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REVIEW ARTICLE

Wnt signalling pathways in chronic lymphocytic leukaemia and B-cell lymphomas

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In this review, we discuss the intricate roles of the Wnt signalling network in the development and progression of mature B-cell-derived haematological malignancies, with a focus on chronic lymphocytic leukaemia (CLL) and related B-cell lymphomas. We review the current literature and highlight the differences between the β-catenin-dependent and -independent branches of Wnt signalling. Special attention is paid to the role of the non-canonical Wnt/planar cell polarity (PCP) pathway, mediated by the Wnt-5–receptor tyrosine kinase-like orphan receptor (ROR1)–Dishevelled signalling axis in CLL. This is mainly because the Wnt/PCP co-receptor ROR1 was found to be overexpressed in CLL and the Wnt/PCP pathway contributes to numerous aspects of CLL pathogenesis. We also discuss the possibilities of therapeutically targeting the Wnt signalling pathways as an approach to disrupt the crucial interaction between malignant cells and their micro-environment. We also advocate the need for research in this direction for other lymphomas, namely, diffuse large B-cell lymphoma, Hodgkin lymphoma, mantle cell lymphoma, Burkitt lymphoma and follicular lymphoma where the Wnt signalling pathway probably plays a similar role.

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Abbreviations

APC, adenomatous polyposis coli; BL, Burkitt lymphoma; BM, bone marrow; CAR, chimeric antigen receptor; *CCND1*, cyclin D1 gene; CELSR, cadherin EGF laminin G seven-pass G-type receptor; CK, casein kinase; CLL, chronic lymphocytic leukaemia; *CSNK1E*, casein kinase ε gene; *CTNNB1*, β-catenin gene; CYLD, cylindromatosis protein; DKK, Dickkopf protein; DLBCL, diffuse large B-cell lymphoma; DVL, Dishevelled; EA, ethacrynic acid; FL, follicular lymphoma; FZD, Frizzled; GC, germinal centre; GSK-3β, glycogen synthase kinase-3 β; HL, Hodgkin lymphoma; HSC, haematopoietic stem cell; IGHV, immunoglobulin heavy chain; LEF, lymphoid enhancer-binding factor; LRP, LDL receptor-related protein; MCL, mantle cell lymphoma; M-CLL/U-CLL, mutated/unmutated immunoglobulin heavy chain status of chronic lymphocytic leukaemia patients; OS, overall survival; PB, peripheral blood; PCP, planar cell polarity; PI3K, phosphoinositide 3-kinase; PORCN, porcupine protein; PRICKLE, prickle-like protein; RAC1, Ras-related C3 botulinum toxin substrate 1; ROR, receptor tyrosine kinase-like orphan receptor; RS, Richter syndrome; RYK, tyrosine-protein kinase; sFRP, secreted frizzled-related protein; TFS, therapy-free survival; VANGL, Vang-like protein

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Introduction

Wnt signalling activity is tightly regulated in time and space and has been considered a cornerstone of mammalian embryonic development as well as adult tissue homeostasis. Wnt signalling is now seen more as a network of interacting pathways instead of a linear signal transduction (Kestler and Kühl, 2008). This can be illustrated by the number and heterogeneity of the processes controlled by the Wnt pathway. These include not only a balance between stemness and cell differentiation, cell-cycle regulation, proliferation and apoptosis but also cytoskeletal rearrangement, cell and tissue polarity, cell adhesion and motility, directed migration and invasion and overall interaction with the micro-environment (Clevers, 2006; Seifert and Mlodzik, 2007). These key developmental processes are important for the normal physiological function of adult tissues and, therefore, commonly impaired in various diseases, including cancer (Clevers and Nusse, 2012).

The Wnt signalling network has been linked to the haematopoiesis mainly *via* its role in the biology of haematopoietic stem cells (HSCs) (Staal *et al.*, 2008; Malhotra and Kincade, 2009; Lento *et al.*, 2013; Staal *et al.*, 2016b). Consequently, the Wnt signalling pathway was shown to be crucial for the leukaemogenesis of malignancies originating from HSCs – namely, acute myeloid leukaemia, acute lymphoid leukaemia and chronic myeloid leukaemia – and these connections have been reviewed extensively (Luis *et al.*, 2012; Laranjeira and Yang, 2016; Staal *et al.*, 2016a). However, not all the principles described in the context of HSCs can be transferred to mature B-cells and their transformed counterparts. Thus, in this review we focus on the role of the Wnt signalling cascade in the mature B-cell-derived haematological malignancies – mainly chronic lymphocytic leukaemia (CLL). In these cells, β-catenin-independent signalling, predominantly the Wnt/planar cell polarity (PCP) pathway, has a prominent role – due to the important function of the Wnt/PCP co-receptor **receptor tyrosine kinase like orphan receptor 1** (ROR1)-driven signalling in CLL (Baskar *et al.*, 2008; Daneshmanesh *et al.*, 2008; Fukuda *et al.*, 2008). Wherever possible, we will extend the findings from CLL to other malignancies originating from the various stages of mature B-cell development, to provide a perspective on the so far poorly characterized links between Wnt signalling and other lymphomas, such as diffuse large B-cell lymphoma (DLBCL), Hodgkin lymphoma (HL), mantle cell lymphoma (MCL), Burkitt lymphoma (BL) and follicular lymphoma (FL) (Ott and Rosenwald, 2008; Frick *et al.*, 2012; Kuppers *et al.*, 2012; Vogt *et al.*, 2017). These diseases are connected by having similar molecular mechanisms involved in their pathogenesis, are clearly dependent on their micro-environment and cell–cell interactions, which suggest analogous roles of the Wnt signalling pathways.

Chronic lymphocytic leukaemia and related lymphomas

Chronic lymphocytic leukaemia – aetiology and treatment

CLL is a lymphoproliferative disease characterized by a progressive accumulation of mature non-functional $CD5^+$ B cells in the peripheral blood (PB), lymphoid tissue and bone marrow (BM). CLL is the most common adult leukaemia in Western countries; with a median age of diagnosis of 67–72 years and an overall incidence of 4–5/100 000 per year in the USA and Europe, which rapidly rises to >30/100 000 above the age of 80 years (Sant *et al.*, 2010; Hallek, 2015). The course of the disease is highly heterogeneous – while some CLL patients remain asymptomatic, others develop an active disease with one or more symptoms requiring therapy, that is, massive lymphadenopathy, BM failure manifested by anaemia and/or thrombocytopaenia and constitutional symptoms (Hallek, 2015). Among the biological markers used for clinical evaluation of patient prognosis, the mutational status of the immunoglobulin heavy chain (IGHV) variable region and recurrent cytogenetic aberrations [del(13q), trisomy 12, del(11q) or del(17p)] were shown to reliably predict the survival of patients with CLL (Delgado *et al.*, 2017). The presence of mutations in *TP53*, *MYD88*, *SF3B1*, *BIRC3* or *NOTCH1* and other genes further help not only to assess the prognosis of patients, but also to understand the biology of the disease and its dependence on different cell-signalling pathways (Lazarian *et al.*, 2017).

CLL patients are typically not treated unless/until they suffer from an aggressive form of the disease. Treatment options involve immunochemotherapy (typically fludarabine/cyclophosphamide/rituximab – regimen) and, more recently, also novel inhibitors that target pro-survival B-cell receptor or anti-apoptotic B-cell lymphoma 2 (BCL2) signalling (Jamroziak *et al.*, 2017). Despite the fact that new treatment options have significantly improved patient response, this therapy needs to be mostly infinite to prevent relapse (Burger *et al.*, 2016; Jain *et al.*, 2017). CLL is thus still considered incurable. This creates a real need for new therapeutic agents that could target the disease on a different signalling pathway to decrease the chance of emergence of a more aggressive and resistant clone.

Richter syndrome and similarities between CLL and lymphomas

CLL patients can develop so-called Richter syndrome (RS), which is when the disease transforms into a high-grade lymphoma (Rossi and Gaidano, 2016), most commonly to DLBCL (2–7% of CLL patients in clinical trials) and HL (0.4–0.7% of CLL patients) (Mauro *et al.*, 2017). An analysis of IGHV-D-J genes revealed that 80% of the DLBCL-RS are clonally related to the preceding CLL phase, while this is true for only 40–50% of HL-RS. These findings indicate that RS is largely an actual transformation of the disease, while the others represent the *de novo* development of lymphoma alongside the CLL clone. The RS prognosis is also highly unfavourable due to the presence of genetic lesions in *TP53*, *NOTCH1*, *MYC* or *CDKN2A*, connected to chemoresistance and rapid disease progression, which are present in 90% of these patients (Rossi and Gaidano, 2016). Transformation of CLL to RS supports the hypothesis that CLL is functionally related to mature B-cell lymphomas on a molecular level and findings from the field of lymphomas should also be considered in CLL pathogenesis and *vice versa*. This standpoint is also applied in this review. The main features of lymphomas and CLL are summarized in Table 1.

Table 1

The main features of lymphomas in comparison with CLL

Leukaemia/lymphoma type	Main features	Origin	Disease-initiating genetic aberration	Reference
CLL	Infiltration of BM and lymphoid organs, high degree of cell migration – recirculation and proliferation in typical pseudofolicles in the lymph nodes	Small mature B cells	—	Hallek *et al.* (2015)
HL	Disseminated lymphoma cells: peripheral lymph nodes, liver, lung and BM	Transformed GC B cells	—	Kuppers *et al.* (2012)
MCL	Systemic dissemination	Small mature B cells	t(11;14), CCND1 translocation	Vogt *et al.* (2017)
DLBCL	Large B cells with a high proliferation index resembling that of germinal centroblasts	B cells in various stage of GC reaction	t(14;18) ectopic expression of BCL2 (45% GC B cell-DLBCL, but not inactivated B cell-DLBCL)	Frick *et al.* (2012)
BL	Actively proliferating differentiating lymphocytes, often detected at site of origin	Transformed GC B cells	MYC locus translocation	Frick *et al.* (2012)
FL	Proliferation of neoplastic GC B cells, with at least a partial follicular pattern	Transformed GC B cells	t(14;18), ectopic expression of BCL2	Ott and Rosenwald (2008)

Wnt signalling pathways

Wnt proteins can drive several distinct pathways that are typically divided into β-catenin-dependent (Wnt/β-catenin) and independent branches. Often, when the Wnt signalling pathway is discussed in the literature in association with leukaemia and lymphoma, the authors refer to the Wnt/β-catenin pathway (Lu *et al.*, 2004; Peiffer *et al.*, 2014; Wang *et al.*, 2014). However, activation of β-catenin-dependent and independent signalling cascades have different functional consequences even though they share several signalling proteins (Figure 1). In this review, we thus distinguish, wherever possible, between the two to avoid confusion and provide a clearer implication.

Wnt/β-catenin pathway

The Wnt/β-catenin pathway has been closely connected to cell proliferation, cell-cycle regulation and stem-cell homeostasis, and therefore, its malfunction is a hallmark of many cancers (Clevers and Nusse, 2012). The pathway (Figure 1, on the left) is activated upon the binding of ligands – Wnt proteins (typical ligands: **Wnt-1**, **Wnt-3**, **Wnt-3a**, **Wnt-8b**, **Wnt-10b** and **Wnt-16**) – to the dedicated receptors and co-receptors – **Class Frizzled** (FZD) and LDL receptor-related protein (LRP) 5/6 (MacDonald *et al.*, 2009). Ligand binding triggers the formation of a complex on the cell surface, which activates cytoplasmic effector proteins Dishevelled (DVL) and **casein kinase 1** (CK1). This leads to destabilization of the AXIN/glycogen synthase kinase-3 β (**GSK-3β**)/adenomatous polyposis coli (APC) destruction complex that is in the absence of the ligand responsible for **β-catenin** degradation by proteasome. Consequently, cytoplasmic β-catenin accumulates and is transported to the nucleus, where it can bind T-cell-specific /lymphoid enhancer-binding factor (LEF) transcription factors and activate the transcription of target genes (*CCND1*, encoding cyclin D1; *MYC*; ***DKK1***, encoding Dickkopf 1; *AXIN2*; and many more).

β-Catenin-independent Wnt signalling – Wnt/PCP pathway

This signalling branch itself contains several pathways that do not require β-catenin stabilization. For the purpose of the review, the Wnt/PCP pathway (Butler and Wallingford, 2017) is the most important. Other β-catenin-independent pathways will not be discussed here, and we refer the interested readers to one of the recent reviews (Semenov *et al.*, 2007; Kestler and Kühl, 2008; van Amerongen, 2012). Compared with the β-catenin-dependent pathway, this signalling network is significantly less explored, and the current understanding of the function of this pathway's components and regulation mechanisms is limited.

The Wnt/PCP pathway (Figure 1, on the right) is implicated in the regulation of cell polarity, migration and invasion (Seifert and Mlodzik, 2007; Butler and Wallingford, 2017). Wnt/PCP is crucial mainly in embryonic development, directing the processes of convergent extension, neurulation or axon guidance. In mammals, the Wnt factors

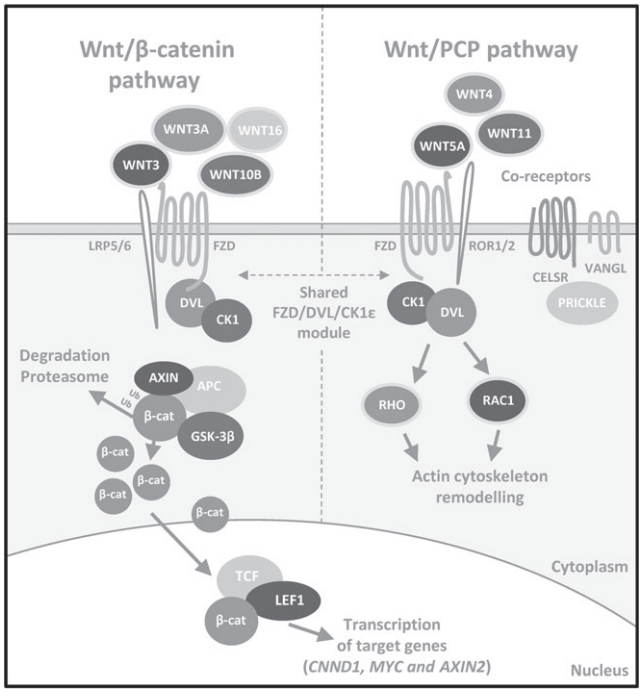

Figure 1

Simplified overview of mammalian Wnt/β-catenin and Wnt/PCP pathways. The Wnt/β-catenin signalling pathway (on the left) is activated upon binding of Wnt proteins (typical ligands Wnt-3, Wnt-3a, Wnt-10b and Wnt-16 are indicated) to dedicated receptors and co-receptors FZD and LRP5/6. This leads to activation of cytoplasmic effector proteins from the DVL family that are phosphorylated by CK1ε, destabilizing the AXIN/GSK-3β/APC destruction complex, normally responsible for β-catenin degradation by proteasome. Cytoplasmic β-catenin accumulates and transports to the nucleus, where it binds TCF/LEF transcription factors and activates transcription of target genes (such as *CCND1*, *MYC* or *AXIN2*). The Wnt/PCP pathway (on the right) is activated by a different set of ligands – typically Wnt-4, Wnt-5a, Wnt-5b and Wnt-11. The Wnt/PCP pathway shares a module composed of FZD, DVL and CK1ε with the Wnt/β-catenin pathway but also contains numerous other transmembrane proteins – ROR1/2, VANGL1/2 and CELSR1–3 (and others not shown on this scheme) and cytoplasmic effectors from the PRICKLE family dedicated to the Wnt/PCP pathway only. This receptor complex conveys the signal downstream *via* a poorly known mechanism involving small G proteins Rho and/or Rac1 and their effectors that remodel the actin cytoskeleton.

able to activate this signalling branch, typically **Wnt-4**, **Wnt-5a/b** or **Wnt-11**, bind to FZD receptors and co-receptors [family of receptor tyrosine pseudokinases – ROR1, **ROR2**, **tyrosine-protein kinase (RYK)** and **protein tyrosine kinase 7**(CCK4)], which transduce the signal. The other core Wnt/PCP components include transmembrane proteins Vang-like protein (VANGL)1/2 and cadherin EGF laminin G seven-pass G-type receptor (CELSR)1–3, cytoplasmic effectors prickle-like protein (PRICKLE)1–4, CK1ε/δ or DVL1–3 and small G proteins RHO/**Ras-related C3 botulinum toxin substrate 1 (Rac1)**. Activation of Rho and/or Rac1 *via* their effectors **ROCK (Rho-associated protein kinase)** and **JNK** leads to the actin cytoskeleton remodelling (Schlessinger *et al.*, 2009).

The role of Wnt signalling pathways in CLL pathogenesis

The Wnt signalling pathway was suggested to be involved in the pathogenesis of CLL (Lu *et al.*, 2004; Gutierrez *et al.*, 2010; Kaucka *et al.*, 2013; Wang *et al.*, 2014; Yu *et al.*, 2016). However, so far, no consensus has been reached about the importance of individual Wnt branches in different physiological processes and cell–cell interactions controlling CLL pathogenesis and CLL response to treatment. The expression of Wnt signalling molecules from both the Wnt/β-catenin and Wnt/PCP pathways (summarized in Table 2 including references) is defective in CLL, but their role in CLL often remains elusive. Below, we describe in detail the reported connections between CLL and the Wnt signalling pathways.

ROR1 – a key player in CLL

ROR1 is a co-receptor acting in the Wnt/PCP pathway. It is a Wnt-5-dedicated receptor that was found to be expressed on the surface of CLL cells and not on mature healthy B-cells (Baskar *et al.*, 2008; Daneshmanesh *et al.*, 2008; Fukuda *et al.*, 2008), with the exception of a subset of non-neoplastic B-cell precursors in the BM, so-called hematogones (Broome *et al.*, 2011). ROR1 can be used as a sensitive marker to analyse residual disease in CLL patients in remission (Kotaskova *et al.*, 2016). Until recently, its mRNA expression and presence on the cell surface of CLL cells was described as uniform; however, a study performed on a larger patient dataset showed that high ROR1 surface levels might distinguish patients with a more aggressive course of the disease (Cui *et al.*, 2016). Due to its unique expression pattern, ROR1 represents an important therapeutic feature for targeting CLL cells, even though the novel data show that some CLL patients may be ROR1-negative (5% of analysed samples) (Cui *et al.*, 2016). Hence, these ROR1-directed therapies may not be applicable for all cases of CLL.

Wnt/PCP pathway governs polarity and migration in CLL cells

Earlier studies proposed that ROR1 might not be the only Wnt/PCP component up-regulated in CLL (Mahadevan *et al.*, 2009; Kotaskova *et al.*, 2010), and currently, this has been ahown to be the case for many other Wnt/PCP pathway components. These include the typical ligands Wnt-5a and Wnt-5b (Lu *et al.*, 2004; Memarian *et al.*, 2009; Janovska *et al.*, 2016), receptors (**FZD_3** and FZD_7, VANGL2 and CELSR1) as well as cytoplasmic effectors (DVL2/3, CK1ε and PRICKLE1) (Kaucka *et al.*, 2013; Khan *et al.*, 2016). High expression of $FZD_{3/7}$, PRICKLE1 and Wnt-5a/b is also associated with a shorter therapy-free survival (TFS) and poor prognosis in CLL patients (Kaucka *et al.*, 2013; Janovska *et al.*, 2016). Functional experiments confirmed the importance of the Wnt/PCP signalling in the pathogenesis of CLL: genetic experiments in mice showed that **FZD_6**, a receptor dedicated to β-catenin-independent Wnt pathway (Golan *et al.*, 2004; Wang *et al.*, 2010), is crucial for the leukaemogenesis in the Eμ-TCL1 CLL mouse model (Bichi *et al.*, 2002; Wu *et al.*, 2009), and the overexpression of ROR1 in the same model leads to earlier disease development (Widhopf *et al.*, 2014).

Table 2

Changes in the expression of Wnt pathway components in CLL

Wnt components		Note	References
Upregulated	ROR1	Wnt/PCP co-receptor Surface marker expressed on CLL B cells Not present on normal mature B cells Described role in CLL cell migration and proliferation	Baskar *et al.* (2008), Daneshmanesh *et al.* (2008), Fukuda *et al.* (2008), Broome *et al.* (2011), Kaucka *et al.* (2013), Cui *et al.* (2016) and Yu *et al.* (2016)
	Wnt-5a and Wnt-5b	High Wnt-5a/b expression associates with adverse prognosis in CLL ROR1 ligands Regulation of CLL cell chemotaxis and proliferation Expression varies in PB/tonsilar B-cell subsets	Kaucka *et al.* (2013), Janovska *et al.* (2016) and Yu *et al.* (2016)
	Wnt-3, Wnt-10a and Wnt-16	Wnt-3 is among the most overexpressed genes in CLL compared with normal B cells Significantly lower Wnt-3 expression in U-CLL Low expression Wnt-3 = independent marker of short TFS in M-CLL Expression varies in PB/tonsilar B-cell subsets	Rosenwald *et al.* (2001), Lu *et al.* (2004), Memarian *et al.* (2009) and Poppova *et al.* (2016)
	LEF1	Absent in normal mature PB/GC B cells LEF1 silencing in primary CLL cells induces cell death Up-regulated in monoclonal B-cell lymphocytosis and CLL High expression associated with adverse prognosis	Lu *et al.* (2004), Memarian *et al.* (2009), Rosenwald *et al.* (2001) and Gutierrez *et al.* (2010)
	DVL1/2/3	Cytosolic components of both Wnt/β-catenin and Wnt/PCP pathways	Kaucka *et al.* (2013) and Khan *et al.* (2016)
	CSNK1E, $FZD_{3/7}$, PRICKLE1, CELSR1 and VANGL2	Wnt/PCP pathway components up-regulated in CLL compared with normal PB B cells $FZD_{3/7}$ and PRICKLE1 high expression associates with adverse prognosis in CLL	Kaucka *et al.* (2013)
Downregulated	DKKs and SFRPs	Soluble inhibitors of Wnt signalling Often epigenetically silenced in CLL	Seeliger *et al.* (2009), Moskalev *et al.* (2012) and Pei *et al.* (2012)

The Wnt/PCP pathway controls cell polarity (Butler and Wallingford, 2017), and there is evidence that the pathway components (VANGL, ROR and DVL) can also be expressed in a polarized manner in the migrating CLL cells, namely, in the CLL-derived MEC-1 cell line (Kaucka *et al.*, 2015) (schematized in Figure 2). It was demonstrated that the Wnt/PCP proteins control chemotactic responses and cell homing in CLL (Kaucka *et al.*, 2013), which are processes that require B-cell polarization (Parameswaran *et al.*, 2011). The migration capacity of primary CLL cells in the CXCL12 gradient is increased after activation of the pathway by recombinant Wnt-5a, an effect that can be blocked by anti-ROR1 treatment (Kaucka *et al.*, 2013; Yu *et al.*, 2016) and is dependent on the activity of Rho activity, not Rac1 (Kaucka *et al.*, 2013; Hofbauer *et al.*, 2014; Yu *et al.*, 2016). This is in line with the importance of Rho-dependent signalling in CLL cell migration that was recognized earlier (Sanchez-Aguilera *et al.*, 2010; Troeger *et al.*, 2012). In a cohort of patients characterized by the autocrine production of Wnt-5a, cell migration and motility of CLL cells was affected *in vitro* (Janovska *et al.*, 2016). These patients showed an aggressive disease course and were mostly from the unmutated IGHV (U-CLL) subgroup.

Another line of evidence comes from *in vivo* studies in mice. The homing of CLL cells *in vivo* can be blocked by

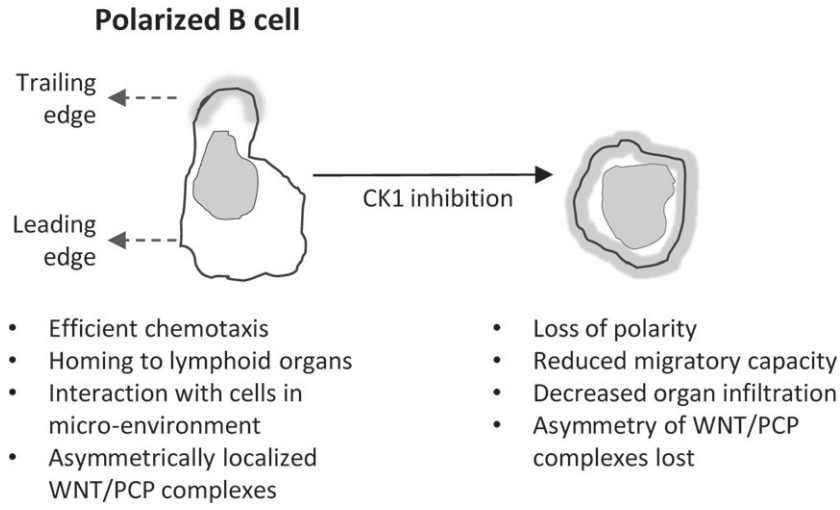

Figure 2

Impact of CK1 inhibition on CLL lymphocytes. Normal morphology of a polarized migrating lymphocyte with clearly distinguishable leading and trailing edge is shown. Wnt/PCP proteins were shown to localize asymmetrically, which can be best demonstrated by trailing edge-specific distribution of VANGL2 (shown in grey). CK1 inhibitor treatment results in B-cell polarity loss. As a consequence, CLL cells are unable to migrate, respond to micro-environmental stimuli or home into distant organs.

inhibition at the level of the Wnt/PCP receptors – ROR1 (Kaucka *et al.*, 2013; Widhopf *et al.*, 2014; Yu *et al.*, 2016) and FZD7 (Kaucka *et al.*, 2013) – as well as downstream at the level of the cytoplasmic effector CK1 (Kaucka *et al.*, 2013). This was shown in numerous experimental models – xenograft of primary CLL cells to immunodeficient NOD/SCID IL2Rγ-null (NSG) mice (Kaucka *et al.*, 2013), adoptive transfer of leukemic splenocytes to ROR1 Tg Eμ-TCL1 mice (Widhopf *et al.*, 2014) or engraftment of MEC-1–ROR1 cell line to Rag2$^{-/-}\gamma_c^{-/-}$ mice (Yu *et al.*, 2016). In addition to the well-defined effects on migration, signalling through Wnt-5a/ROR1/Rac1 (Yu *et al.*, 2016; 2017) and Wnt-5a/ROR1/PI3K/Akt (Daneshmanesh *et al.*, 2015; Cui *et al.*, 2016) pathways has been proposed to have pro-survival and pro-proliferative effects in CLL, which is similar to the ROR1 function in other cancers such as melanoma or breast cancer (Zhang *et al.*, 2012; Fernandez *et al.*, 2016). Future work will determine what the dominant mode of action of ROR1 is in CLL.

Overexpression of Wnts associated with the Wnt/β-catenin pathway in CLL cells

The involvement of the Wnt/β-catenin pathway in CLL was postulated based on the high expression of genes encoding ligands able to activate Wnt/β-catenin signalling in other systems – mainly *WNT3* but also *WNT10A* and *WNT16* (Rosenwald *et al.*, 2001; Lu *et al.*, 2004; Memarian *et al.*, 2009). *WNT3* is among the most up-regulated genes in CLL, and this fact has long been considered one of the strongest arguments supporting an active role of the Wnt/β-catenin pathway in CLL. A recent study performed a detailed analysis of the expression of its ligands in a cohort of 137 patients and correlated the results with the clinical information available (Poppova *et al.*, 2016). This work suggested that in spite of the very high levels of expression of *WNT3* in CLL cells, this was not associated with an aggressive form of this disease. The expression of *WNT3* was significantly lower in U-CLL patients, and moreover, low *WNT3* expression could be used as an independent marker to identify patients with short TFS in the generally indolent subgroup with mutated IGHV (M-CLL). In addition, this study showed that a reduced expression of *WNT3* accompanies the onset of disease activity within U-CLL (Poppova *et al.*, 2016). Importantly, it is currently not clear whether or not Wnt/β-catenin-associated ligands can efficiently activate downstream signalling in CLL – so far, attempts to trigger Wnt/β-catenin pathway by Wnt-3 or Wnt-3a in CLL cells have been unsuccessful (Kaucka *et al.*, 2013; Poppova *et al.*, 2016). This contrasts with the finding that direct inhibition of the β-catenin destruction complex by a GSK-3β inhibitor acting downstream was effective at activating the Wnt/β-catenin pathway (Lu *et al.*, 2004; Poppova *et al.*, 2016). In conclusion, these findings suggest that the ligands produced by CLL cells do not act in an autocrine manner but rather serve to communicate with the other cell types present in the CLL micro-environment.

Somatic mutations in genes encoding Wnt pathway components

Next-generation sequencing techniques, namely, whole-exome sequencing, have recently enabled the discovery of novel genetic aberrations in components of key signalling pathways. Among them, somatic mutations in several Wnt-related genes were demonstrated in CLL and suggested to affect activation of the Wnt pathway and, therefore, disease pathogenesis (Wang *et al.*, 2011; 2014). The authors sequenced 91 samples and discovered 15 mutations in 12 components of the Wnt pathway, totalling 14% of samples (Wang *et al.*, 2014). Interestingly, the mutations were found in genes encoding extracellular factors (*WNT1*, *WNT10*, *DKK2* and *RSPO4*), transmembrane receptors (*FZD5* and *RYK*), cytoplasmic [casein kinase ε gene (*CSNK1E*) and *PRICKLE1*] as well as

nuclear factors (*CHD8*, *BRD7*, *CREBBP* and *BCL9*). The mutations did not associate with any commonly used CLL prognostic factors (IGHV status, ZAP70 expression, age at diagnosis, clinical stage, presence of cytogenetic abnormalities, mutation rate or time to first therapy), and six of the patients were chemotherapy naïve, which ruled out their occurrence as a result of chemotherapy exposure. Functional experiments with overexpression of WT and mutant alleles in HEK293 cells showed that these mutations result in the activation (*BCL9*, *DKK2* and *RYK*), inactivation (*CSNK1E* – encoding for CK1ε, *Wnt1* and *FZD5*) or no (*FZD5* – second mutation) functional change in the Wnt/β-catenin pathway, an effect which was validated in primary CLL carrying the WT or mutated alleles of *BCL9*, *DKK2*, *RYK* and *CSNK1E*. Silencing of these genes showed that these cells were highly dependent on the mutated gene expression for cell survival. Because the study of Wang *et al*. focused on the effect of the mutations on the level of β-catenin-dependent activity in HEK293 cells and cell survival in primary CLL and normal B-cells, no data were obtained regarding the activity of the Wnt/PCP pathway, even though some of the mutated genes encode for proteins that function in either both Wnt pathways (*CSNK1E* and *DVL1*) or in the β-catenin-independent branch only (*RYK* and *PRICKLE1*).

Lymphoid enhancer-binding factor 1 (LEF1)

LEF1 is a critical transcription factor that is activated by the Wnt/β-catenin pathway and drives the expression of its target genes (Behrens *et al*., 1996; Huber *et al*., 1996). LEF1 is required in the early phases of B-cell development (Reya *et al*., 2000; Gutierrez *et al*., 2010), but there is virtually no expression of LEF1 in PB B cells or germinal centre (GC) mature B cells (Reya *et al*., 2000; Lu *et al*., 2004; Gutierrez *et al*., 2010; Kaucka *et al*., 2013). In contrast, LEF1 expression is very high in CLL and also in the pre-leukaemic state of CLL called monoclonal B-cell lymphocytosis (Gutierrez *et al*., 2010). Of note, no such difference was detected in the case of β-catenin (Gutierrez *et al*., 2010; Kaucka *et al*., 2013). Experimental silencing of *LEF1* reduced CLL cell survival (Gutierrez *et al*., 2010; Wang *et al*., 2014), but not the survival of normal CD19+ B cells (Wang *et al*., 2014), in contrast to the silencing of *CTNNB1* (encoding β-catenin) or *DVL1* that caused cell death in both cell types. Higher *LEF1* expression was also associated with adverse prognosis in CLL patients (Erdfelder *et al*., 2010; Wu *et al*., 2016). *LEF1* expression levels, among other CLL-pathogenesis-related factors including ROR1 or PI3K, were shown to decrease when the CLL cells were forced towards differentiation to plasma cells *in vitro* using phorbol myristate acetate or CpG oligodeoxynucleotide, in combination with a CD40 ligand and cytokines (Gutierrez *et al*., 2011; Ghamlouch *et al*., 2015).

One of the candidate gene targets regulated by LEF1 in the CLL context is the cylindromatosis gene (*CYLD*) (Liu *et al*., 2012). Low *CYLD* expression was associated with U-CLL status, and shorter overall survival (OS) in all major CLL cohorts, including the M-CLL subgroup. In this context, LEF1 acts as a transcriptional repressor of *CYLD* – Wu *et al*. (2014) showed that interference with LEF1 binding to DNA restored *CYLD* expression. CYLD acts as a deubiquitinase and a defect in its activity has been implied in several cancers, including CLL (Mathis *et al*., 2015). Interestingly, the $CYLD^{-/-}$ mice exhibited abnormalities in B-cell development, marked by spontaneous B-cell activation and hyperplasia in the periphery, with enlarged lymphoid organs and with cells being hyperproliferative upon stimulation *in vitro* (Jin *et al*., 2007). Overall, the data support the role of CYLD as a tumour suppressor that is directly controlled by LEF1, which acts to repress its activity.

The ambiguous role of Dishevelled proteins

Other components of the Wnt pathway up-regulated in CLL, but practically undetectable in normal B-cells, are DVL proteins (1–3) (Kaucka *et al*., 2013; Khan *et al*., 2016). This finding is interesting, because DVL proteins have been shown to play a key role in both β-catenin-dependent and -independent Wnt pathways (Gao and Chen, 2010; Bryja and Bernatík, 2014). The siRNA knockdown of various DVL isoforms lead to different outcomes; Wang *et al*. (2014) showed that *DVL1* knockdown in primary CLL cells leads to increased CLL cell death, similar to *LEF1* or *CTNNB1* silencing; however, we did not observe such effects with the DVL2 isoform in the CLL-derived cell line MEC-1 (Kaucka *et al*., 2013). However, *DVL2* silencing caused a decrease in chemotaxis in MEC-1 cells, suggesting it has a role in the Wnt/PCP pathway. Similarly, Khan *et al*. (2016) did not observe any effects on viability in the EHEB cell line (also CLL derived) after the silencing of all three DVL isoforms. DVL acts as a switch between the Wnt/β-catenin and Wnt/PCP pathway. Therefore, the recently reported alternative spliced variants of DVL in CLL cells, produced as a result of a mutated SF3B1 (common recurrent mutation in CLL, associated with poor prognosis) (Wang *et al*., 2016), is of considerable interest. The region missing in the SF3B1 mutation-associated spliced variant was shown to regulate the DVL tertiary structure and its role in the individual Wnt signalling branches (Lee *et al*., 2015; Qi *et al*., 2017).

Epigenetic silencing of Wnt inhibitory factors

Another verification for the involvement of Wnt/β-catenin activation in CLL comes from studies using the epigenetic silencing of genes encoding soluble Wnt inhibitors in CLL cells, mainly from the family of secreted frizzled-related proteins (sFRPs) or Dickkopf proteins (DKKs) (Cruciat and Niehrs, 2013), with **sFRP-1** being one of the most hypermethylated genes in CLL (Seeliger *et al*., 2009; Moskalev *et al*., 2012; Pei *et al*., 2012). However, the role of the soluble Wnt inhibitors is not straightforward: recent studies showed that both sFRP and DKK proteins can act as activators and supressors of Wnt/β-catenin or Wnt/PCP pathways (Mii and Taira, 2009; Esteve *et al*., 2011; Kagey and He, 2017). Better insight into the role of epigenetic mechanisms controlling the Wnt pathway in CLL can be obtained by detailed analysis of two recent reports that describe epigenome on large datasets of CLL primary samples and several mature B-cell populations (Kulis *et al*., 2012; Oakes *et al*., 2016). Kulis *et al*. (2012) showed that an epigenetic signature can distinguish the main CLL prognostic subgroups – U-CLL samples resembled the profiles of normal naive B cells and M-CLL related to memory B cells. The data were confirmed and expanded by Oakes *et al*. (2016), who by comparing the epigenome of mature B cells and CLL proposed that CLL cells probably derive from a continuum of B-cell maturation stages. This is of

interest because the expression of Wnt ligands (and probably also of Wnt pathway inhibitors) changes during the B-cell maturation process (see Future directions section).

Similarities between CLL and lymphomas – Wnt-biased view

Similar to CLL, the Wnt pathway was shown to be malfunctioning in HL and non-HL. These malignancies derive from various stages of B-cell maturation, and not surprisingly, their gene expression patterns differ significantly. However, several recent studies suggested a role for Wnt pathways in their disease pathophysiology, mainly *via* effects on the interaction of the lymphoma cells with their micro-environment. The complexity of the cellular composition of lymphomas makes *in vitro* studies more complicated in comparison with CLL – where typically large numbers of homogenous primary cells are available for functional analysis. In most lymphomas, the findings are limited (in contrast to CLL) to immunohistochemical staining and analysis of lymphoma-derived cell lines. For a list of Wnt components that show altered levels in lymphomas, see Table 3. The most important observations are discussed further below.

Hodgkin lymphoma

HL originates from transformed germinal centre (GC) B-cells (Hodgkin–Rees–Sternberg cells) that are rare in the lymphoma tissue (0.1–2%) and depend heavily on the interactions with their micro-environment (Kuppers *et al.*, 2012). This lymphoid malignancy involves peripheral lymph nodes and can also affect liver, lung and bone marrow. Previously, it was reported that only a small subset of primary HL samples express cytoplasmic and nuclear β-catenin and inactivated GSK-3β (Ser^9 phosphorylation) (Morrison *et al.*, 2004), even though HL-derived cell lines show high levels of β-catenin, as well as other components of the Wnt pathway (Sohlbach *et al.*, 2012). It was shown that the majority of classical HL (cHL) samples are positive for the activated form of GSK-3β (Y216 phosphorylation), normally responsible for the inhibition of Wnt/β-catenin signalling (Agostinelli *et al.*, 2017). Recently, a new insight was provided by results of studies that have functionally analysed local Wnt signalling in HL cells' interactions with the micro-environment (Linke *et al.*, 2017a,b). These highlighted the importance of the autocrine Wnt-5a/FZD_5/DVL3/RHOA signalling axis in HL cell motility, chemotaxis and adhesion to endothelial cells (Linke *et al.*, 2017b) and suggested that LEF1 and β-catenin are required for chemotaxis in HL cells and their signalling to endothelial cells (Linke *et al.*, 2017a). The HL cells were shown to produce

Table 3

Changes in the expression of Wnt pathway components in B-cell lymphomas

Lymphoma type	Wnt components	Note	Source of information
HL	LEF1	Up-regulation compared with normal B-cell subtypes Regulation of chemotaxis towards endothelial cells (ECs), adhesion to EC layers and cell invasion LEF1 and β-catenin-regulate cHL secretome – effects on ECs: promoted migration, sprouting and vascular tube formation	Linke *et al.* (2017b)
	β-catenin	Present only rarely and highly expressed in cell lines	Morrison *et al.* (2004) and Sohlbach *et al.* (2012)
	Wnt-5a	Increased Wnt-5a expression compared with normal B cells cHL cell migration, invasion and adhesion depend on autocrine Wnt-5a signalling (Wnt-5a-FZD_5-DVL3-RhoA axis)	Linke *et al.* (2017a)
MCL	CCND1	Up-regulation induced by t(11;14) Initial oncogenic event	Vogt *et al.* (2017)
	ROR1	13–93% positive cells, median 56%	Daneshmanesh *et al.* (2013)
	Nuclear β-catenin	Primary MCL staining Marker of Wnt/β-catenin activity	Gelebart *et al.* (2008)
	LEF1	Expression reported rarely (4–9% of MCL cases)	O'Malley *et al.* (2017)
DLBCL	LEF1	Up-regulated in GC-DLBCL subtype	Cubedo *et al.* (2012)
	ROR1	30–81% positive cells, median 50%	Daneshmanesh *et al.* (2013)
BL	MYC	Locus translocated to Ig enhancer elements 80% patients harbours t(8;14)	Frick *et al.* (2012)
	LEF1	Up-regulated in 'molecular BL' subtype Nuclear localization Transcriptionally active	Walther *et al.* (2013)
FL	ROR1	1–89% positive cells, median 28%	Daneshmanesh *et al.* (2013)

extracellular factors in a LEF1- and β-catenin-dependent manner, which affected the migration, sprouting and tube formation of the endothelial cells normally present in their micro-environment. Secretome analysis revealed that one of these factors is VEGF, whose high expression is associated with a shorter OS in cHL patients, emphasizing the clinical importance of these findings.

Non-Hodgkin lymphomas

Non-HLs represent a heterogeneous group with diverse pathology and several well-defined types of lymphoma. MCL is a rare but aggressive type of lymphoma, where chromosomal translocation t(11;14) is considered an initial tumourigenic event, leading to an up-regulated expression of *CCND1* and impairments in the cell-cycle (Perez-Galan *et al.*, 2011; Vogt *et al.*, 2017). BL is characterized by translocation of the *MYC* gene to one of the three immunoglobulin gene loci, leading to aberrant expression of this proto-oncogene (Frick *et al.*, 2012). FL is a common lymphoma type (Ott and Rosenwald, 2008) marked by the proliferation of the neoplastic cells with the phenotypic features of GC cells – including both centrocyte-like and centroblast-like phenotypes. DLBCL is the most common subtype of malignant lymphoma (Frick *et al.*, 2012). It is characterized by high heterogeneity with respect to clinical presentation, morphology, molecular pathogenesis and patient survival.

In general, compared with CLL, much less is known about the role of Wnt pathways in lymphoma. However, several close similarities exist suggesting the activation of common tumourigenic programmes. This fact can be illustrated by two examples: high expression levels of ROR1 and LEF1. Expression levels of both these genes are virtually negligible in mature B cells – in PB and GC (Gutierrez *et al.*, 2010; Broome *et al.*, 2011), but they are highly expressed in both CLL and lymphomas. Increased ROR1 gene expression, a hallmark of CLL, was observed in non-HLs – MCL (13–93% positive cells in analysed samples), DLBCL (30–81%) and FL (1–89%) (Barna *et al.*, 2011; Daneshmanesh *et al.*, 2013). LEF1 was recently detected at different levels in a large portion of DLBCL samples (Cubedo *et al.*, 2012). Furthermore, *LEF1* gene expression in DLBCLs was comparable with that in CLL. High *LEF1* expression was also identified as a signature gene and possible therapeutic target in the so-called 'molecular BL' subtype (Walther *et al.*, 2013). In MCL, the role of the Wnt/β-catenin pathway is still debatable due to the fact that nuclear β-catenin was detected in the samples (Gelebart *et al.*, 2008). In this case, *DVL2* siRNA silencing and sFRP1 treatment lead to a significant decrease in the proliferation of the MCL cell line and increased apoptosis in the case of knock down. *LEF1* expression was reported only in 4–9% of MCL cases (O'Malley *et al.*, 2017).

Therapeutic possibilities

The maladjusted Wnt signalling pathways in CLL and lymphomas may represent an interesting therapeutic possibility. The candidate targets in the Wnt signalling pathway are summarized in Figure 3 and described in detail below.

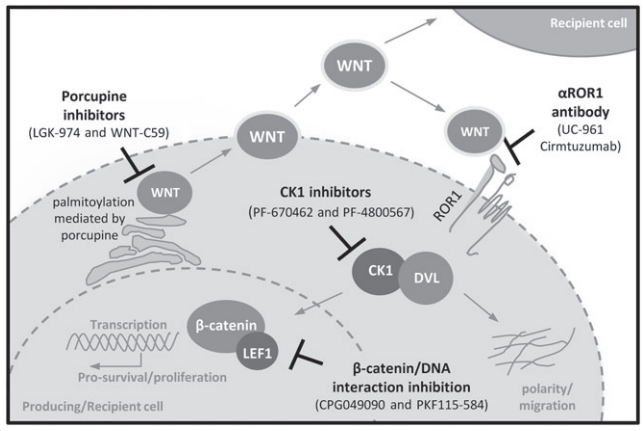

Figure 3

Possible Wnt pathway-related therapeutic targets in CLL. Scheme summarizes the candidate therapeutic targets in CLL and their position in the Wnt pathway. Examples of compounds tested in the preclinical or early phase clinical trials are shown. See text for details.

ROR1 – targeting migration and pro-survival signalling in B-cell malignancies

As mentioned earlier, ROR1 is uniquely expressed in CLL cells and also in other lymphomas. In CLL, higher cell-surface ROR1 is associated with earlier disease progression (Cui *et al.*, 2016) and ROR1 thus represents an attractive target for monoclonal antibodies (mABs)-based therapeutic interventions in aggressive CLL; ROR1 signalling can be blocked by anti-ROR1 mABs (Yang *et al.*, 2011; Kaucka *et al.*, 2013; Janovska *et al.*, 2016). Conflicting data exist as to whether antibodies against ROR1 can (Daneshmanesh *et al.*, 2012; 2015) or cannot (Yang *et al.*, 2011) induce apoptosis in CLL; a discrepancy that can probably be explained by differences in the epitopes recognized by these antibodies. The best characterized anti-ROR1 antibody (UC-961, cirmtuzumab) has undergone preclinical specificity and safety testing (Choi *et al.*, 2015) and has entered phase I clinical trials (ID: NCT02222688). It efficiently blocked Wnt-5a-induced effects such as migration and proliferation and reduced ROR1-triggered CLL development in mice (Yu *et al.*, 2016), which raises hope for its success in the clinical trials. An alternative approach that takes advantage of specific ROR1 expression in CLL, explored the possibility of targeting CLL *via* chimeric antigen receptor (CAR) T-cells that recognize ROR1 (Hudecek *et al.*, 2010). ROR1–CARs were reported to have *in vivo* activity against ROR1+ B-cell lymphoma in a xenograft mouse model (Hudecek *et al.*, 2013) and were well tolerated in preclinical safety studies performed in primates (Berger *et al.*, 2015).

Wnt proteins

Wnt proteins are modified by glycosylation and palmitoylation in order to be secreted from cells in an active form (Willert *et al.*, 2003). This lipid modification is dependent on the activity of porcupine protein (PORCN) (Kadowaki *et al.*, 1996), an enzyme that is present in the membrane of endoplasmic reticulum, and its chemical inhibition leads to disruption of Wnt protein secretion. Wnt modification *via* PORCN can be targeted by small molecules

such as Wnt-C59 and LGK-974 (Liu *et al.*, 2013; Proffitt *et al.*, 2013; Madan *et al.*, 2016), which have already shown their potential in preclinical *in vitro* and *in vivo* investigations. LGK-974 has entered phase I clinical trials to treat Wnt-dependent solid tumours (ID: NCT01351103). The inhibition of PORCN represents an approach that blocks both β-catenin-dependent and -independent Wnt pathways activated by the respective Wnt proteins produced in an autocrine/paracrine manner by tumour cells or released by other cell types into the micro-environment. This feature may be very important in diseases like CLL and B-cell lymphoma, or any metastatic cancers where the tumour progression depends on the interaction of several cell types in the BM stromal niche or lymph nodes.

LEF1

Targeting LEF1 transcription activity was suggested as a therapeutic possibility due to the fact that its inhibition by ethacrynic acid (EA) led to an increase in both primary CLL cell apoptosis and necroptosis (Wu *et al.*, 2016). EA disrupted LEF1 binding to DNA and decreased the expression of its target genes, such as *CCND1* or *MYC*. In another study, the transactivation properties of the LEF1/β-catenin complex were targeted by two small molecule inhibitors (CGP049090 and PKF115-584) that disrupt the interaction between the two proteins (Gandhirajan *et al.*, 2010). Treatment induced apoptosis of primary CLL cells, while healthy B cells were not affected, and these effects were also confirmed *in vivo* in a xenograft mouse model.

Casein kinase 1

Another candidate that could act as a therapeutic target is CK1, an enzyme that phosphorylates various targets in the Wnt pathway – mainly DVLs, VANGL2 and LEF1 (Hammerlein *et al.*, 2005; Bryja *et al.*, 2007; Gao *et al.*, 2011). An increased expression of CK1, together with other Wnt/PCP components, was demonstrated in primary CLL compared with normal PB B-cells (Kaucka *et al.*, 2013). Evidence was presented that CLL cells are sensitive to CK1 inhibition, which leads to CLL cell polarity disruption, blocked chemotaxis *in vitro* both in primary CLL cells and CLL-derived cell lines and diminished primary CLL cell homing *in vivo* in a xenograft mouse model (Kaucka *et al.*, 2013; Kaucka *et al.*, 2015). Because the cell polarity, established as a consequence of directed chemokine signalling or a polarized interaction with T-cells (Troeger *et al.*, 2012; Yuseff and Lennon-Dumenil, 2015), is crucial for the physiological function of B-cells, interference with the process *via* CK1 inhibitors could be explored as a treatment option in future. Selective CK1 inhibitors have not yet reached the stage of clinical trials; however, some compounds (PF-670462 and PF-4800567) were tested and found to be well-tolerated *in vivo* in preclinical studies (Meng *et al.*, 2010; Arey and McClung, 2012) and could be used in a wide variety of Wnt-driven cancers (Cheong and Virshup, 2016). Interestingly, a PI3Kδ inhibitor, TGR-1202, currently in the phases II and III of ongoing CLL and DLBCL clinical trials (ID: NCT02793583, NCT02612311) was shown to affect the activity of CK1ε *in vitro* (Deng *et al.*, 2017), and the upcoming results of these trials will provide important information as to whether targeting CK1 in these patients is beneficial when compared with other PI3K inhibitors.

Future directions

Studies examining the role of Wnt signalling in CLL and B-cell lymphomas reviewed here often present divergent or even contradictory findings. One of the reasons may be that the complex Wnt signalling network often tends to be oversimplified and generalized. Individual Wnt pathways might have a completely different outcome – for example, proliferation versus migration – even in the same cell type. In addition, during the complex process of B-cell maturation/pathogenesis, cells differentiate and gradually change their receptor composition and also their response to Wnt activators/inhibitors from the micro-environment.

A thorough depiction of the expression and role of the components of the Wnt pathway in B-cell differentiation and maturation provides important insights. As an example of this approach, we have summarized in Figure 4 the known expression patterns of *ROR1*, *LEF1*, *WNT3* and *WNT5A/5B* during the B-cell maturation and in the various CLL subgroups. Even this descriptive correlation between the physiological and pathological situation provides a helpful insight and indicates the importance of the dynamic regulation of Wnt signalling activity throughout the B-cell maturation process and the context-dependent switch between the Wnt/β-catenin and Wnt/PCP pathways. It also pinpoints the important, though still unknown, position of ROR1 and LEF1 – the two genes that are very highly expressed in CLL but missing in mature B cells, which either reflects their driving role in CLL or, alternatively, their high expression in the CLL cell of origin.

Another important question related to the function of Wnt signalling in CLL and lymphomas is: are malignant cells receiving or sending the Wnt signals? Are Wnts used by CLL cells to orchestrate their environment or *vice versa*? For example, currently, there is no convincing evidence that CLL cells can respond to Wnt proteins by activation of the Wnt β-catenin-dependent pathway (Kaucka *et al.*, 2013; Poppova *et al.*, 2016). CLL cells can be activated downstream by a GSK-3β inhibitor that inhibits the β-catenin destruction complex, which demonstrates the presence of functional molecular machinery (Lu *et al.*, 2004; Poppova *et al.*, 2016). This suggests that Wnt-3 produced in large quantities by CLL cells can be used to communicate with other cell types, for example, bone marrow stromal cells, which respond strongly to Wnt-3 stimulation (Poppova *et al.*, 2016). This hypothesis is in line with findings from previous studies, which showed that the effects of the CLL–stroma interaction are bidirectional (Ding *et al.*, 2009) and that the expression pattern of both Wnt-associated ligands and receptors in CLL cells changes dynamically – as illustrated in the study by Mittal *et al.* (2014) who performed gene expression profiling in a set of primary CLL samples and compared cells isolated from the PB, BM and lymph nodes (LN). The intraclonal expression changes of Wnt components in primary CLL cells, which emerged from the LN to the PB, also support the idea that the Wnt signalling is modulated in mature B cells in response to different micro-environmental stimuli (Calissano *et al.*, 2011). To determine the functional importance of these Wnt pathway changes and its relevance to the pathogenesis of CLL/lymphoma and response to therapy remains an open question for further investigation.

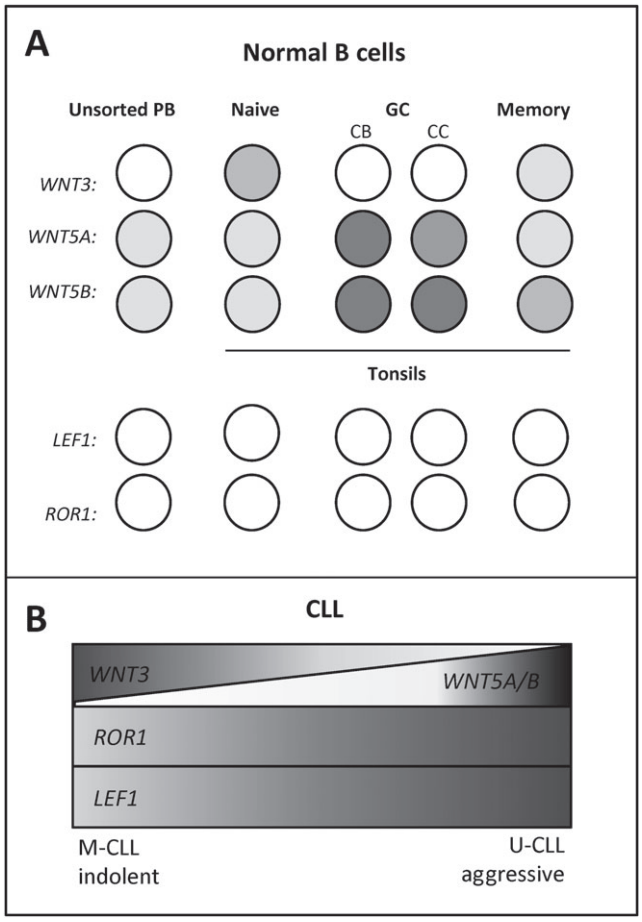

Figure 4

Similarities between CLL and normal B-cell development. (A) Expression of selected Wnt pathway components (ROR1, LEF1, Wnt-3 and Wnt-5a/b) in major mature B-cell subtypes. Wnt proteins associated with β-catenin-dependent or independent Wnt pathways are expressed differentially in mature B-cell development. CB, centroblasts, CC, centrocytes. (B) Expression of the same genes in CLL subtypes. Generally a high expression of LEF1 and ROR1 is a hallmark of CLL; however, additional level of Wnt signalling regulation may be represented by the expression of distinct Wnt proteins, which associate with different CLL subgroups. Based on (*WNT3* – Poppova *et al.*, 2016; *WNT5A/B* – Janovska *et al.*, 2016; *ROR1* – Cui *et al.*, 2016; Kaucka *et al.*, 2013; Broome *et al.*, 2011; *LEF1* – Gutierrez *et al.*, 2010; Wu *et al.*, 2016).

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015a,b,c).

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Conflict of interest

The authors declare no conflicts of interest.

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