Angiogenesis, the growth of new blood vessels, requires dynamic expansion, assembly and stabilization of vascular endothelial cells in response to proangiogenic stimuli. Antiangiogenic strategies have become an important therapeutic modality for solid tumors. While many aspects of postnatal pathological angiogenesis have been extensively studied in the context of nonhematopoietic neoplasms, the precise role of these processes in lymphoma pathogenesis is under active investigation. Lymphoma growth and progression is potentiated by at least two distinct angiogenic mechanisms: autocrine stimulation of tumor cells via expression of vascular endothelial growth factor (VEGF) and VEGF receptors by lymphoma cells, as well as paracrine influences of proangiogenic tumor microenvironment on both local neovascular transformation and recruitment of circulating bone marrow-derived progenitors. Lymphoma-associated infiltrating host cells including hematopoietic monocytes, T cells and mesenchymal pericytes have increasingly been associated with the pathogenesis and prognosis of lymphoma, in part providing perivascular guidance and support to neoangiogenesis. Collectively, these distinct angiogenic mechanisms appear to be important therapeutic targets in selected non-Hodgkin’s lymphoma (NHL) subtypes.

Understanding these pathways has led to the introduction of antiangiogenic treatment strategies into the clinic where they are currently under assessment in several ongoing studies of NHL patients.

**Key words:** angiogenesis, antiangiogenic therapy, microenvironment, non-Hodgkin’s lymphoma, VEGF

**mechanisms of tumor angiogenesis**

**VEGF and the ‘angiogenic switch’**

The neoangiogenic process in cancer is critically influenced by the local tumor microenvironment [1, 2] (Figure 1). The most important mediator of the angiogenic switch is vascular endothelial growth factor (VEGF) [3, 4]. Members of the VEGF family, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF), through interactions with their receptors VEGFR-1, VEGFR2 and VEGFR3, regulate various aspects of vascular angiogenesis and lymphangiogenesis.

VEGF-A (VEGF) is produced by a variety of tumor cells as well as certain tumor-associated stromal cells [5, 6], and binds to two related receptor tyrosine kinases, namely, VEGFR-1 and VEGFR-2 [4]. VEGFR-2 is the primary receptor-transmitting mitogenic VEGF signals in endothelial cells, by activating both the Raf-Mek-Erk [7] and the phosphatidylinositol (PI)-3 kinase-Akt pathways [8]. VEGFR-1 regulates VEGF signaling on vascular endothelium in a tissue-specific manner [4, 9]. VEGFR-1 is also expressed on hematopoietic cells, where it mediates VEGF-directed monocyte chemotaxis, hematopoiesis and recruitment of endothelial progenitors [10]. Binding of VEGFR-1 to PlGF, a homolog of VEGF, can lead to intermolecular transphosphorylation of VEGFR-2, thereby amplifying VEGF-driven angiogenesis through VEGFR-2 [11]. In addition to mediating sprouting angiogenesis, VEGF-A is essential for postnatal vasculogenesis by mobilizing both VEGFR-1-hematopoietic progenitors and VEGFR-2-endothelial progenitors from the bone marrow [2]. The latter can differentiate and incorporate into the growing tumor neovasculature. VEGF-C and VEGF-D are primarily involved in lymphangiogenesis via interaction with VEGFR-3 in addition to VEGFR-2 in the adult [4]. VEGFR-3 signaling is required for both developmental and pathological tumor angiogenesis. Genetic targeting of VEGFR-3 or blockade of VEGFR-3 signaling with mAbs leads to synergistic inhibition of tumor angiogenesis when used in combination with an anti-VEGFR-2 strategy [12]. Certain tumor cells, including acute lymphocytic leukemia and lymphomas, express VEGFR-1 and VEGFR-2, which have been shown to promote survival, proliferation and metastasis via autocrine mechanisms [13–15].

VEGF gene expression is regulated by the concerted action of the transcriptional factor hypoxia-induced factor (HIF)-1 and von Hippel–Lindau (VHL) tumor suppressor gene in response to tissue hypoxia [16]. Under normoxic conditions,
Figure 1. Overview of the lymphoma vascular microenvironment. (A) Tumor cells produce VEGF-A and other angiogenic factors such as bFGF, PDGF and VEGF-C which promote neo-angiogenesis via at least two mechanisms: sprouting angiogenesis of mature resident endothelial cells and vasculogenesis from recruitment of bone marrow-derived progenitor cells. (B) VEGF-A also supports the survival, proliferation and migration of lymphoma cells which express VEGFR1 and VEGFR2 in an autocrine fashion. (C) Malignant stroma, composed of fibroblasts, inflammatory and immune cells, provides additional angiogenic factors. Tumor-associated fibroblasts produce chemokines such as SDF-1, which recruits bone marrow-derived circulating EPCs from the bone marrow [28]. It is conceivable that compounds targeting signaling pathways of both VEGF and other proangiogenic factors such as PDGF could have synergistic antivascular and antitumor activities.

mechanism of neovascular assembly

New vessel growth and maturation are highly complex and coordinated processes, requiring sequential and concerted participation of endothelial cells and perivascular accessory cells in response to proangiogenic signals. Emerging evidence suggests that bone marrow-derived cells contribute to lymphoma neoangiogenesis.

contribution of bone marrow-derived endothelial precursors. Tumor endothelial assembly is presumed to involve two principal processes. The first builds on the sprouting and cooption of preexisting vessels mediated by the migration and proliferation of mature endothelial cells. The second is the recruitment of bone marrow-derived circulating EPCs in response to proangiogenic signals [2]. The latter notion was initially proposed by Asahara et al. [29] in 1997 in the context of animal models of ischemia. Subsequently, multiple studies have addressed the roles of EPC in tumor angiogenesis in mouse models, using myeloablative transplantation techniques which facilitated the temporal and spatial tracking of genetically marked donor bone marrow cells in the recipients. Great variance of bone marrow dependence was reported, ranging from negligible [30] to moderate (1.5%–58%) [31–33], likely reflecting angiogenic responses to different tumor types, organ sites and mouse strains [33]. In angiogenesis-defective 1-mutant mice, bone marrow-derived VEGFR-2+ EPCs constituted a significant proportion of tumor neovessels (>90%) following wild-type bone marrow rescue in a murine xenograft model of aggressive B-cell lymphoma [34]. In a pilot study of six human patients with spontaneous cancers following bone marrow transplantation from opposite sex donors, an average of 4.9% bone marrow endothelial contribution was noted, with Hodgkin’s lymphoma exhibiting the greatest bone marrow component at 12.1% [35].

role of perivascular cells. In addition to endothelial cells, both hematopoietic myeloid cells and periendothelial vascular mural cells have been shown to be important participants in the neovascular assembly process by providing proangiogenic growth factors such as VEGF-A, VEGF-C/VEGF-D, BDNF, PDGF and MMP-9 [2], as well as structural guidance and support to the nascent endothelial structure [36–38].

Activated AKT has been shown to be necessary and sufficient to regulate VEGF and HIF-1 expressions.

Complementary to VEGF signaling pathways, several neoangiogenic pathways participate in the elaborate regulation of angiogenic switch. For instance, the platelet-derived growth factor (PDGF) family is indispensable for its role in vascular remodeling and maturation. PDGF-BB (a ligand of PDGFR-β) produced by endothelial cells recruits pericytes and promotes vascular stability and maturation [26]. Inhibition of PDGFR-β signaling renders tumor vasculature particularly susceptible to VEGF withdrawal [27]. Chemokine SDF-1α has been shown to promote tumor angiogenesis by recruitment of vasculogenic CXCR4+ endothelial progenitor cells (EPCs) from the bone marrow [28]. It is conceivable that compounds targeting signaling pathways of both VEGF and other proangiogenic factors such as PDGF could have synergistic antivascular and antitumor activities.
Corecruitment of wild-type VEGFR1+ proangiogenic myelomonocytic cells and their perivascular association with neovessels were essential to restore murine B6RV2 lymphoma angiogenesis in the angiogenesis-defective Id-mutant mice [34]. When the hematopoietic progenitor cells were genetically targeted and eliminated in a tumor model, neoangiogenesis and tumor growth were both strongly inhibited, underscoring their vital contribution to the bone marrow-derived proangiogenic process [30, 39]. In human subjects, tumor-associated macrophages have been implicated in aggressive disease and inferior outcome in a number of tumors, including follicular lymphoma [40, 41]. In parallel, NG2+ and α-SMA+ pericytes/vascular smooth muscle cells can be recruited and have been shown to proliferate into the interstitial stroma along with vascular endothelial cells under proangiogenic stimuli [42, 43]. Nascent blood vessels with minimal pericyte coverage have been shown to be more sensitive to VEGF inhibition. Coverage by pericytes has been proposed as one of the key events during vessel maturation [27].

Collectively, these data support the participation of bone marrow-derived cells in lymphoma angiogenesis and illustrate the complexity of angiogenic processes, whereby the degree of involvement by bone marrow-derived endothelial and hematopoietic progenitors is dependent on the context of the tumor microenvironment.

role of angiogenesis in human lymphomas

The potential importance of tumor angiogenesis in human lymphoma relates to the association of disease progression with increased angiogenic activity. Emerging evidence, analyzing both the angiogenic properties of the neoplastic cells as well as the vascular microenvironment, suggests that angiogenesis is highly relevant to a number of lymphoma subtypes.

expression of VEGF and VEGF receptors

VEGF expression by neoplastic cells has been demonstrated in aggressive subtypes of lymphoma including peripheral T-cell lymphoma (PTCL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), primary effusion lymphoma [44, 45] and indolent histologies such as chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) [46, 47]. In contrast, only a minority of indolent follicular lymphoma cases show variable expression of VEGF [48, 49]. Increased VEGF expression has been associated with areas of transformation from indolent B-cell lymphoma to aggressive DLBCL and poor prognostic subgroups within DLBCL [50]. Serum VEGF levels are also inversely correlated with event-free survival in NHL patients [51].

Both VEGF and VEGF receptors are expressed by a number of lymphoma cells, particularly CLL and aggressive NHL subtypes, implicating both autocrine and paracrine survival mechanisms (Table 1) [44, 45, 52]. Blockade of VEGFR-1 and VEGFR-2 reduces tumor growth of human lymphoma xenografts in NOD-SCID mice [52]. VEGF signaling via VEGFR-1 and VEGFR-2 in CLL cells confers resistance to apoptosis and promotes survival [53]. Disruption of this autocrine pathway in CLL by epigallocatechin-3-gallate, a receptor tyrosine kinase (RTK) inhibitor, leads to apoptosis and cell death. In DLBCL, VEGF expression was detected in 42%–60% of lymphoma cells [54]. On the basis of immunohistochemical (IHC) evaluation of tissue microarrays, Gratzinger et al. [55] reported that the degree of VEGF expression correlated with the expression level of VEGFR-1 and VEGFR-2 in de novo DLBCL lymphoma cells (non-germinal center subtypes in particular), raising the possibility of VEGF as an autocrine/paracrine growth factor for DLBCL cell survival and proliferation. The same group also reported in a separate study that the concordance of high VEGF and high VEGFR-1 expression identified a subgroup of patients with improved overall survival (OS) and progression-free survival (PFS) when treated uniformly with anthracycline-based chemotherapy (without rituximab), suggesting that autocrine signaling via VEGF-1 may be particularly susceptible to anthracycline-based therapy [56]. Expression of both VEGF and VEGF receptors has also been characterized in MCL [57–59]. These studies collectively provide a strong rationale for targeted therapy using VEGF inhibitors. In correlative studies from the SWOG S0108 trial, which examined the efficacy of single-agent bevacizumab in patients with relapsed DLBCL and MCL, analysis of a limited number of tumor samples demonstrated that VEGF-A and its receptors VEGFR1 and VEGFR2 were expressed on the tumor cells, while VEGF-R2 was mostly seen on the endothelial cells of the tumor vasculature [57]. VEGF-1 expression by MCL cells was confirmed by both IHC and quantitative PCR analysis in relapsed MCL tumors in an independent study [58].

microvessel density

Microvessel density (MVD) measures lymphoma neovascularity, which is generated in response to tumor cells, proangiogenic stromal cells and infiltrating benign T/B lymphocytes and myeloid cells within the tumor microenvironment. Therefore, MVDs vary greatly among different studies due to the heterogeneity of lymphoma stroma, the range of cell surface markers used for staining and differences in scoring methodology. The clinical predictive value of MVDs with respect to underlying lymphoma subtypes remains unclear. In general, MVD scores trend highest in aggressive subtypes including Burkitt’s lymphoma and PTCL, compared with intermediate in DLBCL and lower in indolent follicular lymphoma (FL) [60]. In DLBCL, conflicting data exist on the pathogenic association of MVDs and tumor cell VEGF expression. In a tissue microarray study of 94 cases of de novo DLBCL, Gratzinger et al. [55] reported that the overall MVDs span a wide spectrum reflecting the underlying heterogeneity of the disease, although the average MVDs did correlate with the strength of VEGF staining in a statistically significant manner. In contrast, a subsequent study of 182 patients with de novo DLBCL treated with anthracycline-based chemotherapy showed no correlation between increased MVD and lymphoma cell VEGF expression [56]. However, the investigators did conclude that increased tumor vascularity is associated with poor OS ($P = 0.047$) and is independent of the international prognostic index (IPI) score. Other studies found no correlation between...
baseline MVDs and VEGF expressions, IPI score or clinical outcome [49, 54, 61]. These studies provided a glimpse into the heterogeneity and complexity of the angiogenic processes in DLBCL.

In follicular lymphoma, it is well accepted that MVDs are significantly higher in interfollicular as opposed to intrafollicular regions. Koster et al. [48] reported that increased vascularity pretreatment predicted favorable outcome (improved PFS and OS) in 46 previously untreated FL patients who received CVP chemotherapy combined with IFN-α2b. VEGF expression was conspicuously absent in these FL cells. Jorgensen et al. studied 107 pretherapy FL cases and found that high interfollicular MVDs predicted progressive disease (PD), inferior event-free survival (EFS) and OS and correlated with transformation to DLBCL. The authors acknowledged that shorter follow-up time and nonuniform treatment regimens might account for the discrepancies in their study with regard to clinical outcome compared with the Koster study [49]. Nonetheless, both FL studies highlight the influence of host factors within the tumor microenvironment on neovascularization.

tumor microenvironment

Increasingly, the tumor microenvironment has been recognized to influence neoplastic progression and growth. In follicular
lymphoma and DLBCL, large-scale gene expression profiling studies demonstrated that genetic signatures expressed by stromal and infiltrating immune cells define distinct prognostic groups, thus identifying the analysis of the tumor microenvironment as a molecular tool for risk stratification [62, 63]. In FL, the immune response signature expressed largely by infiltrating nontumor monocytes and dendritic cells predicted inferior clinical outcome. Indeed, the content of the lymphoma-associated macrophages (LAM) has been shown to be an independent predictor of survival in follicular lymphoma [41]. High amounts of intratumoral macrophages have been shown to correlate with poor prognosis in FL patients treated with chemotherapy alone. Importantly, rituximab therapy appears to circumvent the unfavorable outcome associated with high LAM [64, 65]. The precise role of lymphoma infiltrating cells on intrafollicular and interfollicular angiogenesis remains to be defined.

In aggressive subtypes of Burkitt’s lymphoma and DLBCL, VEGF-producing CD68+ and CD133+ cells or mesenchymal (α-SMA+) cells [61]. In aggressive subtypes of Burkitt’s lymphoma and DLBCL, VEGF-producing CD68+ myelomonocytic hematopoietic cells were closely associated with neovessels, lending structural and pericrine support to nascent vasculature which was largely devoid of α-SMA+ pericyte coverage in response to rapid neoplastic growth. In contrast, the perivascular compartment in indolent CLL/SLL is marked by diffuse α-SMA+ pericytic coverage, leading to a more mature vascular composition. Thus, perivascular accessory cells in lymphoma subtypes reflect differential angiogenic responses to distinct proangiogenic growth factors/cytokine milieu within the tumor microenvironment, providing potentially unique targets for antiangiogenic intervention.

circulating endothelial progenitors

Circulating endothelial cells and VEGF levels appear to correlate with tumor volumes in SCID mice bearing human lymphoma [67]. Igreja et al. investigated the presence, differentiation potential and molecular characteristics of CD133+CD34+VEGFR-2+ EPC present in peripheral blood (CEPC) and lymph nodes (LN-EPC) in patients with NHL. They found increased CEPC in younger patients and those with aggressive lymphomas. The levels of CEPC decreased following complete response (i.e., complete disappearance of lymphomatous involvement in lymph nodes, blood and bone marrow) to treatment. In addition, LN-EPCs were detected in vascular structures and in stroma and correlated with lesion size and increased angiogenesis in indolent lymphoma [68]. The presence and relevance of circulating endothelial progenitors in NHL provides potentially useful angiogenesis-specific biomarkers to evaluate the treatment response following antiangiogenic therapy.

genetically modified lymphoma endothelial cells

Lymphoma-specific genetic aberrations have been observed in tumor vasculature. Streubel et al. [69] examined the endothelial cells in 27 B-cell lymphomas for cytogenic alterations that are known to be present in the lymphoma cells and found that an average of 37% of the microvascular endothelial cells in the B-cell lymphomas harbored lymphoma-specific chromosomal translocations. Their findings suggest that microvascular endothelial cells in B-cell lymphomas are in part tumor related and raise the provocative possibility that a proportion of the lymphoma endothelial cells may derive from a common, diseased hematopoietic stem cell/progenitors in the bone marrow.

In aggregate, these studies demonstrate the importance and clinical relevance of neoangiogenesis in the pathogenesis of lymphoproliferative disease, lending support to further exploration of angiogenic mechanisms and novel antiangiogenic therapeutics in specific lymphoma subtypes.

antiangiogenic therapy in human lymphoma

The prototypic antiangiogenic agent, namely the humanized mAb bevacizumab, targets the VEGF-A signaling axis [70–72], thereby disrupting autocrine and paracrine survival mechanisms. When used in combination with chemotherapy, bevacizumab improves PFS in humans with metastatic colorectal, renal, breast and advanced non-small-cell lung cancer [73–75]. A growing list of antiangiogenics is now available, either in various stages of clinical development or as components of standard clinical regimens. The major classes of antiangiogenic therapy include (i) direct anti-VEGF (bevacizumab, VEGF-Trap, VEGF-antisense); (ii) receptor tyrosine kinase inhibitors targeting VEGF receptor signaling as well as receptors for other pro-angiogenic factors; (iii) immunomodulatory drugs (iMiDs) with antiangiogenic properties; (iv) the novel antiendothelial approach of metronomic therapy and (v) other new compounds targeting signaling checkpoints downstream of proangiogenic growth factors, which include mammalian target of rapamycin (mTOR) inhibitors, histone deacetylases (HDAC) inhibitors and proteasome inhibitors. Encouraging preliminary clinical evidence supports the safety, feasibility and clinical efficacy of antiangiogenic therapy in various human lymphoma subtypes, including DLBCL, CLL and MCL (Table 2).

anti-VEGF strategies

In pilot studies, bevacizumab (Avastin™, Genentech and Roche) has shown modest clinical activity in lymphoma patients as a single agent in the setting of relapsed aggressive NHL [76] and has been combined with rituximab-CHOP (R-CHOP) in upfront treatment [54, 77]. Stopeck and the Southwest Oncology Group reported prolonged stabilization of disease in a significant subset (25%) of patients with relapsed aggressive NHL subtypes treated with single-agent bevacizumab at 10 mg/kg every 2 weeks on the SWOG 00108 study [76]. Ganjo et al. [54] examined the safety and clinical efficacy of combination of bevacizumab with R-CHOP in 13 patients with
<table>
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<tr>
<th>Agents</th>
<th>Disease</th>
<th>Phase</th>
<th>Sample size</th>
<th>ORR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
<th>TTP</th>
<th>PFS</th>
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<td>58% @ 6 months</td>
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<td>6.2 months</td>
<td>9.2 months (median)</td>
<td>69% @ 1 year</td>
<td>Fisher [124]</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>MCL</td>
<td>II</td>
<td>30</td>
<td>46.4</td>
<td>3.6</td>
<td>43</td>
<td>43</td>
<td>NR</td>
<td>10 months (median)</td>
<td>NR</td>
<td>Belch [125]</td>
</tr>
</tbody>
</table>

NHL, non-Hodgkin’s lymphoma; CR, complete remission; PR, partial response; SD, stable disease; TTP, time to progression; PFS, progression-free survival; OS, overall survival; VEGF, vascular endothelial growth factor; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; CLL, chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; R, rituximab; AS, anti-sense; T, thalidomide; F, fludarabine; L, lenalidomide; PEPC, prednisone, etoposide, procarbazine, cyclophosphamide; iMiDs, immunomodulatory drugs; mTORi, mammalian target of rapamycin inhibitors; HDACi, histone deacetylase inhibitors; NR, not reported; NA, not applicable, retrospective chart review study.
untreated DLBCL in an upfront setting. Bevacizumab was administered through a central line at 15 mg/kg on day 1 followed by R-CHOP on day 2 for cycle 1 and day 1 for cycles 2–8. Best response included five complete remission (CR), six partial response (PR), one stable disease (SD) and one PD. An overall response rate of 85% and a complete response rate of 38% were demonstrated with 12-month PFS of 77%.

Additionally, there appears to be a marginal correlation between the baseline plasma VEGF-A level and response to treatment. Overall the combination was well tolerated and safe. Treatment with R-CHOP–bevacizumab did not result in any episodes of grade 3 or 4 proteinuria, heart failure or hemorrhage. Currently, phase II trials of R-CHOP–bevacizumab in previously untreated DLBCL and MCL patients are underway at multiple institutions in United States, and phase III trials of R-CHOP–Bevacizumab are ongoing in Europe.

Other anti-VEGF strategies that have been used in lymphoma include VEGF-antisense and VEGF-Trap. Levine reported a phase I study of antisense oligonucleotide against VEGF-A in a small cohort of patients with malignancies and observed a PR in one patient with cutaneous T-cell lymphoma [78]. The VEGF-Trap molecule is currently undergoing a phase I evaluation in association with R-CHOP in France.

immunomodulatory drugs

iMiDs are a series of synthetic compounds that have been developed based on the glutamate-derivative backbone of the prototype drug thalidomide. Thalidomide has wide-ranging functions including antiinflammatory, immunomodulatory, antiangiogenic and direct anticancer activities [79]. The antiangiogenic function of thalidomide was thought to be mediated partially via inhibition of basic fibroblast growth factor-induced angiogenesis [80]. The second-generation compound lenalidomide has enhanced immunological and anticancer properties with fewer side-effects.

thalidomide. Thalidomide has demonstrated clinical activity in both CLL and MCL. Single-agent thalidomide demonstrated a limited and modest overall response rate of 12.5% when given at 200 mg daily escalating to a maximum 800 mg daily to patients with relapsed/refractory indolent NHL (SLL and FL) [81]. In combination with fludarabine, thalidomide was associated with significant therapeutic efficacy in CLL [82, 83]. In treatment-naïve patients, the combination of thalidomide (100–300 mg daily) and fludarabine (standard monthly dosing) achieved a 100% overall response rate (ORR) and 55% CR rate. The median time to disease progression was not reached at 15+ months [82]. In fludarabine-refractory patients, an OR rate of 31% was observed with the FR regimen [83]. Drach et al. have combined thalidomide and rituximab (anti-CD20) in a small phase II trial for patients with relapsed/refractory MCL [84]. Thirteen of 16 assessable subjects demonstrated objective responses (five CR, eight PR, ORR of 81% and CR of 31%), with median time to progression (TTP) at 20 months. These preliminary results are significantly improved over those expected in relapsed MCL with rituximab alone and may relate to antiangiogenic effects or other properties of thalidomide. The main toxic effects of thalidomide include fatigue, somnolence, neuropathy and thromboembolic events.

lenalidomide. Representing a newer generation of iMiDs, lenalidomide has demonstrated significant clinical activity either as a single agent or in combination with rituximab against a variety of lymphoma subtypes, including both aggressive and indolent NHLs, CLL and MCL. Single-agent lenalidomide given at 25 mg orally daily on days 1–21 every 28 days has been studied in a phase II trial setting in relapsed aggressive subtypes (DLBCL, MCL and transformed), as well as indolent subtypes of SLL, FL and nodal marginal zone lymphoma, with a 34% ORR (12% CR) rate in aggressive NHL and a 26% ORR (11% CR) rate in indolent NHL [85, 86]. A recent international study of lenalidomide in aggressive NHL subtypes reported a 28% ORR comparable to previous studies and identified low tumor burden and longer duration from prior rituximab as two favorable predictive factors [87]. Lenalidomide has also been studied in relapsed/refractory CLL. When administered orally at 25 mg on days 1–21 of a 28-day cycle to 45 patients with relapsed disease (51% refractory to fludarabine), lenalidomide achieved an overall response rate of 47% with 9% CR rate [88]. Ferrajoli et al. [89] has reported the clinical outcome and exploratory correlation studies on lenalidomide given as a continuous low-dose treatment of 10 mg daily with dose escalation up to 25 mg daily as tolerated in 44 CLL patients. The overall response rate was 31%, and CR rate was 7%. Over time, a statistically significant decline in plasma basic FGF, but not VEGF, was observed in responders compared with nonresponders. Fatigue and myelosuppression with thrombocytopenia and neutropenia are the most common adverse effects (AEs) with lenalidomide therapy. Wang et al. [90] explored the combination of lenalidomide and rituximab in a phase I/II study in patients with relapsed/refractory MCL. At a 20-mg daily dose of lenalidomide (days 1–21 of 28-day cycle), six of six patients achieved responses including one CR, one PR and one PD, following two cycles of treatment. It remains to be seen whether response rate would continue to improve with longer treatment and follow-up.

metronomic therapy

In contrast to the conventional ‘maximum tolerated dose’ (MTD) chemotherapy, metronomic chemotherapy refers to the administration of low doses of medications on a frequent or continuous schedule without extended drug-free breaks [20, 91]. The main targets of metronomic therapy are the endothelial cells of the growing tumor vasculature [92]. In addition to targeting endothelium preferentially, metronomic therapy also effectively suppresses the surge of bone marrow-derived endothelial cell mobilization following conventional MTD therapy [93], reducing vasculogenesis-dependent lymphoma growth in mouse models [92].

Clinical precedents of metronomic therapy in lymphoma can be found in classical regimens such as COPBLAM III which incorporates nonmyelosuppressive continuous infusional vincristine and bleomycin designed to overcome drug resistance [94, 95]. Another metronomic lymphoma therapy

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which has been used by our group is the PEPC (C3) regimen [96]. PEPC consists of low-dose prednisone (20 mg), etoposide (50 mg), procarbazine (50 mg) and cyclophosphamide (50 mg) administered orally with dosing frequency titrated to hematologic parameters (i.e. absolute neutrophil count >1000). This regimen is convenient, well tolerated and is associated with significant clinical activity in recurrent NHL including MCL [97, 98]. On the basis of the clinical efficacy and tolerability of the PEPC regimen, we have combined PEPC with two agents that are active against MCL, namely rituximab and thalidomide [84], to comprise the RT-PEPC regimen in order to further improve the therapeutic index of metronomic therapy for relapsed/refractory MCL [58].

Our interim analysis of the phase II RT-PEPC indicated significant clinical activity which exceeded the prospectively defined interim efficacy criteria for continued accrual. At a median follow-up of 27.5 months, the overall response rate is 73% (40% CR, 33% PR) for 15 evaluable patients. Nonresponders experienced SD (range 6–11 months). The median TTP was 15 months, with an estimated 2-year PFS of 27% [95% confidence interval (CI) 0.11–0.67] and 2-year OS of 67% [95% CI 0.45–0.99]. Translational studies of the angiogenic phenotypes of primary tumor cells and tumor vasculature are ongoing to correlate angiogenic phenotype with clinical response. Buckstein et al. [99] reported the clinical efficacy of oral combination celecoxib and low-dose cyclophosphamide in relapsed aggressive NHL. In 35 heavily pretreated DLBCL patients, the overall best response rate was 37%, with 22% achieving SD. These observed clinical responses appeared to correlate with declining levels of circulating endothelial cells (CD45+CD31+CD146+) and their precursors (CD45+CD31+CD146+CD133+) in responders, suggesting angiogenesis inhibition as a potential mechanism of action [99].

**other novel antiangiogenic strategies**

**mTOR inhibitors.** The mTOR is a serine/threonine kinase downstream of PI3-kinase/AKT pathway [100]. mTOR-mediated phosphorylation activates two downstream targets, namely p70S6 kinase (p70S6K) and 4E-binding protein 1 (4E-BP1), which are essential in the regulation of cell growth, proliferation and survival [101]. mTOR has emerged as a promising target for cancer therapy in human solid tumors as well as hematological malignancies. Selective mTOR inhibitors have shown antiangiogenic activities by inhibiting HIF-1α-mediated VEGF production downstream of the PI3-kinase/AKT/mTOR pathway, as well as direct inhibitory effect on endothelial cell proliferation [102, 103].

Witzig et al. reported the preliminary results of a phase II trial of the mTOR inhibitor temsirolimus (CCI-779) in patients with relapsed MCL. Temsirolimus was given at 250 mg i.v. every week as a single agent. A 38% response rate was observed in 34 assessable patients, with a median TTP of 6.5 months. Thrombocytopenia was dose limiting, but of short duration [104]. A subsequent randomized, open-label phase III study compared the antitumor activity of temsirolimus with an investigator’s choice of therapy in patients with relapsed MCL. Patients were randomly assigned to one of two schedules of i.v. temsirolimus, 175 mg 3× weekly followed by either 75 mg (175/75 mg) or 25 mg (175/25 mg) weekly or investigators’ choice therapy (IC). Treatment with the temsirolimus 175/75 mg arm resulted in significant improvement in PFS (4.8 months versus 1.9 months, P = 0.0009), objective response rate (22% versus 2%, P = 0.0019) and a trend toward longer OS (10.9 months versus 5.8 months, P = 0.0714), compared with IC. The most common ≥grade 3 AEs were cytopenias [105]. Activity of single-agent temsirolimus in nonmantle cell NHL (DLBCL, FL and SLL/CLL) was reported recently by Smith et al. Of the 56 patients who received two or more cycles of therapy, ORR was 46%. The ORR was 35% in the 74 assessable patients who received at least one cycle of treatment, with 25 patients maintaining SD. The median PFS for all patients, patients completing more than two cycles and patients with PR/CR were 123, 156 and 215 days, respectively [106]. An oral derivative of rapamycin, everolimus (RAD001, Novartis), is currently undergoing phase I/II study to determine its safety and efficacy in relapsed or refractory hematologic malignancies including NHL [107].

**HDAC inhibitors.** HDAC regulate chromatin remodeling and epigenetic modification of gene expression by deacetylation of histones. HDACs are grouped in four classes on the basis of the structures of their accessory domains. Inhibition of HDAC activity causes an accumulation of acetylated proteins including histones and nonhistone substrates, which leads to selective gene transcription alteration [108]. Histone deacetylase inhibitors (HDACi) represent an emerging class of therapeutic agents that induce tumor cell cytostasis, differentiation and apoptosis, in part by angiogenesis inhibition. HDACi has been shown to induce VHL-dependent HIF-1α degradation by acetylation at Lys532 [109]. Further, class II HDACs including HDAC4 and HDAC6 are physically associated with HIF-1α, and their selective inhibition by HDACi can induce HIF-1α acetylation and polyubiquitination in a VHL-independent mechanism [110]. HDACi can also alter VEGF signaling by inhibiting VEGF receptors and neuropilin-1 expression in endothelial cells [111].

To date, pan-HDACi vorinostat (suberoylanilide hydroxamic acid) and panobinostat (LBH589), as well as MGCD0103 which targets class I HDACs, have been evaluated in NHL. Vorinostat given orally at 400 mg daily has demonstrated activity (ORR 24%–29.2%) in heavily pretreated patients with cutaneous T-cell lymphoma (CTCL), which led to its approval by the US Food and Drug Administration (FDA) in 2006 for the treatment of progressive and recurrent CTCL [112–114]. Duvic et al. [114] reported that successful therapy with vorinostat was associated with a reduced CD31+ dermal MVD, and an increase of the antiangiogenic protein thrombospondin-1 following treatment. Limited activity was noted for vorinostat in relapsed DLBCL [115]. Panobinostat was evaluated in 10 patients with progressive CTCL who received treatment (20 mg) three times weekly on a 28-day cycle [116]. Two CRs, four PRs and two SDs were attained in this cohort with a median TTP of 179 days. Microarray analysis of skin biopsies showed distinct gene expression response profiles over time following panobinostat treatment, including consistent downregulation of proangiogenic genes guanylate cyclase 1A3 (GUCY1A3) and
angiopoietin-1 (ANGPT1). MGCD0103, a selective class I HDAC inhibitor, has shown activity in both DLBCL and FL [117]. An interim analysis of 50 patients (33 DLBCL and 17 FL) on two dosing schedules (110 mg and 85 mg 3x/week) showed one CR, three PRs and five SDs in the assessable 17 DLBCL patients and one PR in the assessable 10 FL patients. In general, HDACs can be safely administered with class-related common toxic effects including transient cytopenia, nausea, diarrhea, fatigue, dehydration and QTc prolongation, which appear to be schedule, route and dose dependent.

**proteasome inhibitors.** Inhibition of the ubiquitin-proteasome pathway in tumor cells hinders tumor growth by inducing cell cycle arrest, apoptosis and inhibiting tumor metastasis and angiogenesis. Bortezomib (PS341, Velcade™), a dipeptidyl boronic acid derivative, selectively inhibits the 26S proteasome which is essential in the degradation of intracellular proteins including p53, nuclear factor kappa B, bcl-2 and cyclin-dependent kinase inhibitors such as p21 and p27 [118]. Bortezomib was shown to mediate antiangiogenesis in myeloma patients via dose-dependent inhibition of VEGF and IL-6 secretion by endothelial cells [119]. Bortezomib has direct inhibitory effects on HIF-1α activation by reinforcing the factor-inhibiting HIF-1-mediated inhibition of p300 coactivator recruitment [120]. Induction of HIF-1α (which was common in tumors under hypoxia) was shown to render endothelial cells particularly sensitive to the proapoptotic and antiangiogenic effects of bortezomib [121], suggesting that bortezomib can selectively target tumor-associated vessels.

In non-Hodgkin’s lymphoma, several phase II studies [122–125] confirmed activity of single-agent bortezomib in follicular lymphoma, MCL and marginal zone lymphoma. A multicenter pivotal trial determined that the overall response rate in relapsed MCL was 33%, including 8% CR/unconfirmed CR, with a median duration of response of 9.2 months and TTP of 6.2 months [124], leading to FDA approval of bortezomib for this indication. The most common 2grade 3 AEs were peripheral neuropathy, fatigue and thrombocytopenia. Bortezomib also has activity in other B-cell processes, including Waldenström’s macroglobulinemia and amyloidosis [118]. Currently multiple trials are ongoing to further examine its efficacy either as a single agent or in combination with other active regimens including CHOP plus rituximab in NHL.

**receptor tyrosine kinase inhibitors.** Sunitinib (SU11248) is an orally bioavailable inhibitor that affects receptor tyrosine kinases including VEGF receptors 1, 2, 3 and PDGF receptors α and β. Its therapeutic effects are at least partially mediated via antiangiogenesis, with activities demonstrated in gastrointestinal stromal tumors (GIST), renal cell carcinoma (RCC) and acute myeloid leukemia, among other tumor types. On the basis of phase II and III studies in patients with metastatic RCC and GIST, sunitinib is FDA approved for metastatic RCC and imatinib-resistant GIST. Sunitinib is currently being studied in relapsed and refractory DLBCL patients in a prospective phase II clinical trial sponsored by the National Cancer Institute of Canada. Sunitinib is given at 37.5 mg po daily continuously, and the result of this study is awaited [126].

**conclusion**

Neoangiogenesis has increasingly been recognized to play potentially important pathogenic roles in lymphomagenesis, by mediating autocrine stimulation of proliferation and survival of lymphoma tumor cells via the VEGF–VEGF receptor axis and through recruitment of bone marrow-derived VEGF receptor axis and through recruitment of bone marrow-derived progenitors to support neovascular assembly and metastasis. Antiangiogenic therapy with bevacizumab is safe and has modest activity in DLBCL. The combination of bevacizumab and the standard chemoimmunotherapy R-CHOP is currently being investigated in both DLBCL and MCL. iMiDs including thalidomide and lenalidomide, either as single agent or in combination with fludarabine or rituximab, have demonstrated clinical activity against a wide variety of NHL including DLBCL, CLL and MCL. Metronomic low-dose chemotherapy appears to have broad clinical applicability in lymphoma, particularly in relapsed and refractory settings. Novel biological agents, targeting RTK including VEGF receptors and PDGF receptors, and various downstream targets along the angiogenic signaling pathways which regulate HIF-1α and VEGF expression, are in various stages of clinical development and investigation in human lymphoma patients.

Given this array of agents, which at least in part target the tumor vasculature, one can envision a large number of combinations with other drugs that target more directly the malignant tumor cells. For example, RTK, HDAC, mTOR or proteasome inhibitors can be combined with antilymphoma chemoimmunotherapy regimens for synergy in either upfront or salvage settings. Combinations of biological agents, such as upstream RTK inhibitors plus downstream inhibitors (HDAC inhibitors or mTOR inhibitors), or inhibitors combination targeting divergent yet synergistic downstream pathways (HDAC inhibitors with proteasome inhibitors or mTOR inhibitors), can also be contemplated, as long as there are no significant overlapping toxic effects. Metronomic regimens provide an appealing clinical feasibility of combining low-dose chemotherapy with many of the new biological compounds to augment antiangiogenic effects, thus maybe particularly effective in resistant disease or maintenance setting. The list of possible agents, doses and sequences and the resulting evaluation process present significant challenges. Further understanding of the lymphoma specific and drug-specific mechanisms underlying antiangiogenic therapy is contingent upon a better grasp of the complex biology of lymphoma angiogenesis and validation of clinically useful biomarkers which reflect the dynamics of drug and target interaction. Correlative assay development for various blood and tissue angiogenesis biomarkers is actively being pursued in human lymphoma patients. This information will provide much-needed insights for the rational design of future effective antiangiogenic therapy and schedules that are tailored to appropriate clinical settings.

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