo* in primary B cells promoted their CD40-ligation-dependent survival but did not affect their proliferation.[78] However, in this study, siRNA-dependent FOXP1 silencing in multiple DLBCL cell lines had no effect on viability, caspase-3/7 activity or proliferation. Cotransduction of primary B cells with constitutively active inhibitor of nuclear factor kappa-B kinase subunit beta (CA-IKK2) was used to demonstrate that FOXP1 cooperates with NF- $\kappa$ B activity to enhance B-cell survival, with FOXP1 repression of proapoptotic genes complementing NF- $\kappa$ B induction of antiapoptotic genes. Thus, Van Keimpema et al. proposed that lymphoma therapies targeting NF- $\kappa$ B will also overcome the cell survival benefits derived from aberrant FOXP1 expression.

### **FOXP1 promotes Wnt signaling in DLBCL**

A gain-of-function screening approach using a mass spectrometry-coupled lentiviral 'CD-tagging' mutagenesis approach identified FOXP1 as an activator of Wnt/ $\beta$ -catenin signaling in melanoma cells.[79] This was validated in other models including the DB and KARPAS-422 DLBCL cell lines and zebrafish. Interestingly, while full-length FOXP1 and some smaller isoforms activated Wnt signaling, other potentially dominant-negative FOXP1 isoforms (including one lacking the DNA-binding domain) inhibited Wnt signaling. FOXP1 formed a complex with TCF7L2 and CBP at Wnt target gene promoters, and enhanced  $\beta$ -catenin acetylation by CBP. Consequently, Wnt inhibition preferentially sensitized FOXP1-expressing GCB-DLBCL cell lines to chemotherapy drugs such as doxorubicin. Furthermore, Wnt inhibition significantly reduced *in vivo* xenograft growth of DB cells (although FOXP1 shRNA was less effective), and so the authors proposed that Wnt inhibitors may have therapeutic benefit in FOXP1-positive DLBCL. Interestingly,  $\beta$ -catenin expression was elevated not in ABC-DLBCL cell lines but in only the two GCB-DLBCL cell lines tested having abundant full-length FOXP1 protein,[79] suggesting that abundant full-length FOXP1 protein in a proportion of GCB-DLBCL may have different roles to abundant FOXP1 in ABC-DLBCL.

### **FOXP1 contributes to plasmacytic differentiation and loss of MHC class II expression in DLBCL**

Double immunolabeling studies in tonsil enabled us to identify that terminally differentiated plasma cells

generally lacked FOXP1 protein expression.[80] Normal bone marrow plasma cells, multiple myeloma cell lines and primary myeloma cases also rarely expressed the FOXP1 protein, although abundant transcripts implicated post-transcriptional silencing of FOXP1 in plasma cells. We proposed that FOXP1 downregulation might be important during plasma cell differentiation. Sagardoy et al. also implicated FOXP1 in terminal B-cell differentiation on discovering its activation of the key plasma cell regulator *PRDM1*. [77] Furthermore, whole-genome sequencing of the lymphoplasmacytic lymphoma Waldenström's macroglobulinemia identified *FOXP1* gene copy number losses in 37% of patients.[81]

Disruption of a plasma cell gene signature was identified following overexpression of FOXP1 in primary human memory B cells and OCI-Ly7 DLBCL cells. Specifically, FOXP1-mediated direct transcriptional repression of important regulators such as *PRDM1*, *IRF4*, and *XBPI*. [82] Analysis of FOXP1 in tonsillar B-cell subsets confirmed the downregulation of FOXP1 protein and transcripts in CD38<sup>+</sup> plasma cells relative to other B-cell subsets. Differences between these transcript data and our findings may reflect the tissue source of plasma cells, (those in our study being derived from bone marrow), [80] and/or, given the many FOXP1 transcript isoforms, slightly different methods of transcript detection. *FOXP1* transcript maintenance is also consistent with the discovery of microRNA regulatory hubs that modulate plasma cell differentiation, including miR-365a-3p, miR-34a-5p, miR-148a-3p, and miR-183-5p that suppressed endogenous FOXP1 in SKW6.4 cells.[83] Importantly, FOXP1 overexpression inhibited terminal differentiation of IgG<sup>+</sup> but not IgM<sup>+</sup> memory B cells, the former having 2-fold lower FOXP1 expression than the latter.[82] Tsai et al. also subsequently reported repression of *PRDM1* expression and plasma cell differentiation by FOXP1.[83] Overall these data demonstrate that FOXP1 expression levels are sufficient to determine B-cell fate and consequently that maintained FOXP1 expression in ABC-DLBCL likely contributes to lymphomagenesis by disrupting terminal B-cell differentiation.

Plasmacytic differentiation has been linked to the ABC-DLBCL subtype and reduced MHC class II expression, which is known to correlate with a poor outcome.[84] We silenced FOXP1 transiently using siRNA in GCB-DLBCL cells (expressing full-length protein) and ABC-DLBCL cells (expressing smaller isoforms) and microarray-profiled gene expression changes. In common with earlier studies, we identified repression of GC signature genes (e.g. *LPP*,

VNN2, and *NEIL1*) and multiple FOXP1-regulated pathways including immune response, cell activation, chemotaxis, and apoptosis. Importantly, we found regulation of MHC class II genes as among the most significant differences between the two DLBCL subtypes and confirmed that in ABC-DLBCL cells FOXP1 can repress surface expression of both HLA-DR and CD74.[85] Pan MHC class II restoration following FOXP1 shRNA was also independently reported.[43] Reduced HLA-DRA expression correlated with a poor response to R-CHOP, a non-GCB subtype and reciprocal expression with FOXP1 in non-GCB DLBCL.[85] FOXP1 thus represents a novel regulator of genes targeted by the class II MHC transactivator (CIITA) and of CIITA itself [43] and so its targeting represents one potential strategy to restore immune surveillance in high-risk DLBCL.[85]

### ***FOXP1 promotes DLBCL survival by repressing S1PR2 signaling***

To understand FOXP1 function Flori et al. [86] used genomewide ChIP-sequencing combined with gene expression profiling of DLBCL cell lines (SU-DHL-6, U-2932, and RIVA) transfected with siRNAs targeting either total FOXP1 or preferentially the high-molecular-weight isoform. They observed that apoptotic cell death, and regulation of most target genes, was more potently induced by targeting all FOXP1 isoforms. Twenty-seven biologically relevant target genes with cancer or B-cell associations in the literature were selected for functional characterization by gain- or loss-of function screening as appropriate. Ten of 25 FOXP1 transcriptionally repressed genes affected DLBCL viability but few correlated inversely with *FOXP1* transcript expression in primary DLBCL. The G-protein-coupled receptor *S1PR2*, which restricts GC B cells to lymph nodes, showed strong reciprocal relationship with *FOXP1* expression in DLBCL and normal B-cell subsets, and was validated as a direct FOXP1 target gene. Experimentally, *S1PR2* exhibited proapoptotic activity in DLBCL and impaired growth of SUDHL6 xenografts, while high *S1PR2* transcript levels correlated with favorable outcome in DLBCL patients treated with CHOP or R-CHOP. As previously observed for HIP1R,[28,68] combining high *FOXP1* with low abundance of *S1PR2* was a superior predictor of patients' survival.[86] In addition to the transcriptional *S1PR2* repression by FOXP1 occurring predominantly in ABC-DLBCL, recurrent mutation of *S1PR2* in GCB-DLBCL [87] probably represents another mechanism to inactivate this DLBCL tumor suppressor.

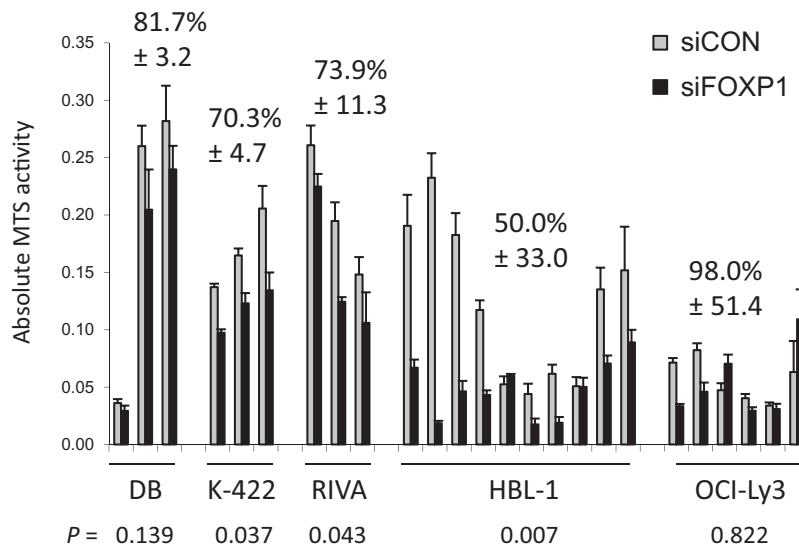
### ***ABC-DLBCL addiction to FOXP1***

In contrast to some transient siRNA studies, maintained FOXP1 knockdown using inducible shRNA induced apoptotic cell death in two ABC-DLBCL cell lines (TMD8 and HBL-1) but not in GC-DLBCL cell lines.[43] Global gene expression profiling was performed prior to cell death and combined with FOXP1 ChIP-sequencing on a wider panel of ABC and GCB-DLBCL cell lines identified binding not only at many proximal promoters, but also at putative intronic and long-range enhancers. As in other studies, FOXP1 targets distinguished ABC- and GCB-DLBCL cell lines and were highly correlated with FOXP1 expression and cell-of-origin subtypes in primary DLBCL. Strikingly, FOXP1 enforced many ABC-DLBCL hallmarks including MYD88, JAK2, and classical NF- $\kappa$ B pathways, activated *MYC* expression and IKK phosphorylation. While FOXP1 promoted the partial plasma cell differentiation phenotype in ABC-DLBCL, it repressed BCL6 signatures determining GCB-cell identity.[43]

### ***Conclusions and future directions***

Targeting by genetic aberrations, correlation with poor survival and recent functional studies demonstrate an important role for FOXP1 in DLBCL pathogenesis. However, it remains necessary to consider complexities arising from the expression of multiple FOXP1 isoforms, multiple FOXP family members and a range of cellular contexts including GC and non-GC subtype. Particularly, abundant expression of a FOXP1<sub>AL</sub> variant with an alternate N-terminus in the HBL-1 cell line,[58] is associated with sensitivity to FOXP1 depletion (Figure 2). Further characterization of rare strongly FOXP1-positive GC B cells lacking an epitope encoded by exon 7 (possibly FOXP1<sub>S</sub> or FOXP1<sub>AL</sub>) may define a normal GC counterpart for ABC-DLBCL.[58] Also, we have recently described FOXP2 coexpression and coimmunoprecipitation with FOXP1 in DLBCL and its association with inferior survival and immune response signatures.[88] We are currently investigating potential redundancy between FOXP1, FOXP2, and FOXP4 in DLBCL.

It is important to understand why there have been discrepancies in reports as to whether FOXP1 depletion affects DLBCL cell growth or survival, even when using the same cell lines for example U-2932 and S-UDHL-6.[44,78] Studies should consider that U-2932 contains two clones from the original patient (R1 and R2) displaying differential FOXP1 isoform profiles and expression of B-cell differentiation molecules,[58] and variability between studies may reflect differing



**Figure 2.** Variable effect of acute FOXP1 depletion on DLBCL cell line viability. DLBCL cell lines DB, Karpas-422 (GC-DLBCL) and RIVA, HBL-1, OCI-Ly3 (ABC-DLBCL) were maintained in RPMI media supplemented with L-glutamine and 10% fetal bovine serum. They were subjected to electroporation (Amaxa, Slough, UK) in the presence of 1  $\mu$ M control (siCON) or siRNA oligonucleotides depleting all FOXP1 isoforms (siFOX P1). Viability was determined by MTS assay (Promega, Southampton, UK) 72 h after transfection according to the manufacturer's instructions. Each bar represents mean  $\pm$  SD of triplicate biological samples within a single experiment, and numbers above represent overall percentage viability  $\pm$  SD in FOX P1 depleted relative to control cultures. When FOXP1 expression was determined by JC12 Western blotting, effective FOXP1 depletion was confirmed in all cell lines (data not shown).

proportions of these clones. In contrast, OCI-Ly10 cell viability and proliferation is reliably unaffected by FOXP1 silencing, indicating that not all ABC-DLBCL cell lines are FOXP1 dependent.[43,78] This might be explained by FOXP1/FOXP2 redundancy and strong FOXP2 expression in OCI-Ly10, [88] although another strongly FOXP2<sup>+</sup> ABC-DLBCL cell line, RIVA, is partially responsive to FOXP1 silencing (Figure 2). In our experience efficient siRNA-mediated FOXP1 depletion from DLBCL cells is highly reproducible, but reductions in cell viability considerably less so (Figure 2). However, regulation of immune response signatures and MHC class II expression suggest that important FOXP1-depletion phenotypes such as improved immune surveillance may be successfully studied *in vivo* even when FOXP1 silencing *in vitro* has variable effects on viability.

The importance of FOXP1 extends beyond lymphoma, and we proposed initially that FOXP1 was a tumor suppressor in epithelial malignancies.[2] Loss of tumoral FOXP1 expression correlates with poor prognosis in breast cancer,[55] and in neuroblastoma where it regulates tumor cell proliferation, viability and colony formation in soft agar,[89] while in prostate cancer, FOXP1 inhibits cell proliferation and migration.[90] However, oncogenic FOXP1 activities in epithelial malignancies are emerging including regulation of cancer stem cell-like characteristics in ovarian cancer,[91] and promotion of cell survival in colon cancer

via modulating FOXO transcription factor activity.[92] Interestingly, in this colon cancer model system, FOXP1 depletion impaired cell growth only after FOXO3 activation.[92] Thus, specific cooperating events, including activation of other transcription factors, can enable certain aspects of FOXP1 functionality. This could explain why *Foxp1* overexpression alone was insufficient to cause lymphomagenesis in mice.[77] Furthermore, studies of *FOXP1* translocation in sequential lymphoma biopsies have raised important questions concerning the role of FOXP1 in disease initiation versus progression.[60] In MALT lymphomas, translocations affecting *MALT1* and *BCL10* maintain NF- $\kappa$ B activation independently of *Helicobacter pylori*-induced inflammation,[93] and *FOXP1* translocations may reinforce a distinct stage of differentiation and/or key biological pathways in DLBCL. FOXP1 expression within the tumor microenvironment may also play critical roles in cancer biology, upregulated *Foxp1* expression in T cells having been linked to their unresponsiveness in advanced ovarian and breast tumors.[94] Furthermore, expression of *Foxp1* in endothelial cells regulates neovascularization and its depletion inhibits angiogenic phenotypes such as proliferation, tube formation, and migration.[95]

In DLBCL, FOXP1 and its target genes contribute to both GCB and ABC signatures and it is likely that FOXP1<sub>L</sub> and FOXP1<sub>S</sub> proteins, and also the related FOXP2 transcription factor have oncogenic roles.



Understanding differences between FOXP1 expression and function in normal tissues and DLBCL cells will be critical to identify novel FOXP pathway-based therapeutic strategies in DLBCL.

## Acknowledgements

We thank patients who have contributed to our studies and collaborators who have provided reagents and useful discussions. In particular, we are indebted to the late Dr. Phil Brown whose work contributed significantly to our understanding of FOXP1 biology.

**Potential conflict of interest:** Disclosure forms provided by the authors are available with the full text of this article at <http://dx.doi.org/10.1080/10428194.2016.1228932>.

## Funding

DMG is supported by a Bloodwise Specialist Program grant, [13047], awarded to AHB.

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