

# New targets for lymphoma treatment

P. W. M. Johnson<sup>1</sup>

<sup>1</sup>CR UK Clinical Centre, Cancer Sciences Division, University of Southampton, Southampton, UK

The rapid development in understanding the biology of lymphoid cells has led to the identification of multiple pathways and targets for therapy in recent years. These range from cell surface interactions involving the B-cell receptor (BCR), through signalling pathways such as that mediated via the mammalian target of rapamycin (mTOR) and NF- $\kappa$ B, modulation of gene expression by histone acetylation, control of protein degradation by the proteasome or heat shock proteins, and regulation of apoptosis by both surface death receptors and intracellular proteins. In addition, a variety of strategies for driving immune responses to lymphoma have been investigated, including the use of small molecule immunomodulators, and agonistic antibodies which target immune cells rather than the malignant cells themselves. Finally, the idiotype remains an attractive target for B-cell lymphoma, being wholly patient specific, and vaccination strategies aimed at raising humoral and cellular responses are at an advanced stage of clinical study. The development of all these agents remains at an experimental stage, with none having as yet delivered clear advantages in terms of overall survival in phase III trials, although some are getting close. In many cases novel combinations are in clinical testing, with either conventional cytotoxics, monoclonal antibodies or several new agents together. A deeper understanding of their mechanisms of action should allow the rational development of such combinations in the near future.

## cell surface targets

The biology of the B-cell surface continues to yield interesting targets for therapy. Investigation of the mechanisms of action for anti-CD20 antibodies suggests strongly that the localization of antibody-bound CD20 in membrane lipid rafts has an important influence on their therapeutic effect [1]. At the same time, studies of the patterns of somatic mutation in the B-cell idiotype, in follicular lymphoma especially, have shown a very high frequency of mutations creating N-glycosylation motifs, suggesting that interaction of the surface immunoglobulin (sIg) with a specific ligand may be important in the maintenance of the cells [2]. These observations together suggest that BCR signalling in the context of membrane lipid rafts may be a potent target for novel small molecule or antibody reagents. The intracellular signalling pathways beyond the BCR depend upon the Src family kinases phosphorylating immunoreceptor

tyrosine-based activation motifs (ITAMs) on the peptides associated with the sIg. This in turn results in signalling via phosphoinositide 3-kinase (PI 3-kinase) in concert with a variety of co-receptor molecules such as CD19, CD21 and CD81. This activation process may be suppressed if the inhibitory co-receptor Fc $\gamma$ RIIB is engaged, binding the SH2 domain-containing inositol phosphatase (SHIP), which dephosphorylates the phosphatidylinositol 3,4,5-triphosphate (PIP3) produced by PI 3-kinase. The balance between these pathways suggests several potential means to influence the survival of B-lymphoma cells.

## the mammalian target of rapamycin

The mTOR protein is a key component in the pathways signalling between external conditions such as BCR activation, growth factor levels or nutrient supply, and cell growth, metabolism and angiogenesis. mTOR is upregulated by Akt following activation of PI 3-kinase, and suppressed via the AMPK system in conditions of low ATP supply. mTOR stimulates the synthesis of several proteins at the translation level through its phosphorylation of S6K1 and 4E-BP1. This in turn raises levels of cyclin-D1, driving the cell cycle. It also raises levels of glucose and amino acid transporters, increasing nutrient uptake and metabolism, and induces angiogenesis via up-regulation of hypoxia-inducible factors [3]. A variety of abnormalities may be present in lymphoma upstream of mTOR, including upregulation of p-AKT or PI 3-kinase, loss of the Akt inhibitor PTEN, and in mantle cell lymphoma the t(11;14) results in constitutive upregulation of cyclin-D1 mRNA transcription. All of these suggest that inhibition of mTOR may result in cytotoxicity or cell cycle arrest in lymphoma cells. The clearest application is in mantle cell lymphoma, where inhibition of cyclin-D1 translation through disruption of the initiation complex dependent upon mTOR can be expected to exert profound effects. Several clinical studies in lymphoma have indicated that mTOR inhibitors have activity as single agents. A phase II study in recurrent mantle cell lymphoma yielded a response in 13 of 34 subjects with recurrent lymphoma, most of whom had stage IV disease. Patients received 250 mg of temsirolimus once a week until disease progression. Most responses were seen after the first cycle of therapy and the median time to progression was 6.5 months with a median survival of 12 months [4]. Further trials are in progress now to test the possibility of reducing doses during prolonged treatment, with other mTOR inhibitors such as everolimus, and to combine them with other agents such as rituximab.

The author reports no relationships with companies whose products or services are mentioned in this manuscript.

## NF- $\kappa$ B as a target

The NF- $\kappa$ B family comprises five members: p65 (RelA), RelB, c-Rel, NF- $\kappa$ B1(p105/p50) and NF- $\kappa$ B2(p100/p52). These can form homodimers or heterodimers, and are retained in the cytoplasm in an inactive state, bound to I $\kappa$ B proteins. NF- $\kappa$ B activation can occur through two pathways: the classical (also called the canonical pathway) and the alternative (non-canonical) pathways. These lead to activation of I $\kappa$  kinase and the degradation of I $\kappa$ B proteins, resulting in the release of sequestered NF- $\kappa$ B proteins, followed by translocation into the nucleus and activation of the target genes. Activation of the canonical NF- $\kappa$ B pathway mainly releases active p50/p65 and p50/C-Rel dimers, while the alternative pathway leads to nuclear accumulation of the p52/RelB complex. Abnormal activation of NF- $\kappa$ B, for example by overexpression of upstream kinases, has been shown to contribute to lymphoma development and progression, as well as resistance of malignant cells to chemotherapy and radiation. In particular, the activated B-cell (ABC) subtype of diffuse large B-cell lymphoma, characterized by its pattern of gene expression, shows constitutive I $\kappa$  kinase activity, with accumulation of NF- $\kappa$ B proteins in the nucleus [5]. This is mediated by the CARD11/MALT1/BCL10 signalling complex which activates I $\kappa$ B kinase  $\beta$  (IKK  $\beta$ ). Recent analysis of CARD11 sequences in diffuse large B-cell lymphoma has shown the presence of activating mutations in 7 of 73 cases [6]. Several NF- $\kappa$ B-regulated proteins block programmed cell death, including members of the Bcl-2 family (BCL2, BCLXL and A1/Bfl-1) and the inhibitor of apoptosis (IAP) family (c-IAP1, c-IAP2 and XIAP). The NF- $\kappa$ B pathway also influences proliferation, which may involve target genes such as cyclin-D2 and c-myc. Production of interleukin (IL)-6 and IL-10 is induced by NF- $\kappa$ B in ABC type lymphoma, and there appears to be a positive feedback loop via JAK and STAT3 signalling. This makes these pathways attractive targets for novel therapies, and preliminary work with I $\kappa$  kinase inhibitors and JAK signalling inhibitors has shown interesting activity against ABC lymphoma *in vitro* [7]. The ubiquitous nature of NF- $\kappa$ B signalling, particularly in the immune system, may, however, make it hard to find truly selective agents.

## histone deacetylases

Histone binding to DNA suppresses gene transcription, while the acetylation of histones on their lysine residues by histone acetyltransferases opposes this effect, rendering the histones incapable of DNA binding and loosening the chromatin structure to allow efficient transcription. Deacetylation of histones results in suppression of gene expression, and is abnormally active in a variety of lymphoma types. Using inhibitors of histone deacetylases might be expected to relieve this block, and thereby restore normal patterns of gene activity in lymphoma cells. The results of de-repression with histone deacetylase (HDAC) inhibitors include increased expression of the cyclin-dependent kinase inhibitors p21 and p27, which leads in turn to upregulation of a variety of pro-apoptotic proteins such as Bax, and death receptor proteins such as DR4 and CD95, and reduced expression of the apoptosis repressors XIAP and survivin, Bcl-2 and Mcl-1 [8]. There are

more than a dozen human HDAC enzymes, which can be divided into three main classes based on structure and functional characteristics through homology to yeast HDACs. Class I and II HDACs are metalloenzymes, with a conserved catalytic domain containing a zinc atom. The class III HDACs (sirtuins) comprise a distinct set of enzymes with homology to yeast Sir2 (silent information regulator 2). These do not contain a catalytic zinc, using nicotinamide adenine dinucleotide (NAD)<sup>+</sup> as the cofactor. It is worth noting that HDAC inhibitors may also modulate several other proteins which are affected by acetylation, for example p53, NF- $\kappa$ B, heat-shock protein (Hsp) 90 and  $\beta$ -catenin [9]. A variety of HDAC inhibitor molecules have entered clinical testing, including relatively simple hydroxamates, and a variety of other molecules such as cyclic peptides or aliphatic acids. The different classes of molecule show different patterns of HDAC inhibition, with hydroxamates and bicyclic peptides relatively specific to classes I and II. In lymphoma there is most evidence for the use of vorinostat, an orally available hydroxamate, and Romidepsin, a bicyclic peptide given intravenously, in cutaneous T-cell lymphoma (CTCL). Response rates to single-agent therapy have been between 25 and 30%, with a substantial number of non-responders also showing disease stabilization for several months [10]. Phase II studies in recurrent diffuse large B-cell lymphoma have been less encouraging, but MGCD0103, an oral isotype-selective inhibitor has shown significant activity in patients with recurrent Hodgkin lymphoma [11].

## the proteasome

The proteasome is a ubiquitous enzyme complex essential to the degradation of proteins involved in cellular growth and survival, particularly cell cycle control. It modulates levels of cell cycle proteins such as the cyclins, cyclin-dependent kinases and their inhibitors p21 and p27, as well as p53. The orderly degradation of these proteins is essential to cell cycle transition. The proteasome is also central to the regulation of transcription, through its control of NF- $\kappa$ B levels. Inhibition of the proteasome can thus produce an antineoplastic effect by affecting several regulatory mechanisms including induction of apoptosis, inhibition of cell growth and survival pathways and inhibition of gene expression central to cellular adhesion, migration and angiogenesis [12]. Bortezomib is an inhibitor of the proteasome, acting by binding to the enzyme's active site. It has shown anti-lymphoma effects, probably using several different mechanisms, including inhibition of cell cycle progression, induction of apoptosis, NF- $\kappa$ B blockade and inhibition of angiogenesis. Bortezomib inhibits constitutive NF- $\kappa$ B expression and cyclin-D1 expression, and upregulates the pro-apoptotic Noxa protein, which interacts with Mcl-1 and promotes the release of pro-apoptotic Bak. The best evidence of single-agent activity is in patients with mantle cell lymphoma. Responses have been seen in ~30–40% of cases, including some heavily pre-treated individuals [13]. The most common significant toxicities are peripheral neuropathy (13%), fatigue (12%) and thrombocytopenia (11%). There are theoretical reasons to think that bortezomib may have activity against ABC-type large B-cell lymphoma as well, and the

combination of bortezomib with chemotherapy is being tested with this in mind. Further combination studies with HDAC inhibitors, apoptosis inhibitors and other targeted agents are underway, and more potent inhibitors of the proteasome such as carfilzomib are also entering the clinical domain.

### heat shock proteins

Hsp90 is a ubiquitous, evolutionarily conserved molecular chaperone, required for the stability and function of proteins implicated in cell growth, differentiation and survival. Several mutated proteins involved in lymphoma cell survival are dependent upon Hsp90 activity. In the inactive state, an adenosine triphosphate (ATP) molecule is bound to the N-terminal. When this ATP molecule is hydrolysed, conformational changes mediate a series of association–dissociation cycles between Hsp90 and substrate proteins, termed ‘client proteins’. These include receptor-type tyrosine kinases, serine–threonine kinases, signal transducer proteins, mutated oncogenic proteins, chromosome translocation products including chimeric protein kinases, steroid receptors, cyclin-dependent kinases, transcription factors and the telomerase catalytic subunit, h-TERT. Inhibitors of Hsp90 can increase degradation of such client proteins by the ubiquitin/proteasome pathway. Treatment of lymphoma cells with Hsp90 antagonists may result in cell cycle arrest, differentiation and apoptosis through client protein degradation, with malignant cells more dependent upon these than their normal counterparts, so-called oncogene addiction.

### apoptosis

The dysregulation of apoptosis is a central feature of the pathology of several lymphoma types, and many approaches have been taken to restoring the intrinsic tendency of lymphocytes to programmed cell death. Anti-sense oligonucleotides provided some proof of concept data, but more potent small molecule inhibitors the Bcl-2 family proteins are now in early phase trials, and appear to have considerable promise. The best-characterized target is the BH3 domain of the anti-apoptotic Bcl-2, Bcl-XL and Mcl-1 proteins, with several small molecule inhibitors being tested for their potential as enhancers of the cytotoxicity of conventional anti-lymphoma drugs. AT-101, a derivative of the natural compound gossypol, is one such molecule which has shown promising results *in vitro*. Obatoclax is a pan-BCL-2 inhibitor that has shown efficacy against several haematologic malignancies *in vitro*, and against chronic lymphocytic leukaemia and acute myeloid leukemia in early clinical studies. Innovative constructs such as hydrocarbon ‘staples’ inserted into native BH3 peptide sequences have been used to stabilize  $\alpha$ -helices of Bcl-2 domains (SAHBs) to explore the pharmacodynamic effects of ‘BH3 replacement’ in cancer cells and mouse models of deregulated apoptosis, although these are some way from clinical application. Another potential target in the apoptotic pathway is the survivin molecule. A recent study of 3.5 million human ‘transcriptomes’ found that survivin is one of the top four transcripts uniformly upregulated in cancer cells [14]. It is a member of the ‘Inhibitor of Apoptosis’ (IAP) family of proteins that has been implicated in both preservation of cell

viability and regulation of mitosis in tumor cells. Approaches to survivin blockade have involved both anti-sense oligonucleotides and small molecule inhibitors. One of the latter is a molecule called YM155, selected in a high-throughput screen using a surviving promoter assay, which has already shown some activity in phase I trials, and is now in a large phase II study using a 7-day continuous i.v. infusion.

### immunomodulation

Apart from antibodies, the most extensively studied immunomodulatory agents in lymphoma are the oral immunomodulatory drugs (IMiDs), thalidomide and lenalidomide. Their mechanisms of action remain uncertain, but appear to involve direct cytotoxic action in some cell types, the modulation of immunity via altered cytokine production and cellular changes both on the malignant cell and reactive T and NK cells, and the suppression of angiogenesis by downregulation of vascular endothelial growth factor. Lenalidomide has been the most extensively tested, and has shown activity against small lymphocytic, mantle cell, follicular, peripheral T-cell and diffuse large B-cell lymphoma in phase II studies [15]. The dose-limiting toxicity is myelosuppression, and an increased risk of thromboembolism has been noted in some of the myeloma trials. In general the activity seen in patients with recurrent and refractory lymphoma has been moderate, with response rates of 25–50%, but these agents hold considerable promise for both combination and maintenance use, given their oral availability.

### references

1. Cragg MS, Morgan SM, Chan HT et al. Complement-mediated lysis by anti-CD20 mAb correlates with segregation into lipid rafts. *Blood* 2003; 101: 1045–1052.
2. Zhu D, McCarthy H, Ottensmeier CH, Johnson P, Hamblin TJ, Stevenson FK. Acquisition of potential N-glycosylation sites in the immunoglobulin variable region by somatic mutation is a distinctive feature of follicular lymphoma. *Blood* 2002; 99: 2562–2568.
3. Costa LJ. Aspects of mTOR biology and the use of mTOR inhibitors in non-Hodgkin’s lymphoma. *Cancer Treatment Rev* 2007; 33: 78–84.
4. Witzig TE, Geyer SM, Ghobrial I et al. Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. *J Clin Oncol* 2005; 23: 5347–5356.
5. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. *J Exp Med* 2001; 194: 1861–1874.
6. Lenz G, Davis RE, Ngo VN et al. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. *Science* 2008; 319: 1676–1679.
7. Lam LT, Wright G, Davis RE et al. Cooperative signaling through the STAT3 and NF-(kappa)B pathways in subtypes of diffuse large B cell lymphoma. *Blood* 2008.
8. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev* 2006; 5: 769–784.
9. Peart MJ, Smyth GK, van Laar RK et al. Identification and functional significance of genes regulated by structurally different histone deacetylase inhibitors. *Proc Natl Acad Sci USA* 2005; 102: 3697–3702.
10. Duvic M, Talpur R, Ni X et al. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). *Blood* 2007; 109: 31–39.
11. Younes A, Pro B, Fanale M et al. Isotype-selective HDAC inhibitor MGCD0103 decreases serum TARC concentrations and produces clinical responses in

- heavily pretreated patients with relapsed classical Hodgkin lymphoma. *Blood* 2007; 110: 2566.
12. Rajkumar SV, Richardson PG, Hideshima T, Anderson KC. Proteasome inhibition as a novel therapeutic target in human cancer. *J Clin Oncol* 2005; 23: 630–639.
  13. Strauss SJ, Maharaj L, Hoare S et al. Bortezomib therapy in patients with relapsed or refractory lymphoma: potential correlation of in vitro sensitivity and tumor necrosis factor alpha response with clinical activity. *J Clin Oncol* 200; 24: 2105–2112.
  14. Velculescu VE, Madden SL, Zhang L et al. Analysis of human transcriptomes. *Nat Genet* 1999; 23: 387–388.
  15. Chanan-Khan AA, Cheson BD. Lenalidomide for the treatment of B-cell malignancies. *J Clin Oncol* 2008; 26: 1544–1552.