



## Review article

## Nanomedicines for the treatment of hematological malignancies

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## ABSTRACT

Hematological malignancies (HM) are a collection of malignant transformations originating from cells in the primary or secondary lymphoid organs. Leukemia, lymphoma, and multiple myeloma comprise the three major types of HM. Current treatment consists of bone marrow transplantation, radiotherapy, immunotherapy and chemotherapy. Although, many chemotherapeutic drugs are clinically available for the treatment of HM, the use of these agents is limited due to dose-related toxicity and lack of specificity to tumor tissue. Moreover, the poor pharmacokinetic profile of most of the chemotherapeutics requires high dosage and frequent administration to maintain therapeutic levels at the target site, both increasing adverse effects. This underlines an urgent need for a suitable drug delivery system to improve efficacy, safety, and pharmacokinetic properties of conventional therapeutics. Nanomedicines have proven to enhance these properties for anticancer therapeutics. The most extensively studied nanomedicine systems are lipid-based nanoparticles and polymeric nanoparticles. Typically, nanomedicines are small sub-micron sized particles in the size range of 20–200 nm. The biocompatible and biodegradable nature of nanomedicines makes them attractive vehicles to improve drug delivery. Their small size allows them to extravasate and accumulate at malignant sites passively by means of the enhanced permeability and retention (EPR) effect, resulting from rapid angiogenesis and inflammation. Moreover, the specificity to the target tissue can be further enhanced by surface modification of nanoparticles. This review describes currently available therapies as well as limitations and potential advantages of nanomedicine formulations for treatment of various types of HM. Additionally, recent investigational and approved nanomedicine formulations and their limited applications in HM are discussed.

## 1. Introduction

Presently, multiple chemotherapeutic and molecular targeted agents are available for the treatment of hematological malignancies (HM). Nevertheless, only a subset of patients will achieve long-term remission or complete cure of the disease. This is at least partly due to the lack of specificity of these agents for the disease site and their short biological half-lives in the circulation [1]. Lack of specificity results in

exposure of healthy organs to the drugs that gives off-target adverse effects. High doses and frequent dosing to maintain the therapeutic levels at the malignant site further increase the magnitude of these adverse effects [2,3]. Therefore, drug delivery systems could be essential not only to decrease exposure of healthy tissues to the drug, but also to retain the active substance in the circulation and deliver it to the malignant cells. Thereby, nanomedicines can significantly improve efficacy and safety profiles of encapsulated chemotherapeutic agents.

**Abbreviations:** BMSC, Bone marrow stromal cell; BM, bone marrow; VCAM1, Vascular cell adhesion molecule 1; VLA-4, Very late antigen-4; HM, hematological malignancy; CLL, Chronic lymphocytic leukemia; ICAM1, Intercellular adhesion molecule 1; VEGF, Vascular endothelial growth factor; bFGF, basal fibroblast growth factor; Ang-1, Angiopoietin 1; TGF- $\beta$ , Transforming growth factor beta; PDGF, Platelet-derived growth factor; HGF, Hepatocyte growth factor; IL, interleukin; OPG, Osteoprotegerin; IGF-1, Insulin-like growth factor 1; LFA-1, Lymphocyte function-associated antigen 1; ECs, Endothelial cells; AML, Acute myeloid leukemia; CXCL, Chemokine (C-X-C motif) ligand; CXCR, Chemokine (C-X-C motif) receptor; RANK(L), Receptor activator of nuclear factor kappa B (ligand); CML, Chronic myeloid leukemia; MM, Multiple myeloma; NLCs, Nurse-like cells; BAFF(R), B cell activating factor (receptor); APRIL, A proliferation-inducing ligand; BCMA, B cell maturation antigen; TACI, Transmembrane activator and CAML interactor; CAM-DR, Cell adhesion-mediated drug resistance.

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In past few decades, nanocarriers such as liposomes and polymeric nanoparticles received considerable attention for the treatment of various types of solid tumors, leading to several successful formulations that entered the clinic [2,4]. However, less work has been done to develop nanomedicines for the treatment of hematological malignancies [1]. In this review, currently available (chemo)therapies for HM and their shortfalls are discussed. Nanomedicines are presented as an attractive approach to improve treatment, which is elucidated by an overview of investigational and approved drug formulations. Finally, challenges associated with development of novel nanomedicine formulations and their clinical translation, are discussed.

## 2. Hematological malignancies

Hematological malignancies (HM) comprise a variety of cancers derived from the blood, bone marrow (BM) and lymphatic system. Of all cancers diagnosed in the United States in 2017, 10.2% was estimated to be categorized as a hematological malignancy [5]. In children, adolescents and young adults, leukemia causes more deaths than any other cancer [6]. In majority of HM, bone marrow is the predominant site of tumor localization together with peripheral blood and secondary lymphoid organs, such as spleen and lymph nodes. [7,8]. In BM, normal hematopoietic stem cells differentiate into cells of the myeloid or lymphoid lineage. Granulocytes, monocytes, mast cells, erythrocytes and thrombocytes differentiate from myeloid precursor cells (myelopoiesis), whereas T cells, B cells, Natural Killer (NK) cells and plasma cells are produced by the lymphoid lineage (lymphocytopoiesis). Hematological malignancies can be subdivided into leukemia, lymphoma, and multiple myeloma, based on the cell-of-origin. Table 1 provides an overview of different types of HM.

## 3. Target sites in hematological malignancies

To improve therapeutic intervention of hematological malignancies, delivery of drugs at the site of disease is one of the goals. Fig. 1 illustrates various target sites in HM.

### 3.1. Bone marrow microenvironment

The BM microenvironment consists of cellular and non-cellular compartments. The importance of bone marrow vasculature was recently discussed [36–39]. The cellular compartment includes bone marrow stromal cells (BMSC), endothelial cells, osteoclasts, and osteoblasts, whereas non-cellular compartment includes extracellular matrix and important cytokines like interleukin (IL)-6, IL-21, and tumor necrosis factor alpha (TNF $\alpha$ ), and growth factors such as insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF) [40]. Interaction of BMSC and neoplastic cells plays a crucial role in proliferation and survival of neoplastic cells and progression of the disease [37,38]. This interaction can also lead to drug resistance [39,41]. The interaction of malignant cells with BMSC and other cells in the bone marrow microenvironment is depicted in Fig. 2 together with their key signaling factors. BMSC can interact with malignant cells via cell surface molecules; for example, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) on BMSC connect to lymphocyte function-associated antigen 1 (LFA-1) and very late antigen 4 (VLA-4) on malignant cells, respectively. These interactions trigger activation of the nuclear factor kappa B (NF- $\kappa$ B) pathway, and secretion of IL-6 from BMSC, which enhances the production and secretion of VEGF from malignant cells.

The abnormal proliferation of osteoblasts and osteoclasts results from the progression of the disease. In MM, for instance, the balance between osteoclast activation and osteoblast proliferation is lost, causing bone lesions. Bone formation and resorption is normally a controlled process regulated by mainly two molecules, receptor activator of NF- $\kappa$ B ligand (RANKL) and osteoprotegerin (OPG) [42]. RANKL

produced by BMSC and macrophage inflammatory protein 1 alpha (MIP-1 $\alpha$ ) produced by MM cells stimulates osteoclast activation in the disease. OPG would limit osteoclast formation, but OPG is decreased by high local IL-3, Dickkopf-related protein 1 (DKK-1) and hepatocyte growth factor (HGF) concentrations [32]. Notch signaling induces production of IL-6, VEGF, and IGF, leading to malignant cell proliferation and survival [38]. The intensive signaling via direct cell-cell interaction and through soluble mediators leads high vascularization and angiogenesis in leukemic bone marrow, which is responsible for enhanced proliferation and survival of malignant cells and hence increased resistance [43].

Cross talk of malignant cells with BMSC and other cells in the BM microenvironment is shown in Fig. 2. The interaction between VCAM1 on BMSC and VLA-4 on malignant cell is found in all kind of HM. In multiple myeloma and in CLL, ICAM1 on BMSC interacts to the LFA-1 present on myeloma cell and CLL cell. In MM, Notch-1 binds to DLL1. Soluble factors such as VEGF, bFGF, Ang-1, TGF- $\beta$ , PDGF, HGF, IL-1, and IL6 secreted by BMSC; IL-6 and OPG by osteoblasts; IL17 by Th17 cells; VEGF and IGF-1 by myeloma cells increase proliferation of myeloma cells and increase angiogenesis. VCAM1 – VLA-4 and ICAM1 – LFA-1 bindings take place between BMSC and ECs in AML. Soluble factors IL-6, IL-8 and CXCL12 from BMSC increase proliferation and survival of leukemic cells in AML through binding to IL-6R, CXCR2 and CXCR4, respectively. RANK present on osteoclasts reacts with RANKL on AML cells and myeloma cells. Osteoblasts increase activation and survival of CML cells via Ang-1 – Tie2 interaction. CD44 and VLA-4 on CML cells help homing and adhesion of these cells by interacting to HA and fibronectin in the extracellular matrix in BM microenvironment. In CLL, NLCs and T cells increase proliferation of the leukemic cells. NLCs secrete CXCL12 and CXCL13 that binds to CXCR4 and CXCR5 respectively; BAFF/APRIL on NLCs interacts with BCMA/TACI/BAFFR on CLL cells. T cells react via CD40 – CD40L interaction. Together these complex interactions between malignant, stromal and other cells in the BM result in enhanced proliferation and survival of malignant cells, resistance to apoptosis, and CAM-DR.

### 3.2. Secondary lymphoid organs

Secondary lymphoid organs such as lymph nodes and spleen provide a distinct microenvironment for tumor cells in hematological malignancies.

#### 3.2.1. Lymph nodes

Lymph nodes are the primary organ where immune responses are initiated and are therefore rich in immune cells such as dendritic cells, B, T and NK cells as well as macrophages. Notably, immune responses can prevent malignant transformation. For example, downregulation of MHC class I molecules on the surface of transforming cells is detected by NK cells and induces lysis of the respective cells. However, transformed cells have developed mechanisms to circumvent effective immune responses and often mediate immune suppression. Functional NK cells in lymph nodes of patients, which produce antitumor cytokines like TNF- $\alpha$  and interferon gamma (IFN- $\gamma$ ), correlate with a favorable prognosis of multiple types of HM [44,45]. This indicates that anti-tumor immune responses are still active in some malignancies, impacting progression and outcome. On the other hand, a proinflammatory microenvironment can also support the expansion of HM. Follicular dendritic cells (FDC) are key players in secondary lymphoid organs comprising approximately 1% of all germinal center (GC) cells. FDC interfere with apoptosis and promote survival of B cells in GC by secretion of cytokines such as IL-15, thereby supporting the proliferation and expansion of the malignant cells [46].

#### 3.2.2. Spleen

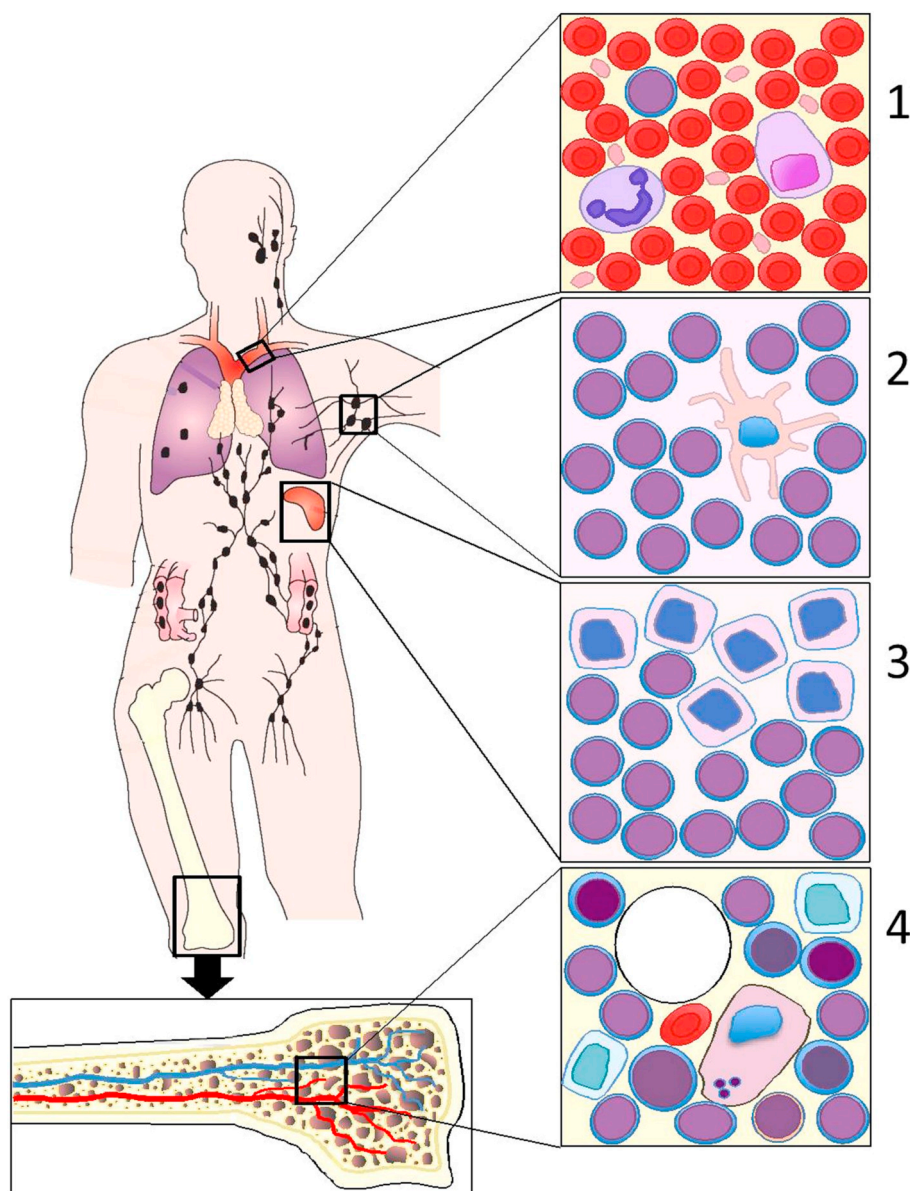
As the largest lymphoid organ of the body, the spleen plays an important role in immunological defenses. Involvement of spleen is

**Table 1**

An overview of different types hematological malignancies. The classification is based on origin of the disease and cell types involved. Chromosomal abnormalities and/or mutations frequently associated are highlighted but not limited to the list. Due to the scope of this review the somewhat rarer forms are not discussed here.

Type of HM	Subtype	Origin	Cancer statistics 2017 (USA) [5] Estimated new cases/deaths epidemiology	Cell types involved and infiltrated sites	Frequently associated chromosomal abnormalities/mutations/ translocations (t)	Ref
1. Leukemia Originate from bone marrow precursor cells from either the myeloid or the lymphoid lineage.	i. Acute Lymphoblastic Leukemia (ALL) For detailed information see reference 4	Hematopoietic precursors of the lymphoid lineage.	5970/1440Most common leukemia in people younger than 20 years.	Clonal population of lymphoid cells. 85% of ALL arise from the B cell lineage and 15% from T cell precursors. Infiltration of bone marrow, blood and extramedullary sites	t(12;21): <i>ETV6-RUNX1</i> t(1;19) (q23;p13): <i>E2A-PBX1</i> t(9;22) (q34;q11): <i>BCR-ABL1</i> t(4;11) (q21;q23): <i>MLL-AF4</i>	[5,7,9–11]
	ii. Acute Myeloid Leukemia (AML) For detailed information see reference 8	Hematopoietic precursors of the myeloid lineage.	21,380/10,590 Median age of diagnosis is 64 years.	Abnormally or poorly differentiated blasts of the myeloid system. Infiltration of the bone marrow, peripheral blood and other organs.	Chromosomal mutations in 97% of cases inv(3)(q21;q26.2) t(3;3)(q21;q26.2) occasionally t(9;22)(q34;q11) t (8;21), inv (16), t (15;17): part of diagnosis	[7,12] [5,13,14]
	iii. Chronic myeloid Leukemia (CML) For detailed information see reference 11	Hematopoietic stem cells	8950/1080 Median age of diagnosis is 60 years.	Myeloid cells and their precursors in the bone marrow. Involvement of bone marrow, peripheral blood and spleen.	Driven in almost all cases by the Philadelphia chromosome: t(9;22) (q34;q11): <i>BCR-ABL1</i>	[5,15–17]
2. Lymphoma Lymphomas are defined as a group of malignancies derived from mature lymphoid cells. The majority arises from B cells and are therefore called B cell lymphomas	i. Hodgkin's Lymphoma (HL) For detailed information see reference 15	B cells (majority of cases)	8260 /1070 Two age groups: early adulthood (age 15–40, usually 25–30) and late adulthood (after age 55). 72,240/20,140	Reed-Sternberg (HRS) cells in classical HL (95% frequency) and lymphocytic and histiocytic (L&H) cells in LPHL. Involvement of spleen and lymph nodes.	Rare events affecting <i>p53</i> , <i>Fas</i> , <i>IkBa</i> Frequent microsatellite instability Novel translocation: t(2;14)(p13;q32.3): <i>BCL11a</i>	[5,7,18–21] [2,5,7,8,22,23]
	ii. Non-Hodgkin's Lymphoma (NHL) For detailed information see reference 19	Arise from lymphocytes that are at various stages of development				
	(a) Chronic Lymphoid Leukemia (CLL) For detailed information see reference 22	B cells	20,110/4660 Average age of diagnosis at 70 years.	Monoclonal CD5 positive B cells. Accumulation of malignant B cells in the blood, bone marrow and lymphocytic tissue	del13q14 trisomy 12 del11q22-q23 del17p13.	[5,7,24–26]
3. Multiple myeloma Malignancy of B-lymphocytes characterized by clonal proliferation of a single plasma cell resulting in monoclonal immunoglobulin production.	(b) Follicular Lymphoma (FL) For detailed information see reference 23	B cells	n.d. Follicular lymphomas account for 20% of NHL Average age of diagnosis at 60 years.	Germinal center (GC) B cells. Infiltration of cervical, axillary, inguinal and femoral lymph nodes. Bone marrow is involved in 50% of patients.	t(14;18)(q32;q21): ectopic <i>Bcl2</i> expression; in 85% cases of FL. Inactivating mutations of the <i>MLL2</i> gene is found in over 80% of FL.	[6,7,27]
	(c) Diffuse large B cell lymphoma (DLBCL) For detailed information see reference 25	B cells	n.d. Most common subtype of NHL (40%) Middle age	Heterogeneous group of B cell malignancies, characterized by large cells. Develop at either nodal or extra-nodal sites.	3q27: <i>Bcl6</i> t(14;18): <i>Bcl2</i>	[7,28]
	For detailed information see reference 32	B cells	30,280/12,590 Median age of disease onset is 65–70 years	Monoclonal post-GC plasma cells. Infiltration of bone marrow and extramedullary sites.	Trisomies: trisomic MM 14q32: IgH translocated MM Or combination of both Secondary cytogenetic abnormalities: gain (1q), del(1p), del(17p), del(13), <i>RAS</i> mutations, and secondary translocations involving <i>MYC</i>	[5,7,29–35]

n.d. = not determined



**Fig. 1.** Target sites in hematological malignancies. The peripheral blood (1), secondary lymphoid organs (2 & 3) and bone marrow (4) are the primary target sites for drug delivery in hematological malignancies

seen in all types of HM, most prominently in lymphomas. Spleen involvement is found in approximately 30–40% cases of NHL and in one third of HL [47]. In HL, spleen involvement can also upstage the disease. Splenomegaly is also found in other HM such as CML. The spleen is organized in two regions; the red pulp and the white pulp, which are separated by the marginal zone [48]. Filtration of blood and iron recycling take place in the red pulp. The white pulp contains T and B cells zones. Spleen is responsible to regulate innate as well as adaptive immune responses and plays a key role in tumor immunity by recruiting monocytes and macrophages to the tumor tissues [48]. Because of the phagocytic activity of monocytes and macrophages, a significant amount of intravenously administered nanoparticles tends to accumulate in the spleen [49]. Though this property of spleen “eating” the nanoparticles could be advantageous [50]. Number of inflammatory monocytes has been shown to have negative correlation with lymphoma patient survival [51]. As inflammatory and non-inflammatory monocytes use distinct mechanisms for recruitment, targeting inflammatory monocytes in spleen and bone marrow by using siRNA containing nanoparticles, and thereby altering their recruitment to the

tumors has been evident to reduce tumor growth *in vivo* [50]. The spleen can be considered as one of the major target sites in HM [47,52].

### 3.3. Peripheral blood

The cellular compartment of peripheral blood consists normally of leukocytes, red blood cells (RBC) and platelets. Detection of circulating malignant cells by complete blood count (CBC), flow cytometry and blood smears is applied in the diagnosis of HM as well as in monitoring therapeutic efficacy [53–55]. Moreover, peripheral cytopenias such as anemia, thrombocytopenia, and pancytopenia are common in HM. The mature blood cells are replaced by immature blast cells. In multiple myeloma, for instance, the plasma cells are replaced by plasmablasts, which are rapidly dividing cells that secrete high amounts of immunoglobulins [31]. There is also an increase in calcium levels in the blood because of the bone resorption due to local osteoclast activation (Section 3.1). The accumulation of immunoglobulins and calcium in the kidneys can cause inflammation and subsequent renal failure [56]. In leukemias including CML, presence of > 20% blast cells in peripheral



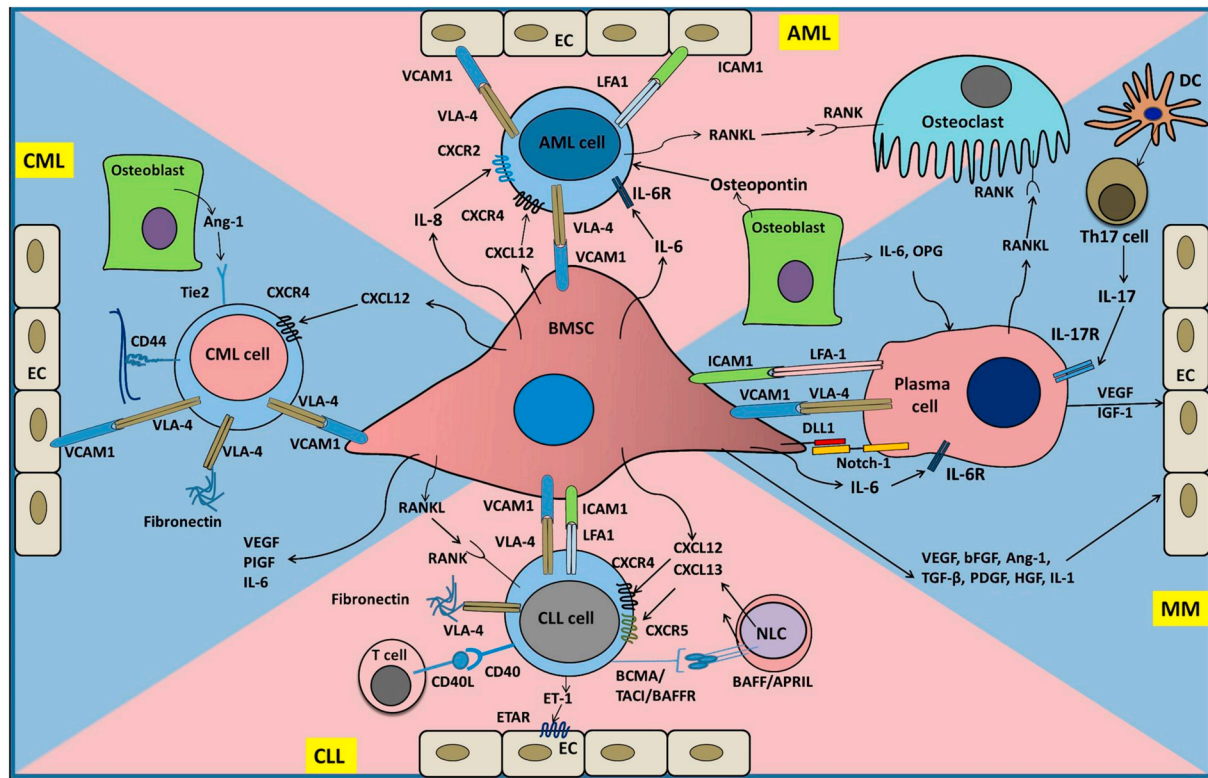


Fig. 2. Bone marrow microenvironment (BM) in hematological malignancies (HM). Four different types of malignancies are chosen as examples to show the complexity of BM microenvironment in HM.

blood (and bone marrow) is classified as a blast crisis, which is difficult to treat [57].

### 3.4. Liver

The liver is the second largest organ of the body and a part of reticuloendothelial system. In hematological malignancies, liver is a site of malignant cells infiltration which produce hepatomegaly or formation of multiple nodules in the liver [58,59]. Although life-threatening complications such as acute liver failure in patients with HM is rare [60–62], clinical manifestations are often seen due to hepatic involvement [58]. Lymphomatous infiltration of the liver has been seen in lymphomas, more commonly in non-Hodgkin lymphoma (NHL) (16–43% patients). In Hodgkin disease, malignant infiltration of Reed-Sternberg cells have been described in up to 14% of patients [58,59]. Jaundice and Cholestasis are also sometimes found in lymphoma patients [59]. In ALL and AML hepatic involvement has been described to be approximately 95% and 75% cases, respectively [58]. Liver infiltration and mild to moderate hepatomegaly can also be seen in chronic phase as well as blast crisis in CML. Chemotherapeutic-induced hepatotoxicities are also common in HM patients [59]. This makes liver an important target site in HM.

## 4. Current and novel drugs for the treatment of hematological malignancies

In many solid tumors, the malignant tissue, including infiltrated surrounding tissue, can often be removed surgically. Excision can be further combined with radio- and chemotherapy to eradicate potential residual cancer cells. However, in case of HM, the malignant cells are not restricted to a specific site and treatment needs to be effective in multiple tissues, including bone marrow, secondary lymphoid organs and the blood stream. Treatment is variable and dependent of the specific type and stage of HM that the patient is diagnosed with.

Treatment strategies consist of bone marrow transplantation, radiotherapy and chemotherapy. Recently, the potential of immunotherapeutic approaches has been demonstrated for the first time in HM [63–65]. More than 70 drugs are currently available for the treatment of HM. However despite these therapeutic options not all HM are curable up to now.

### 4.1. Currently available drugs and treatment combinations

#### 4.1.1. Radiotherapy

With radiotherapy, the patient is exposed to a specific dose of ionized radiation targeted to the region of the malignant neoplasm. The DNA of the cells exposed to the radiation is damaged. Radiotherapy is often used prior to stem cell transplantation as a part of a conditioning regimen or in combination with chemotherapy for a synergistic effect [66]. There are several side effects associated with radiotherapy. Damage to healthy tissue that is in proximity to the target site is the most frequently encountered adverse effect causing dose-limiting toxicity. Based on the diffuse distribution of the malignant cells in HM, radiotherapy is only successful in a small subset of patients.

#### 4.1.2. Hematopoietic stem cell transplantation (HSCT)

Stem cell transplantation is a treatment option for durable remissions of HM [67]. After conditioning via high dose therapy with cytotoxic drugs and/or radiation, which aim for temporary yet complete destruction of the hematopoietic system, the patient's bone marrow is restored with healthy hematopoietic stem cells by autologous or allogeneic stem cell transplantation [68]. In autologous HSCT the patient's own healthy cells are collected prior to the conditioning regimen and re-infused afterwards. Although autologous HSCT has become a treatment choice for HM such as MM and some lymphomas, however, relapse is often inescapable. Relapse may come from sporadic tumor cells that remain after chemotherapy or by contamination of cancer cells in the transplanted cells. The latter can be avoided by using another

approach of HSCT, i.e. allogeneic HSCT. In allogeneic HSCT, stem cells from a human leukocyte antigen (HLA)-matched healthy donor are transplanted after a conditioning regimen designed to completely eradicate the recipient's hematopoietic system. However, allogeneic HSCT is associated with an increased risk of graft-versus-host disease, which may also induce anti-tumor immune responses, known as the 'graft-versus-leukemia' effect.

#### 4.1.3. Chemotherapy and targeted therapies

To date, multiple chemotherapy and antibody-based drugs are available for the treatment of hematological malignancies. These include various classes of cytotoxic agents, small molecules, immune-modulators, and immunotherapeutics (Table 2). Combination therapy is often applied with multiple drugs and/or other treatment modalities such as radiation. The rationale is that it will improve treatment of HM by simultaneously targeting multiple pathways. Advances in understanding the pathogenic pathways have led to the development of several targeted approaches in HM. These include modulators of epigenetic alterations, i.e. histone deacetylase inhibitors (HDI), proteasome inhibitors, immune modulators and small molecules such as tyrosine kinase inhibitors (TKI, Table 2). TKI inhibit active signaling pathways and aim to target the malignant cells' growth and survival pathways. With imatinib mesylate the proof-of-principle for the successful treatment using TKI in HM was provided. By directly binding to and inhibiting the constitutively active kinase Abelson murine leukemia viral oncogene homolog 1 (ABL1), imatinib treatment resulted in long-term remission and almost doubled the 5-year survival rate in CML. However, in CML and breakpoint cluster region-ABL1-driven ALL, resistance towards imatinib frequently develops via mutations in ABL1 that prevent binding of the TKI. For this reason, several second generation TKI have been developed to target imatinib resistant clones. Second generation TKI such as nilotinib and bosutinib show better potency against most mutated forms of ABL1 but still failed to overcome the T315I gatekeeper mutation [69]. Recently, a third generation TKI, ponatinib, has been developed to successfully treat the T315I mutation containing disease [69]. Furthermore, several TKI target more than one kinase and can be used for the treatment of additional kinase-driven malignancies. One example is dasatinib, initially designed to target ABL1, which also inhibits the function of Src, c-Kit and PDGFR kinases. Based on this effect, dasatinib therapy for the treatment of other (solid) malignancies is under investigation [70].

#### 4.1.4. Antibody and immunotherapy

**4.1.4.1. Monoclonal antibodies.** Monoclonal antibodies naturally possess a long circulatory half-life. This property helps to overcome the pharmacokinetic challenges of many small molecules. By humanizing antibodies, immune responses against subsequent doses can be minimized so that the long-circulating property is maintained. In general, monoclonal antibodies target specific surface molecules of the malignant cell population although frequently the surface molecules are also expressed, albeit to a lesser extent on healthy counterparts. After binding, cells can be killed via direct cytotoxic effects as well as complement-mediated cell lysis (CDC) and antibody-dependent cytolytic effects (ADCC) by NK cells. Modifications can further increase the efficacy as described in the following section. Rituximab was the first monoclonal antibody approved for treatment of HM. It binds to the large loop of CD20 and causes cell polarization as well as CDC and ADCC. In addition, a number of other CD20 targeting antibodies are used for the treatment of HM, including ofatumumab. Ofatumumab is a fully humanized CD20 antibody with increased cytolytic effect and a slow dissociation rate, and is particularly suitable for cells with low target expression [73]. Obinutuzumab is another humanized anti-CD20 antibody with increased binding affinities towards FcγR on NK cells by afucosylated Fc segments that may be beneficial as compared to Rituximab [74]. Based on their (semi-)specific binding to the malignant cells, monoclonal antibodies can also

be used for specific site delivery of coupled cytotoxic agents or radioisotopes: For example, ibritumomab targets the same epitope on the CD20 molecule as rituximab but that antibody is of murine origin. It is covalently bound to tiuxetan, which is a chelator bound to the radioactive element yttrium-90. Due to its high beta energy, the radiation can kill even bulky lymphomas [75,76]. Tositumomab also targets CD20 and is often used in combination with tositumomab labeled with the radioisotope iodine-131 [77]. However, despite promising response rates, tositumomab was discontinued in 2014 due to the decline in usage [78].

**4.1.4.2. Bispecific antibodies.** Blinatumomab is the first bispecific T-cell-engager antibody (BiTE) approved for the treatment of relapsed/refractory Ph-negative ALL [79]. It is a single chain protein that targets CD3 and CD19. Thereby, it brings CD3+ T cells and (malignant) CD19+ B cells in close proximity. This activates T cells that subsequently lyse the target B cells via release of cytotoxic granules and activation of the perforin–granzyme pathway in the B cell. Although the activated T cells are able to engage multiple leukemic cells, the optimal administration schedule requires continuous infusion because of the short plasma half-life of the single chained antibody derivative. Potential improvements of this therapeutic strategy include bispecific antibody-drug conjugates that could enhance the T-cell activity. Alternatively, the physical connection between the two epitopes could be made through a coupling of both epitopes to a nanoparticle surface. However, identification of two expressed targets on the same tumor cell, different tumor cells or cells in the microenvironment, for which bispecificity is actually beneficial, remains challenging.

**4.1.4.3. Chimeric antigen receptor (CAR) T-cell therapy.** Ex vivo engineering of T cells is a highly promising treatment option for HM. In this approach, T cells from the patient are isolated and engineered to recognize the target tumor cells via a chimeric antigen receptor (CAR) that binds characteristic surface receptors on these tumor cells such as CD19. The genetic information for the expression of chimeric receptor is delivered via lenti- or retroviral transduction of the T cells. These CAR T cells are reinfused into the patient to destroy their target cells [80]. Despite the impressive clinical responses found in initial trials, serious therapy-associated toxicities can occur. A recent report provided evidence that CAR T cells could also act as targeted delivery vehicles for precise delivery of therapeutic cargoes, such as herpes virus entry mediator (HVEM). HVEM is a protein that binds and activates the negative regulator B-and T-lymphocyte attenuator (BTLA) in normal B cells that limits proliferation. However, its function is frequently blocked in B cell lymphoma. Using CD19-directed engineered CAR T cells, functional HVEM protein can be delivered directly to lymphomas *in vivo*. In addition to directly attacking malignant cells, CAR T cells can thereby be used as 'micro-pharmacies' for precise therapeutic delivery [81].

**4.1.4.4. Anti-angiogenic therapies.** Angiogenesis is a hallmark of cancer. However, also in HM a role of angiogenesis has been strongly evident. Antibodies and small molecules interfering vessel formation are in clinic for solid tumors as well as HM [82,83]. The role of VEGF in angiogenesis is very well documented. Approaches to inhibit VEGF or VEGF receptors (VEGFRs) have proven successful [84]. Anti-VEGF antibody bevacizumab is approved for solid tumor treatments and is currently in clinical trials for HM, including AML, CLL, CML, NHL, and MM [82]. Midostaurin is an inhibitor of VEGFR2, PKC, PDGFR, Flt3, and c-Kit. It is recently approved for FLT3-mutated AML [85]. Other receptor tyrosine kinase (including VEGFRs) inhibitors such as vatalanib, semaxinib, sorafenib, sunitinib, cediranib are approved for various solid tumor treatments and are currently being investigated for HM [82,86]. Proteasome inhibitors have been shown to have antiangiogenic properties by downregulating VEGF expression via

**Table 2**  
Currently available treatments for hematological malignancies.

Category (MoA) <sup>a</sup>	Drug	Trade name	Indication [71,72]
Antitumor antibiotics	Bleomycin	Cerubidin®	HL, NHL
	Daunorubicin	Adriamycin®, Rubex®	AML, ALL
	Doxorubicin	Blenoxane®	AML, ALL, HL, NHL
	Idarubicin	Idamycin®	AML
	Mitoxantrone	Novantrone®	AML, NHL
Antimetabolites	Azacitidine	Vidaza®	CML
	Cladribine	Leustatin®	CLL
	Clofarabine	Clolar®	AML, ALL
	Cytarabine	Cytosar-U®	AML, ALL, HL, NHL
	Decitabine	Dacoge®	AML
	Fludarabine	Fludara®	CLL, NHL
	Hydroxyurea	Hydrea®, Droxia®	CML
	Mercaptopurine	Purinethol®	ALL
	Methotrexate	Emthexate®	AML, ALL, CLL, CML, HL, NHL
	Pralatrexate	Foloty®	NHL
	Thioguanine	Thioguanine®, Tabloid®	AML, ALL, CML
	Pamidronate	Aredia®	MM
	Zoledronic acid	Zometa®	MM
Biphosphonates <sup>b</sup>	Arsenic trioxide	Trisenox®	AML
	Tretinoin	Vesanoid®	AML
Cell-maturing agents	Bendamustine	Treanda®	CLL, HL, NHL
	Busulfan	Myleran®, Busulfex®	AML, ALL, CLL, CML, HL, NHL
DNA-damaging drugs	Carboplatin	Paraplatin®	HL, NHL
	Carmustine	BiCNU®	MM, HL, NHL
	Chlorambucil	Leukeran®	CML, NHL
	Cisplatin	Platinol®	AML, ALL, CLL, CML, HL, NHL
	Cyclophosphamide	Cytosan®, Neosar®	AML, ALL, CLL, CML, HL, NHL
	Dacarbazine	DTIC-Dome®	HL
	Ifosfamide	Ifex®	HL, NHL
	Lomustine	CeeNU®	HL
	Mechlorethamine	Mustargen®	CLL, CML
	Melphalan	Alkeran®	MM
	Nelabrine	Arranon®, Atrience®	ALL, NHL
	Procarbazine	Matulane®	HL
	Etoposide	VePesid®, Etopophos®, Toposar®	AML, ALL, CLL, CML, HL, NHL
	Teniposide	Vumon®	ALL
	Dexamethasone	Decadron®	MM
	Methylprednisolone	Medrol®	ALL
	Prednisone	Deltasone®	ALL, HL, NHL, MM
Histone deacetylase inhibitors	Belinostat	Beleodaq®	NHL
	Panabinstat	Farydak®	MM
	Romidepsin	Istodax®	NHL
	Vorinostat	Zolinza®	NHL
	Lenalidomide	Revlimid®	MM
Immune Modulators	Pomalidomide	Imnovid®, Pomalyst®	MM
	Thalidomide	Thalomid®	MM
	Paclitaxel	Taxol®	ALL
Mitotic inhibitors	Vinblastine	Velban®	HL
	Vincristine	Oncovin®	ALL, HL, NHL
Monoclonal antibodies	Alemtuzumab	Campath®, Lemtrada®	CLL, NDL
	Blinatumomab	Blinicyto®	ALL
	Brentuximab vedotin	Adcetris®	HL, NHL
	Daratumumab	Darzalex®	MM
	Gemtuzumab ozogamicin	Mylotarg®	AML
	Ibritumomab	Zevalin®	NHL
	Obinutuzumab	Gazyva®, Gazyvaro®	CLL
	Ofatumumab	Arzerra®	CLL
	Rituximab	Rituxan®	AML, ALL, CLL, CML, HL, NHL
	Tositumomab	Bexxar®	FNHL
Phosphoinositide 3-kinase inhibitors	Idelalisib	Zydelig®	CLL, NHL
Proteasome inhibitors	Bortezomib	Velcade®	MM, NHL
	Carfilzomib	Kyprolis®	MM
	Ixaomib	Ninlaro®	MM
	Bosutinib	Bosulif®	CML
Tyrosine kinase inhibitors	Dasatinib	Sprycel®	ALL, CML
	Ibrutinib	Imbruvica®	CLL
	Imatinib mesylate	Gleevec®, Glivec®	ALL, CML
	Nilotinib	Tasigna®	CML
	Omacetaxine mepesuccinate	Synribo®	CML
	Ponatinib	Iclusig®	CML

MoA = mechanisms of action, HL = Hodgkin's lymphoma, NHL = non-Hodgkin's lymphoma, AML = acute myeloid leukemia, ALL = acute lymphoid leukemia, CML = chronic myeloid leukemia, CLL = chronic lymphoid leukemia, MM = multiple myeloma, FNHL = follicular non-Hodgkin lymphoma.

<sup>a</sup> Some Chemotherapeutic agents possess multiple mechanisms of action. Classification here is based on their primary mechanism.

<sup>b</sup> Bisphosphonates are not part of cytotoxic treatment protocols, but are used as part of supportive care in selected malignancies, multiple myeloma for instance.

p53 induction and inhibition of NF $\kappa$ B pathway [82,86,87]. Proteasome inhibitors bortezomib, carfilzomib and ixazomib are approved for multiple myeloma. Immune modulatory drugs thalidomide and its analogs lenalidomide and pomalidomide are in clinic for treatment of multiple myeloma. They have antiangiogenic properties by downregulating VEGF secretion and inhibiting PI3K-Akt signaling pathway [86,88]

#### 4.2. Novel drugs

Novel treatment approaches based on recent insights into the molecular mechanisms of malignant transformation is used to design molecules that specifically target specific steps in disease pathogenesis. On average, only 1 in 1000 of these molecules will make it to human testing. This high attrition rate is primarily due to tox/PK/PD and is eventually reviewed by regulatory authorities. Of those, that do make it this far, only a 1 of 5 will be approved, (as a result of) patient variability, disappointing efficacy, and limited predictability of *in vitro* and *in vivo* models. Therefore, new molecules have only a 1:5000 chance of reaching regulatory approval [89].

##### 4.2.1. Novel kinase inhibitors

Second and third generations of kinase inhibitors aim to overcome the development of resistance generated by the first line drugs. MK-0457 (tozasertip/VX680) is an example [90,91]. MK-0457 not only overcomes mutant T315I of ABL1 but also potently inhibits aurora kinases. Unfortunately, this drug exhibited strong cardiac toxicity [92]. Danusertib (PHA-739358), is a potent inhibitor of all three Aurora kinases with additional inhibition of both wild type and mutant ABL as well as several other kinases. The drug has been tested in a phase II study in patients with CML who relapsed on imatinib. The study recorded two complete hematologic responses in patients carrying the T315I mutant. Dosing was via a 6h infusion, once weekly, which was well tolerated. In a phase I study, adults with either accelerated or blastic phase CML or BCR-ABL1<sup>+</sup> ALL resistant cases were enrolled. 20% of the patients responded to the treatment, however, adverse events included anemia, diarrhea, and febrile neutropenia [93]. PIM kinases promote proliferation and accelerate downstream cytokine and growth factor signaling networks and are frequently upregulated in leukemia and lymphoma. [94]. AZD1208 is a potent and highly selective pan-PIM kinase inhibitor that showed efficacy in AML in preclinical models. The phase I study in AML patients was, however, terminated before completion because of dose limiting toxicities and no clear evidence of antitumor activity [95–97].

##### 4.2.2. Negative feedback inhibitors

Insights into regulatory mechanisms have revealed new treatment targets: signals from the B cell receptor (BCR) are essential for survival and proliferation of normal B cells. This would argue for inhibition of the BCR as therapeutic strategy. Surprisingly, however, in specific B-cell malignancies this signaling is disrupted. Depending on the developmental stage and (costimulatory) context, also hyperactive signaling from a self-reactive BCR by the ubiquitous presence of a self-antigen can induce negative selection and cell death. We have recently shown that in BCR-ABL1-driven ALL, not only a reduction of signaling strength in pre B ALL (i.e. by TKI) results in significant cell death but also an increase of (pre-)BCR signaling [98]. Therefore, agonist and antagonists of this signaling pathway may represent novel treatment targets. Careful dosing and timing is of particular relevance for this class. Improved drug delivery that promoted optimal concentration and sufficient serum half-life may be critical for this strategy.

##### 4.2.3. Targeting apoptosis

The hematopoietic system has a high turnover rate and strict control of cellular apoptosis is essential for homeostasis. In HM the apoptosis program is frequently deregulated resulting in uncontrolled

proliferation and accumulation of malignant cells. In addition, resistance to apoptosis is a major cause for treatment failure. Thus, the deregulated apoptosis pathway in HM is a promising therapeutic target. A variety of cell death promoting and inhibiting proteins that constitute potential targets comprise death receptors and ligands, including DR4/5, TRAIL, the inhibitor of apoptosis (IAP) family or the second mitochondria-derived activator of caspase (SMAC). Furthermore, the pro-survival Bcl-2 protein family, provide attractive targets. Death receptor agonists and ligands include mapatumumab a monoclonal antibody targeting DR4 (Glaxo Smith Kline/Human Genome Science), drozitumab, a monoclonal antibody for DR5 (Genentech), and the soluble ligand rhApo2L/TRAIL for DR4/5 (Genentech and Amgen). Furthermore, the IAP survivin can be inhibited by the small molecule antagonist YM155 (Astellas Pharma) [99,100]. All compounds are in Phase II clinical trials. YM155 competes for the binding sites of the transcription factor Sp1 on the survivin promoter and thereby prevents its transcription. Additionally, SMAC mimetics, such as LCL161 by Novartis, are also under clinical investigation. Different strategies to inhibit Bcl-2 proteins have been developed and comprise small molecule inhibitors, drugs targeting Bcl-2 mRNA and BH3 peptidomimetics, the natural inhibitor of Bcl-2 proteins. Despite overall encouraging progress, the therapeutic window is small. For example the poor pharmacological properties of BH3 mimetics has prevented their entry in clinical trials [101]. A small molecule inhibitor obatoclax mesylate, in phase 1-2 (Gemin X/Teva Pharmaceutical Industries) is limited in dose through transient neurotoxicity as most common adverse event [102]. Another small molecule inhibitor ABT-199 showed efficacy in ALL and lymphoma mouse models and also a clinical trial with CLL patients has been initiated, with success in reduction of lymphadenopathy and peripheral blood lymphocytes, however scheduling and dosing of ABT-199 needs to be improved, as the tumor lysis syndrome frequently occurred [103]. Approaches to activate caspases are under pre-clinical evaluation but have not yet entered clinical trials.

##### 4.2.4. Other agents

Screening of approved drugs for activity in HM has resulted in surprising activities [104]. For example a NO modification to the first approved HIV protease inhibitor saquinavir (Saq-NO) resulted in activity against ALL, AML. The intracellular p70S6 kinase of the mTOR pathway might be the target of Saq-NO [105]. The nuclear export receptor, exportin 1 (XPO1) is hyperactive in aggressive lymphomas, AML and CLL. It mediates, for example, the transport of tumor suppressors. KPT-330 (selinexor), an XPO1 blocker, entered clinical phase I/II, but systemic toxicities occur. A next-generation XPO1 inhibitor, termed KPT-330 was designed, possessing similar potency *in vitro* and increased tolerability, even when dosed daily. Also enhanced survival in AML and CLL mouse models compared to KPT-300 was observed [106].

Taken together, the newer generation chemotherapeutics are potent drugs, however, their off-target kinase inhibiting activity frequently lead to severe adverse effects. The balance between on- and off-target is of crucial importance in order to achieve a wide therapeutic window. Most conventional therapies, including chemotherapy, generally lack specificity and selectivity towards the molecular process of transformation or towards the site of the disease. Therefore, healthy organs are exposed with the cytotoxic drugs, which result in off-target adverse effects. Moreover, most of the chemotherapeutic drugs as well as several small molecules are associated with pharmacokinetic challenges, i.e. poor solubility, short biological half-life, large volume of distribution, and rapid clearance. Furthermore, binding to plasma proteins such as albumin and  $\alpha$ -1 acid glycoprotein (AGP) results in low bioavailability of the drugs [44]. This may result in resistance to the therapy and disease relapse. Resistance may also occur by drug efflux by e.g. p-glycoproteins [45]. Consequently, escalated doses and more frequent dosing are often required to maintain the therapeutic concentrations of the drug in the target tissue. To overcome these challenges associated with conventional chemotherapy and to improve the safety profiles,



there is a currently unmet need for better therapy selectivity: either based on molecular pathways or based on targeted localization, or both. Using drug delivery systems, which are more specific to the tumor site (s) and minimally affect the healthy tissues, therefore offer great potential in the treatment of HM. Drug delivery approaches by using nanomedicines offers a tool to ameliorate the therapeutic index of the potent anticancer drugs by improving overall pharmacological properties i.e. tox/PK/PD. Nanomedicines have proven to be successful in overcoming the shortfalls of conventional therapy. The following section focuses on the rationale of using nanomedicines in HM. Additionally, an overview is given of the different types of nanoparticles that currently are being used in the clinic or that are in various stages of the (pre-) clinical development.

## 5. Nanomedicines for drug delivery in hematological malignancies

Nanomedicines are small sub-micronized particles that, when delivered intravenously, tend to accumulate passively at the site of inflammation. Solid tumors display a chronic inflammatory phenotype featured by angiogenesis and infiltration of immune cells. Tumors require angiogenesis to fulfill high nutrition and oxygen demands to be able to multiply. The ability of tumors to stimulate this process leads to rapid and uncontrolled neovascularization resulting in an irregular shape, dilated lumen, and leaky architecture of the blood vessels walls. Basement membrane in these vessels is also abnormal and poorly organized. In addition, the deranged inflammatory signaling in and around tumors leads to a chronic pro-inflammatory state further increasing capillary permeability. These abnormal anatomical features allow macromolecules and nanoparticles below a size of several hundreds of nanometers to passively extravasate into tumor tissue (enhanced permeability). At the same time, poor lymphatic drainage in tumors and extracellular matrix hindrance limits clearance (enhanced retention). The EPR effect in solid tumors was described more than 30 years ago by Maeda and colleagues [107]. In contrast to solid tumors, this EPR phenomenon is not very much appreciated in hematological malignancies. The diffuse localization of HM and its vascular phase might appear to make EPR less relevant. However, there is strong evidence of increased angiogenesis in these “liquid” tumors as well. Increased microvessel density and/or infiltration of inflammatory cells is seen in bone marrow of patients suffering from virtually all types of HM [84,108–114]. Increased microvessel density and endothelial cell mass plays an important role in providing nutrients and oxygen to malignant cells. In addition, malignant cells also produce angiogenic factors and express cognate receptors that support angiogenesis and tumor cell proliferation and expansion in HM [115]. CLL is one example where proliferation is occurring primarily in secondary lymphoid organs with an inflammatory signature. CLL cells mediate activation of the proinflammatory transcription factor NFκB and this activation is critical for CLL engraftment and disease progression in mouse models [116]. Since HM also rely on angiogenesis to rapidly develop and expand, anti-angiogenic therapy, which is extensively studied for solid tumor treatment, has also been proved successful for the treatment of HM [84]. Apart from accumulating at the tumor site, nanomedicines are also inclined to accumulate in organs of the mononuclear phagocytic system (MPS) such as liver, spleen and bone marrow. These organs contain large numbers of phagocytes and interestingly also are primary and/or secondary target sites in most of the hematological malignancies (see Section 3). Hence, targeting these organs can further improve the therapeutic efficacy. As described in previous sections, peripheral blood is one of the prominent target sites in HM. Long circulation properties would enhance the probability of NPs to encounter malignant cells in peripheral blood. Furthermore, most NPs are less than 200 nm in diameter [1]. Due to their small size, accumulation of NPs increases in the bone marrow, the site of origin in most HM [36,117]. Several reports have shown accumulation of small, long circulating liposomes in bone

marrow by passive diffusion [118,119]. A more detailed overview of specific properties of NPs for BM targeting is discussed in Section 6. The next sections describe nanomedicines.

### 5.1. Liposomes

Liposomes were first described by Alec Bangham in 1965 [120]. In the early 1970s, it was hypothesized that liposomes could have potential as drug delivery systems. Since then, several liposomal drugs have received regulatory approval for treatment of solid tumors as well as HM. Liposomes are composed of membrane forming phospholipids and cholesterol that form, in aqueous solutions, metastable spherical structures with a lipid bilayer surrounding an internal aqueous compartment. The structure is stabilized by hydrophobic interactions and additionally by hydrogen bonds, van der Waals forces, and electrostatic interactions. The amphiphilic property of liposomes offers an excellent opportunity to encapsulate a wide range of hydrophobic and hydrophilic drug molecules in the lipid bilayer and in the aqueous compartment, respectively. Conventional liposomes contain a phospholipid bilayer, which often includes cholesterol. Use of cholesterol improves the stability of the membrane bilayer and prevents leakage of drugs encapsulated in the aqueous core. Second generation liposomes provide a longer circulation time by reducing opsonization by poly(ethylene glycol) (PEG) coating. PEG is a hydrophilic polymer that provides steric stabilization to liposomes that prevents opsonin adherence to the surface. A third generation of liposomes is additionally decorated with targeting ligands specific to proteins or receptors present on the target cell surface (Fig. 3). Many malignant cells express increased levels of certain receptors such as growth factor receptors, as compared to normal cells, which can be targeted by specific peptides and/or antibodies.

#### 5.1.1. Clinically available liposomal formulations for the treatment of hematological malignancies

Currently there are four liposomal formulations available in the clinic that are used for the treatment of HM (Table 3).

**5.1.1.1. Liposomal doxorubicin (Doxil®/Caelyx®/LipoDox®).** Doxorubicin is an anthracycline used as a first line therapy in many cancers. Doxorubicin shows antitumor activity by intercalating into the DNA double helix. It interferes with DNA and RNA synthesis and inhibits the DNA topoisomerase II enzyme in tumor cells. Doxorubicin treatment is limited by side effects; the dose-limiting toxicity is cumulative dose-related cardiotoxicity. Other side effects include myelosuppression, nausea, vomiting, and mucocutaneous effects. The safety profile of doxorubicin has been improved by liposomal encapsulation. Liposomal doxorubicin shows reduced cardiotoxicity allowing intensified treatment schedules. The pharmacokinetic profile has been improved distinctly with extended circulation time and small volume of distribution limited to the plasma volume, as compared to free drug. The EPR effect can result in enhanced accumulation in tumor tissue. Nevertheless, the ground for regulatory approval was mainly based on reduced cardiotoxicity. A recent case report described a patient that has received 115 cycles of liposomal doxorubicin without apparent cardiotoxicity. The basis for this improvement is the non-permeable vasculature in the heart, reducing exposure. Although cardiotoxicity is reduced, new toxicities can emerge as a result of the changed tissue distribution e.g. liver, spleen, bone marrow toxicities and hand-foot syndrome etc. Although tumor accumulation is higher, this does not automatically translate into improved efficacy. The liposomal membrane prevents toxicity but also prevents bioactivity of the doxorubicin. Therefore, the doxorubicin is not active until the membrane integrity is compromised. This can happen in the tumor interstitium, in the tumor cells or in stromal cells. As a result the peak concentrations of doxorubicin may be substantially lower than for free doxorubicin, which may impact its therapeutic effect. Two types of liposomal doxorubicin have been developed and approved for clinical

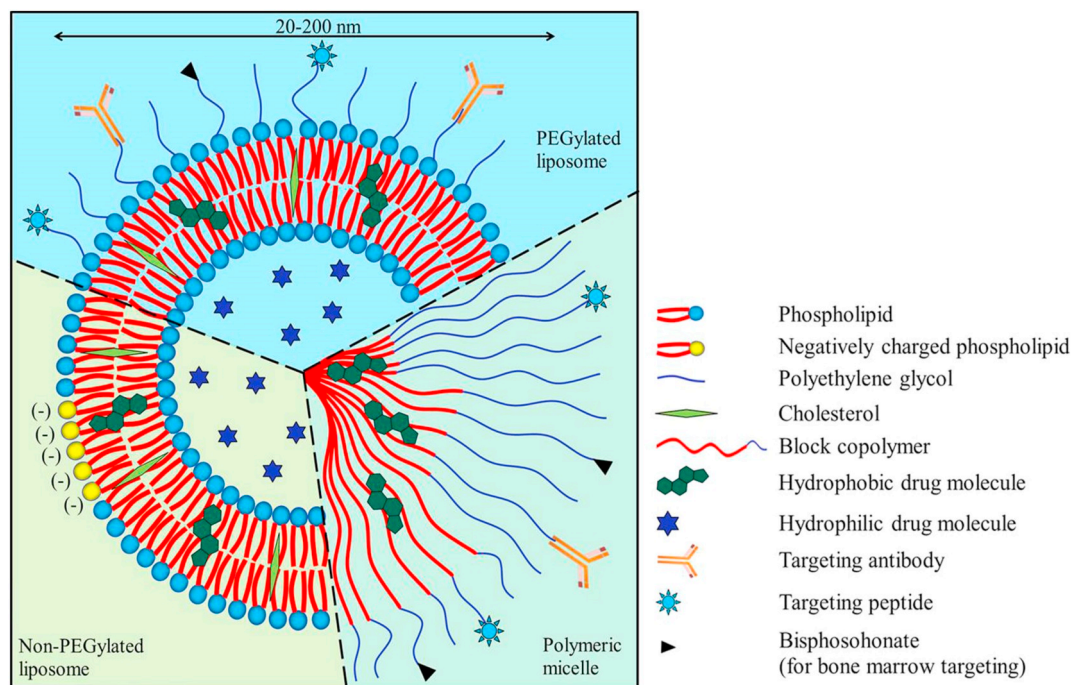


Fig. 3. Schematic representation of a nanoparticle showing liposomes and polymeric micelles.

use, PEGylated liposomal doxorubicin (PLD) and Non-PEGylated liposomal doxorubicin (NPLD). PLD is indicated for the treatment of AIDS-related Kaposi's sarcoma and hematological malignancies whereas NPLD (Myocet®) is approved in Europe and Canada for the treatment of metastatic breast cancer in combination with cyclophosphamide. Doxil®, PEGylated liposomal doxorubicin hydrochloride (PLD) received accelerated approval in 1995 for the treatment of AIDS-related Kaposi's sarcoma. In 2007, Doxil® received approval in combination with proteasome inhibitor bortezomib for the treatment of multiple myeloma patients who have received at least one prior therapy not including bortezomib. This approval was based on a randomized phase III clinical trial with 646 patients having progressive multiple myeloma [121]. PLD with bortezomib was found to be superior to bortezomib monotherapy in this study effectuating increased median time to progression (9.3 months vs. 6.5 months), improved 15-month survival rate (76% vs. 65%), and increased median duration of response (10.2 vs. 7.0 months). However, Grade 3/4 adverse events were more frequent in the combination group (80% vs. 64%). The safety profile was consistent with the known toxicities of the two drugs. An increased incidence in the combination group was seen of grade 3/4 neutropenia, thrombocytopenia, asthenia, fatigue, diarrhea, and hand-foot syndrome. Despite these toxicities, PLD has been successfully used for the treatment of multiple myeloma. Interestingly, only liposomal doxorubicin is used for the treatment of multiple myeloma but not the free drug.

**5.1.1.2. Liposomal daunorubicin (DaunoXome®).** Daunorubicin is another antineoplastic anthracycline closely related to doxorubicin with a similar activity profile. Daunorubicin, in combination with other drugs, is given for the treatment of hematological malignancies including acute non-lymphocytic leukemia (myelogenous, monocytic, erythroid) in adults and acute lymphocytic leukemia in children and adults. DaunoXome® is a non-PEGylated liposomal formulation of daunorubicin. Similar as for doxorubicin, liposomal encapsulation of daunorubicin improved the therapeutic index of this drug. A favorable pharmacokinetic profile was observed for DaunoXome®. A recent phase III clinical trial demonstrated improved long term survival with DaunoXome® in comparison to the free drug in patients with AML

aged 60 and above [122]. Also in pediatric relapsed AML patients, DaunoXome® showed improved therapeutic outcome when combined with fludarabine, cytarabine and granulocyte colony-stimulating factor (FLAG) compared to FLAG treatment alone [123].

**5.1.1.3. Liposomal vincristine (Marqibo®).** Vincristine is a semisynthetic vinca alkaloid. Vincristine induces apoptosis, primarily by inhibition of mitosis at the metaphase through its binding to tubulin [152]. Marqibo®, the liposomal formulation of vincristine, also termed vincristine sulphate liposome injection (VSLI), comprises a sphingomyelin and cholesterol bilayer, encapsulating vincristine sulfate in the aqueous core. Marqibo® received accelerated approval by the FDA in 2012 for the treatment of patients with Philadelphia chromosome-negative ALL [153]. This approval was based on the results of a single-arm trial (HBS407 trial). In this trial, 65 ALL patients older than 18 years received Marqibo® [154]. The complete remission was 4.6% (3/65 patients), and the percentage of complete remission with incomplete blood count recovery was 10.8% (7/65 patients). A recent phase II clinical trial also supports Marqibo® as a better treatment choice for the treatment of ALL in adults [155]. In a phase I study using Marqibo® in pediatric ALL patients, an improved therapeutic outcome was also found [156]. Marqibo®, as part of a regimen containing cyclophosphamide, doxorubicin, prednisolone and rituximab, or just with or without rituximab, for treatment of patients with (relapsed and refractory) aggressive Non-Hodgkin lymphomas, also showed favorable phase II results [157,158].

**5.1.1.4. Liposomal cytarabine + daunorubicin (CPX-351/VYXEOS™).** VYXEOS™ or CPX-351 has been recently approved after positive results in a Phase III trial. Cytarabine and daunorubicin combination therapy, known as “7+3” in the field, is a standard regimen for the treatment of AML. CPX-351, a liposomal formulation co-encapsulating cytarabine:daunorubicin in a synergistic molar ratio of 5:1 showed the largest therapeutic index over a range of free drug combinations in preclinical *in vivo* experiments [159]. A phase III randomized open label study of CPX-351 in 309 elderly patients with newly diagnosed high-risk secondary AML showed significantly

**Table 3**  
Approved and (pre-)clinical liposomal formulations for the treatment of hematological malignancies.

Product [Company]	Type of nanomedicine	Drug	Indication (Hematological malignancy(ies))	Development stage	Reference(s)
Doxil®/Caelyx® [Johnson & Johnson]	Liposome	Doxorubicin	Kaposi's sarcoma, ovarian cancer, breast cancer, multiple myeloma, lymphoma	Market	[1,124–126]
DaunoXome® [Gilead Sciences, Inc.]	Liposome	Daunorubicin	Acute myeloid leukemia, non-Hodgkin lymphoma, leukemia and solid tumors	Market	[124]
Marqibo® [Talon] [Spectrum Pharmaceuticals]	Liposome	Vincristine	Acute lymphoid leukemia, non-Hodgkin lymphoma	Market	[124,126–128]
CPX-351 [Celator]	Liposome	Cytarabine + daunorubicin	Acute myeloid leukaemia	Market	[1,2,126,129]
Onco-TCS [Inex/Enzon]	Liposome	Vincristin	Non-Hodgkin lymphoma	Phase II/III	[4,130]
Oncocort® [Enceladus Pharmaceuticals]	Liposome	Dexamethasone phosphate	Multiple myeloma	Phase I/IIa	[131]
L-Annamycin [Callisto]	Liposome	Annamycin	Acute lymphoid leukemia, acute myeloid leukemia,	Phase I/II	[124,132]
Liposomal tretinoin (ATRA-IV) [MD Anderson/NCI]	Liposome	Tretinoin	Refractory Hodgkin disease, kidney cancer, solid tumors, Acute promyelocytic leukemia	Phase I/II	[124,133]
DCR-MYC (DCR-M1711) [Dicerna Pharmaceuticals]	Lipid nanoparticle <sup>a</sup>	Dicer substrate RNAi (DsiRNA) targeting MYC oncogene	Solid tumors, multiple myeloma, lymphoma	Phase I	[4,134]
LEM-ETU [NeoPharm/Insys]	Liposome Cationin liposome	Mitoxantrone	Solid tumors, lymphoma, acute myeloid leukemia, multiple sclerosis, and prostate cancer	Phase I	[135–137]
MRX34 [Mima Therapeutics]	Liposome	miR-RX34	Liver cancer, solid tumors, lymphoma, myeloma	Phase I	[138–140]
PNT2258 [ProNAi Therapeutics]	Liposome	DNAi targeting BCL-2	Non-Hodgkin lymphoma, solid tumors	Phase I	[141,142]
Liposomal Grb-2 [MD Anderson/Bio-Path]	Liposome	Grb2 antisense nucleotide	Leukemia	Pre-clinical	[4,143]
Nanobins	Liposome	Arsenic trioxide	Lymphoma	Pre-clinical	[1,144]
-	Liposome	Bortezomib	Chronic myeloid leukemia, , neuroblastoma	Pre-clinical	[145–148]
-	Liposome	Carfilzomib	Multiple myeloma	Pre-clinical	[149–151]

<sup>a</sup> Not a liposomal formulation.

improved overall survival, event free survival, and response. There were equal Grade 3-5 adverse events with similar frequency and severity in both CPX-351 and “7 + 3” arms [160–162].

### 5.1.2. Liposomal formulations in (pre-)clinical stages for the treatment of hematological malignancies

Apart from the successful clinical translation of above-mentioned liposomal formulations, there are several other liposomal formulations being tested in pre-clinical and clinical stages (Table 3).

Glucocorticoids (GCs) are a class of molecules that inhibits tumor growth by multiple mechanisms of action including antiangiogenic, anti-inflammatory and antitumor activities [163–166]. The clinical outcome of GCs treatment is limited due to their short half-life in circulation and serious off-target adverse effects [167]. We have previously shown that liposomal encapsulation of (GCs) substantially improved the therapeutic index in various solid tumor models. Liposomal prednisolone phosphate (LCL-PLP) showed tumor growth inhibition in melanoma and colon carcinoma mouse models [168–170]. In a spontaneous breast carcinoma model, LCL-PLP showed enhanced accumulation into tumors, and superior efficacy compared to free drug [165]. Oncocort® (Enceladus Pharmaceuticals), a liposomal formulation of dexamethasone phosphate is currently in phase I/IIa as a monotherapy in patients with progressive multiple myeloma [131].

Proteasome inhibitors induce apoptosis in the malignant cells by inhibiting 26S proteasome activity [171]. Moreover, they also possess antiangiogenic properties [87]. Bortezomib, carfilzomib, and ixazomib are approved proteasome inhibitors for MM treatment. Although these small molecules have improved overall responses, dose-related toxicity often remains a challenge. Bortezomib treatment resulted in minimal benefits and considerable toxicity in a pilot study in patients with imatinib-refractory CML [172]. Liposomally encapsulated bortezomib, however, showed prolonged blood circulation and decreased clearance compared to the free drug, and better efficacy/toxicity profiles in CML animal model [145]. In MM and neuroblastoma, liposomal bortezomib showed remarkable tumor growth inhibition with reduced systemic toxicity compared to free drug *in vivo* [146,147]. Similarly, liposomal carfilzomib showed significant tumor growth inhibition and less toxicity in MM animal models. Synergy was observed when liposomal carfilzomib was combined with free doxorubicin, or co-encapsulated liposomal doxorubicin [149,150]. This data strongly suggest that liposomal encapsulation would increase the therapeutic index of potent chemotherapeutics by improving their tox/PK/PD profiles.

### 5.2. Polymer-based nanomedicines

Polymer-based nanomedicines offer an infinite variety of building blocks to tailor the system's characteristics to meet the needs of different compounds. Polymers can be biocompatible, the release of the active compound can be tailored and they can respond to specific stimuli, such as pH or temperature. Polymeric micelles (PM) made of block copolymers with an amphiphilic nature, enable them to load hydrophobic compounds in the core. The development of PM as drug delivery systems started more than three decades ago with seminal work of Bader and co-workers [173]. PM form spontaneously when amphiphilic block copolymers are brought in an aqueous environment. During PM formation, the hydrophobic interactions between the hydrophobic building blocks will form the core whereas the hydrophilic chain engages with H-bridges with water to form the shell of the micelles. The core-shell structure enables the segregation of hydrophobic compounds in the core of the micelles while the hydrophilic shell guarantees colloidal stability and a prolonged circulation *in vivo*; the small average diameter of PM, 10–100 nm, and a near neutral surface charge are advantageous for efficient accumulation in target tissues by means of the EPR effect. The chemical composition, molecular weight of the polymer blocks and the ratio between the blocks can be tailored in order to design formulations with favorable size, drug loading and



**Table 4**  
Investigational polymer-based nanomedicines for the treatment of hematological malignancies.

Product [company]	Type of nanoparticle	Drug	Indication (Hematological malignancy(ies))	Development stage	Reference
ABI-011 (nab5404) [Abraxis BioScience/Celgene]	Albumin nanoparticle	Thiocolchicine dimer (IDN 5404)	Solid tumors, lymphoma	Phase 1	[192,193]
Abraxane® [Mayo Clinic]	Albumin-stabilized nanoparticles	Paclitaxel	Multiple Myeloma	Phase 2	[194]
CD19-DOX-NPs	Polymeric nanoparticle	Doxorubicin	ALL	Pre-clinical	[195]
SP1049C [Supratek Pharma]	Pluronic-based micelles	Doxorubicin	AML	Phase 3	[182,183]
CFZ-PM	Polymeric micelle	Carfilzomib	Lung cancer, Multiple Myeloma	Pre-clinical	[196]
Folate and retinoic acid grafted/dextran (FA-RA/DEX)	Polymeric micelle	Doxorubicin	AML	Pre-clinical	[197]
NK012 [Nippon Kayaku Co., Ltd.][180]	Polymeric micelle	Prodrug of 7-ethyl-10-hydroxy camptothecin (SN-38)	Multiple Myeloma	Phase 1/2 JapicCTI-111652	[185]
NC-4016 [NanoCarrier]	Polymeric micelle	Oxaliplatin	Solid tumors, lymphoma	Phase 1 NCT01999491	[198]
Polybutylcyanoacrylate nanoparticles	Polymeric nanoparticle	Imatinib mesylate	Leukemia	Pre-clinical	[199]
PEG-PCL bearing pendant cyclic ketals	Polymeric nanoparticle	Dexamethasone	ALL	Pre-clinical	[200]
Alendronate-modified PEG-PLA	Polymeric micelle	Ponatinib and SAR302503	CML	Pre-clinical	[201]

release. The shell of the PM provides the possibility of decoration with specific antibody or peptide ligands to target the particles towards specific cell population.

#### 5.2.1. Polymeric nanomedicines in (pre-)clinical stage for the treatment of hematological malignancies

HM are not often investigated first when developing polymeric nanomedicine therapies. For solid tumors, two polymeric micelles-based formulations are currently on the market. Genexol®-PM is a paclitaxel-encapsulating micelle for the treatment of various types of cancers such as ovarian, small-cell lung, pancreatic, breast and bladder cancer [174,175], based on enabling work by Kataoka and co-workers [176]. Several other PM nanoparticle formulations are currently under clinical evaluation for the treatment of solid tumors such as breast cancer (phase III) and colorectal cancer (phase II) [177–181].

PM formulations showed promising results in pre-clinical studies and are presently being evaluated clinically for the treatment of hematological cancers (Table 4). SP1049C is a formulation composed of doxorubicin loaded in a blend of two non-ionic Pluronic® block copolymers. SP1049C has an average diameter of 30 nm and a drug loading capacity of 8%. In a murine leukemia model animals treated with the SP1049C formulation showed a decreased ability of the tumor cells to form colonies, and decreased tumor growth rate [182]. Moreover, SP1049C also prevented multidrug resistance in leukemic cells [183].

NK012, is a PM loaded with the prodrug of SN-38 (7-ethyl-10-hydroxycamptothecin) [184]. SN-38 is chemically conjugated to the block copolymer, PEG-b-poly(L-glutamic acid), via esterification of the phenol group present in the drug molecule and the carboxylic acid group in the polymer backbone. The freeze-dried product contains 20% (w/w) of SN-38 and has an average diameter after reconstitution of 20 nm. The anti-myeloma activity of this formulation was assessed in an orthotopic model of multiple myeloma in immunodeficient mice [185]. Mice were treated with NK012 at 9.4 mg/kg/day every week for 6 weeks. Bortezomib was also included in this study and was injected in combination with NK012 at a dose of 0.5 mg/kg/day every 4 days in a total of 8 injections. The dosing schedule of NK012 and bortezomib was selected based on their clinically recommended dose. NK012 decreased the percentage of myeloma cells in a dose-dependent manner. Plasma levels of M protein in untreated animals were significantly higher. In animals treated with NK012 M protein concentrations in plasma were diminished and were similar to those of animals treated with bortezomib. Both treatments also decreased bone destruction compared to untreated mice. The combination of NK012 with bortezomib prolonged survival to 83.5 days, while survival of untreated animals was 42.5 days on average. NK012 is currently clinically evaluated for the treatment of breast, lung and colorectal cancer [186–188]. Also a phase I/II clinical

trial is initiated in patients with relapsed or refractory MM in Japan [189].

NC-4016 is a polymeric micelle formulation loaded with the platinum-based drug (1,2-diaminocyclohexane)platinum(II) (DACHPt) [190]. Platinum-based drugs are extensively used for ovarian cancer, melanoma and lymphoma. Their cytotoxic effect is due to the formation of a chelate-complex with DNA, forming platinum-DNA adducts that alter the conformation of DNA. The administration of the free drug leads to systemic toxicity including nephro- and neurotoxicity. DACHPt is a platinum drug with less cytotoxicity than its parent compound oxaliplatin. NC-4016 is formulated by loading DACHPt in PEG-poly( $\gamma$ -benzyl-L-glutamate) block copolymers via metal-complex formation between the drug and the carboxylic group of the polymer backbone. The micelles have an average diameter of 40 nm and a drug loading capacity of 75% (w/w). NC-4016 efficiently targets the primary tumor in an orthotopic model of scirrhous gastric cancer as well as the lymphatic metastases. NC-4016 efficiently inhibited tumor growth. This formulation is currently being tested in a phase I trial in patients with advanced solid tumors or lymphoma, [191].

#### 5.3. Other drug delivery systems for the treatment of hematological malignancies

Drug conjugates include protein-drug conjugates, antibody-drug conjugates, lipid-drug conjugates, PEG-protein conjugates, and PEG-drug conjugates. Abraxane® is an albumin-based protein-drug conjugate formulated with paclitaxel. Paclitaxel is an antimitotic agent that exerts its function by stabilizing the microtubules and preventing their disassembly during cell division. Abraxane® is approved for the treatment of advanced non-small cell lung cancer, metastatic breast and pancreatic cancers and is currently under clinical evaluation for the treatment of multiple myeloma and lymphomas [202]. The formulation was tested in a clinical phase II trial in relapsed or refractory MM patients [203]. Patients received the formulation intravenously at day 1, 8 and 15. Treatment was repeated every 28 days up to 12 dosages when no adverse effects were present. To date, no results are available as the study is still ongoing. The same formulation was tested in MM patients in combination with lenalidomide in a phase I/II trial [202]. Abraxane® was dosed weekly at 100 mg·m<sup>-2</sup> for 3 weeks together with 10 mg of lenalidomide daily for 21 days, with a dose escalation for lenalidomide up till 25 mg. Also here results on efficacy have not been published. Nab5404 (formerly ABI-011) is a formulation of albumin nanoparticles loaded with a thiocolchicine dimer (IDN 5404). IDN 5404 was selected for its dual activity as an anti-tubulin agent and topoisomerase-I inhibitor. It acts as a vascular disrupting agent (VDA) and is able to lead to rapid collapse of vascular tissues. This formulation was tested pre-



**Table 5**  
Clinically available and in (pre-)clinical development drug-conjugates nanomedicines for HM.

Product [Company]	Type of conjugate	Drug	Indication (Hematological malignancy (ies))	Development stage	Reference(s)
Brentuximab vedotin (Adcetris®) [Seattle Genetics]	Antibody-drug conjugate	MMAE	Hodgkin lymphoma	Market	[205]
Ibritumomab tiuxetan Zevalin® [IDEC/Spectrum]	Antibody-drug conjugate	Yttrium-90 or Indium-111	Non-Hodgkin lymphoma	Market	[206]
Oncaspar™ (PEG) [Baxalta]	PEG protein conjugate	L-asparaginase Asparaginase paclitaxel	ALL	Market	[2,126,209]
Elacytarabine (Clavis Pharma)	Lipid-drug conjugate	Cytarabine	AML	Phase III	[1,210–213]
PegAsys [Hoffmann-La Roche]	PEG protein conjugate	IFNα2a/-IFNα2b	CML	Phase III	[2,214]
PEG-SN38 (EZN-2208) [Belrose Pharma/Enzon]	PEG drug conjugate	SN38 (irinotecan derivate)	lymphoma, solid tumors, breast cancer, colorectal cancer	Phase II	[215,216]

clinically in tumor xenograft models of ovarian and colon cancer showing promising results [98,99]. A phase I trial started in 2010 for the treatment of advanced solid tumors and lymphomas [204]. The study has been completed, however no results are available yet. Antibody-drug conjugates like, brentuximab vedotin (Adcetris®), and ibritumomab tiuxetan (Zevalin®) are approved for the treatment of HL and NHL, respectively [205,206]. Brentuximab vedotin is an anti-CD30 antibody conjugated via a valine-citrulline peptide linker to the anti-mitotic agent monomethyl auristatin E (MMAE). After CD30-mediated internalization, proteolytic enzymes cleave off MMAE, leading to tubulin polymerization and G2/M-phase growth arrest followed by apoptosis [205,207,208]. Similarly, ibritumomab tiuxetan is an anti-CD20 antibody conjugated to a radioactive isotope: either Yttrium-90 or Indium-111 [206]. Finally, Oncaspar™ is a PEG conjugate of asparaginase, which is approved for first line treatment of children with ALL [209]. Table 5 depicts approved and investigational drug conjugates for HM.

## 6. Targeting the bone marrow in hematological malignancies with particulate delivery systems

It is clear that the bone marrow microenvironment is a crucial site in the development and progression of hematological malignancies. Targeting the bone marrow microenvironment can be improved by drug delivery systems and can be achieved passively or actively. Various factors play roles in targeting of nanomedicines to the bone marrow, including particle size, composition, and surface charge of nanoparticles.

### 6.1. Particle size

Nanomedicines are naturally inclined to accumulate in the bone marrow. Reticuloendothelial sinusoidal blood capillaries consist of pores as large as 60 nm in diameter. Therefore, nanoparticles below this size can penetrate and distribute into the bone marrow interstitial space [217]. Notably, like in solid tumors, the bone marrow also displays local inflammation and angiogenesis during HM [108]. Also here, tumor development is dependent on growth factors such as VEGF that facilitate rapid formation of angiogenic blood vessels. However, these blood vessels are abnormally structured and tortuous causing blood flow to be heterogeneous [218]. Additionally, the endothelial cell-cell junctions are less tightly coupled leading to increased permeability and leakiness [219]. As a result, the abnormal vessel wall structure is characterized by a large diversity in inter-endothelial junction widths with maximum pore diameters as large as several hundred nanometers, allowing even nanoparticles of this size to extravasate into the tumor [218,220,221]. Size is also important because of the fact that smaller liposomes < 100 nm in diameter circulate longer in the circulation, have less interaction with plasma proteins, and escape uptake by MPS. Larger liposomes would be cleared more rapidly from the circulation.

On the other hand liposomes smaller than 50 nm will limit the drug encapsulation efficiency [222].

### 6.2. Composition

Lipid composition affects the uptake of liposomes by bone marrow. Cholesterol plays an important role in the stability of liposomal formulations. Liposomes containing a molar cholesterol content < 30% are unstable in the circulation resulting in rapid release of encapsulated drug [118,223]. Apart from its direct role in membrane stability, cholesterol content also affects the uptake of liposomes by various tissues including bone marrow. High cholesterol content in hydrogenated egg phosphatidylcholine (HEPC) and egg phosphatidylcholine (EPC) liposomes markedly increased accumulation in bone marrow. Different uptake mechanism and involvement of serum components was hypothesized for cholesterol-dependent uptake of HEPC liposomes by bone marrow. It is likely that selective opsonization by serum components such as C3 complement protein plays a role in the increased uptake of cholesterol-rich liposomes by phagocytic cells in the bone marrow. This also fits with the earlier findings that high cholesterol containing liposomes activate complement system [224,225]. Regarding polymer-based nanoparticles, the polymer molecular weight has been shown to influence the bone targeting capacity. For instance, *in vivo* behavior of aspartic acid-HPMA copolymers was evaluated by Wang and co-workers and they found that higher molecular weight leads to higher bone accumulation, which also correlated to prolonged circulation times of the conjugates [226].

### 6.3. Surface charge

Surface charge of liposomes plays a major role in bone marrow uptake. Negatively charged phospholipids like phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidic acid (PA) increased the uptake of liposomes by bone marrow macrophages [227]. Targeting and/or depleting bone marrow macrophages has a great therapeutic potential in HM (see Section 7.2.1) but also means indirect competition with organs such as liver and spleen that are also rich in macrophages. Additionally, receptors on the macrophages can be targeted to further enhance the uptake by active targeting approaches. These receptors include, for instance, mannose receptors, galactose receptors, folate receptors, nicotinic acetylcholine receptors, and scavenger receptors A and B (CD36). These receptors can be targeted by specific ligands coated on the surface of nanoparticle. Negatively charged liposomes found to be accumulated in the bone marrow. The presence of 20% DSPG in the bilayer of CPX-351 liposomes resulted in higher accumulation in the mouse bone marrow [118]. In recent studies, succinic acid-coated liposomes have been shown to accumulate in the bone marrow. It is believed that succinic acid and other anions are ligands of scavenger receptors of the macrophages. Also degree of PEGylation plays a major role in uptake by

bone marrow. They found optimal amount of PEG to be 0.6% to get the highest uptake by rabbit and monkey bone marrow. Another important factor is the extent of total lipids. Above the dose of 50 mg/kg of lipids, bone marrow was found to be the first organ to be saturated, and most of the liposomes are taken up by liver and spleen. In later studies it was revealed that uptake of succinic acid liposomes by bone marrow is species dependent as only large animals such as rabbit and monkey were found to accumulate major portion of liposomes to the bone marrow while in small animals such as mouse and rat the results were opposite [119]. Unfortunately, similar studies have not been performed in humans yet. Another strategy to target bone marrow is the use of bisphosphonates. Molecules from this category are known to be calcium-chelating agents. Since bone marrow, especially sites that display high bone turnover, actively recruit calcium. This feature of bone marrow, found in HM malignancies such as multiple myeloma, provides opportunity to target by bisphosphonates as has been shown for polymeric-based particulate systems. N-(2-Hydroxypropyl) methacrylamide (HPMA) is a hydrophilic polymer that has been extensively used for bone targeting purposes. For instance, HPMA copolymer-PTX-alendronate (ALN) conjugate showed antitumor efficacy against mammary adenocarcinoma inoculated into the tibia, when compared to PTX alone or in combination with ALN [228]. Instead of PTX, TNP-470, which is a potent anti-angiogenic agent used as a therapeutic drug. HPMA copolymer-ALN-TNP-470 conjugate showed 65% tumor inhibition in a murine osteosarcoma model, when compared to 50% inhibition for free TNP-470 together with ALN [229]. PLGA nanospheres have been used for bone targeting via alendronate functionalization of the nanoparticle surface. A PEG-PLGA copolymer was functionalized with ALN and was demonstrated that by increasing the ALN density the adsorption to Hap increases, as expected. The effect of the molecular weight of PEG used was also assessed, and by increasing PEG molecular weight the adsorption of targeted nanoparticles to Hap decreased, probably due to the shielding of the targeting moieties by the longer PEG chains [230]. In another study, ALN-PEG-PLGA nanoparticles loaded with bortezomib were tested in a MM mouse model. The optimal ALN percentage at the NPs surface was found to be 20%, and further increase in ALN content up to 60% led to a plateau on the binding capacity to HA. Bortezomib-loaded untargeted and targeted NPs were incubated with MM1S cells to assess the ability to induce apoptosis. It was shown that ALN did not show any improvement on the ability of bortezomib NPs to induce apoptosis. This result demonstrates that the setup of the experiment does not translate the *in vivo* scenario where different types of cells are present and the targeted NPs are of great value in specifically deliver the content to the multiple myeloma cells. Fluorescently labeled NPs were intraperitoneally injected and 24h after mice injected with targeted NP showed increased retention at spleen, femur, skull and lymph nodes, compared to untargeted formulation. Contrariwise to *in vitro* results, the bone accumulation of targeted nanoparticles was 9.6-fold higher than in the untargeted counterpart [231]. PLGA nanoparticles functionalized with ALN were also prepared by others and evaluated their toxicity profile, which showed not to have any cytotoxicity and satisfactory blood compatibility [232]. *In vivo* efficacy of these nanoparticles was evaluated in an orthotopic mouse model of breast cancer with bone metastases. Drug loaded and unloaded targeted nanoparticles decreased the number of osteoclast in the tumor area, which was attributed to be due to the alendronate activity [233]. PLGA-PEG-ALN micelles were also applied for vancomycin delivery to bone with osteomyelitis [234]. Similarly, PEG-PLA nanoparticles functionalized with different percentages of ALN (ranging from 0 to 40%) were prepared. The higher the ALN percentage, higher was the accumulation in bones. At 40% of ALN the accumulation was 3.2 fold higher compared to untargeted nanoparticles. However, the targeting moiety only contributed to a higher accumulation in bone when was present at the nanoparticle surface at a ration higher than 10% [201]. Poly(Y-benzyl-L-glutamate) nanoparticles, with average size below 80 nm, were also

functionalized with PEG-ALN to target bone marrow showed adequate delivery towards the bones [235].

#### 6.4. Targeting moieties

Active targeting increases specific delivery to the tumor tissues. Many cell surface biomarkers which are specifically or extensively expressed to the malignant cells surface such as receptors and other proteins, peptides, antibodies, and polysaccharides can be specifically targeted by coupling ligands to the surface of liposomes and other nanoparticles for drug delivery [236]. Several types of ligands can be used for active targeting of tumor cells, including antibodies, peptides, and oligonucleotide aptamers being the mostly used for hematological malignancies. Targeting via antibodies is a very straightforward approach. Antibody conjugated liposomes are called immunoliposomes. Various techniques can be used to modify antibodies and subsequently to couple them to stealth liposomes. These techniques include the use of antibodies modified with several reactive groups such as 2-Iminothiolane (Traut's reagent), N-Succinimidyl 3-(2-pyridyldithio)propionate (SPDP), Succinimidyl-oxycarbonyl- $\alpha$ -methyl- $\alpha$ -(2-pyridyldithio)toluene (SMPT), N-hydroxysuccinimide Sacetylthioacetate (SATA), S-Acetylmercaptosuccinic anhydride (SAMSA), and Succinimidyl acetylthiopropionate (SATP) [237]. Monoclonal antibodies against specific biomarkers on malignant cells can be used to target liposomes for HM. An additional advantage of immunoliposomes is that in most cases the antibody itself has cytotoxic effects to the malignant cells. For example CD38 is uniformly expressed antigen on myeloma cells [238]. Daratumumab, an anti-CD38 antibody is effective against multiple myeloma and was approved as a monotherapy last year by FDA [239]. Coupling anti-CD38 antibody to liposomes could not only provide the specificity to the malignant B cells but could also be used as a combination therapy. Immunoliposomes coated with specific antibody and loaded with a chemotherapeutic drug against specific cell surface marker might be a promising targeted combination therapy for HM. CD19-targeted and DOX loaded micelles showed increased survival time, compared to controls, when injected in a human ALL xenograft model [195]. Transferrin targeted and DOX loaded polymeric nanoparticles composed of Pluronic85/lipid were developed for the treatment of childhood leukemia [240]. These nanoparticles were able to inhibit tumor growth up to 90%, whereas control groups inhibit tumor growth in around 10%. Transferrin was also conjugated to PEG-PLL-PLGA for the preparation of targeted micelles loaded with edelfosine for the treatment of leukemia [241]. Micelles size was of 120 nm and when functionalized with transferrin 190 nm. Circulation time of the loaded drug was assessed and found to be similar for targeted and untargeted formulations.

Targeting nanoparticles using peptides is another strategy investigated extensively in drug delivery research. In case of HM, bone marrow is the primary target. Interaction with specific adhesion molecules expressed by human bone marrow endothelial cells (HBMEC) is a potential method for bone marrow homing of nanoparticles. Therefore, targeting bone marrow endothelium by using peptides that bind to these molecules offers a useful tool for drug delivery. To target molecules that are overexpressed on angiogenic endothelial cells such as  $\alpha_v\beta_3$  integrins, liposomes can be coated with Arg-Gly-Asp (RGD) peptides [242,243]. Another well-known example of endothelial targeting is very late antigen-4 (VLA-4), also known as  $\alpha_4\beta_1$  integrin. Interestingly, VLA-4 is a heterodimer not only expressed on the surface of angiogenic endothelial cells but also by abnormal white blood cells in many hematological malignancies including lymphomas, leukemias, and multiple myeloma [244–248]. It has been shown that liposomes conjugated with a cyclic pentamer peptide, called VLA-4 peptide, can be used to target HM [149,245]. Other targets also facilitate targeting of bone marrow endothelium, for instance, E-selectin, a cell adhesion molecule [249] which is expressed in response to inflammatory stimuli

but absent in normal cells [250,251]. Targeting bone marrow by use of a thioaptamer that is specific to E-selectin increased bone marrow accumulation of nanoparticles [250,252].

## 7. Challenges in the development of nanomedicine formulations and their clinical translation

Despite the acknowledgment that nanomedicines offer many advantages in drug delivery over free drugs, it remains difficult to develop successful nanoparticle-based formulations that are approved and used in the clinic. For example, to date only 11 liposomal formulations are approved for clinical use [38]. With regard to polymeric-based nanomedicines, only two formulations have received approval for use in clinical practice – one micellar formulation and one albumin-based nanoparticle.

### 7.1. Technical challenges

#### 7.1.1. Liposomal nanomedicines

Loading therapeutic compounds efficiently and stably in liposomes is one of the major challenges. Working on a small-scale formulation in a laboratory is rather simpler approach than large-scale production to fulfill the formulation demand at industrial level. Furthermore, the formulation must be stable to allow long term storage, while at the same time, once administered, the encapsulated drug must be available at the right site and at right time to exert therapeutic effects. Non-PEGylated conventional liposomes have shorter half-lives than their stealthy counterparts, conceivably narrowing their therapeutic window. Slow release from long circulating PEGylated liposomes could contribute to systemic effects, however, it also diminishes drug concentrations at the (primary) site of disease. Moreover, long circulation properties may result in non-specific accumulation in the skin causing serious side effects such as hand-foot syndrome, or palmar-plantar erythrodysesthesia, as reported for Doxil® [253]. Particle size is one of the very important characteristics that defines the opportunity of nanomedicines to be used as drug delivery vehicles. Conventional small scale, batch-based laboratory methods for production of nanoparticles are usually highly dependent on the operator's experience and are not suitable for reproducible large-scale production [254]. The use of advanced techniques, however, enables to not only produce nanoparticles of narrow size distribution but also results in precise control over chemical composition, drug loading and surface properties of nanoparticles that is operator independent. Examples of such techniques include microfluidics technologies and particle replication in non-wetting template (PRINT) technology [255–258]. In general, in order to develop a successful liposomal formulation optimization of loading efficiency and stability must be carefully monitored.

#### 7.1.2. Polymer-based nanomedicines

One key aspect in the development of polymeric nanomedicines is the stability of the loaded drug inside the nanoparticle. In the design of polymeric drug delivery systems one can tune the polymers properties and introduce specific side groups for instance to increase the compatibility between polymer and drug to be loaded. Therefore, the properties of a nanocarrier such as molecular weight, ratio of hydrophobic/hydrophilic block, concentration of drug carrier in relation to the drug will all influence its performance as a drug delivery system. These properties should be tuned accordingly to each drug and purpose.

### 7.2. Biological challenges

#### 7.2.1. Circulation times and clearance

Besides the technical challenges, there are some biological hurdles associated with drug delivery. Once nanomedicines are administered, the defense system of body recognizes them as foreign. Opsonins present in plasma, such as immunoglobulins (Ig), fibronectin, lipoproteins

and complement proteins adsorb to the surface of nanocarriers [222,259]. The opsonized nanoparticles are subsequently taken up by the MPS (previously called reticuloendothelial system (RES)). The organs involved in clearance are liver, spleen, kidney, lung, and bone marrow with liver and spleen being the major source of particle uptake and clearance [260]. Macrophages and other phagocytes present in these organs are responsible for both opsonization-dependent and opsonization-independent clearance of nanoparticles. For this reason, various surface coatings have been developed to decrease recognition by host cells and thereby initiating increasing circulation times in blood. The most abundantly applied coating is PEG. This highly hydrophilic molecule makes the particles more 'stealthy' by reducing protein absorption and affecting the composition of the proteins that are absorbed on the surface, thereby evading immune cells [261,262]. Although, the stealth property of PEGylated nanoparticles can delay and partly reduce the clearance by the MPS, repeated injections of PEGylated nanoparticles may also trigger anti-PEG IgM production, altering the pharmacokinetics and biodistribution [263,264]. This process, also known as accelerated blood clearance (ABC) phenomenon, depends on several factors including composition, coating/PEG density and the chain length, size and surface charge of nanoparticles, route and time interval of administration, and animal species [263–268]. Apart from the IgM production, PEGylated nanoparticles may also activate innate immune responses leading to immediate, non-IgE mediated complement activation resulting in faster blood clearance and pseudoallergic reactions. This complement activation related-pseudoallergy (CARPA) is associated with clinical symptoms such as anaphylaxis, facial flushing and swelling, headache, chills, and cardiopulmonary distress [269]. To circumvent all these biological challenges and in order to make a clinically translational formulation, careful optimization of various aspects such as size, composition, density and length of coating/PEG moiety, dosing frequency, total dose and animal model (and strain), is required. Additionally, a different strategy to avoid early clearance of nanomedicines from the peripheral blood has been studied; depletion of phagocytes. Temporary (partial) depletion of the immune cells that are mainly responsible for nanoparticle clearance could be facilitated by clodronate-loaded liposomes. Using clodronate liposomes significantly increased plasma residence times and changed the biodistribution of nanomedicines in animal models [78,270]. Clodronate is a first generation bisphosphonate that is approved for the prevention and treatment of osteoporosis. When encapsulated in liposomes it specifically kills tissue macrophages of the MPS [271,272]. Macrophage depletion in the organs from the MPS by clodronate liposomes not only supports prevention of nanoparticle clearance from the peripheral blood; it also is a tool to study the role of macrophages and other phagocytes in health and disease [273–275]. Finally, administration of clodronate liposomes has therapeutic potential in a variety of diseases including CLL [276–280].

#### 7.2.2. Animal models and clinical translation

The first step to evaluate novel pharmaceuticals including nanomedicines is *in vitro* testing in order to identify the biocompatibility and efficacy towards cancer cells. Conventional *in vitro* cell culture models, however, lack the complexity of the tumor microenvironment [281]. To address this issue, some advanced *in vitro* models have been recently developed including 3D co-cultures and organ-on-a-chip technologies. In 3D co-culture systems, tumor cells are cultured together with supporting cells, such as bone marrow stromal cells, that have direct cell-to-cell interaction and/or paracrine interactions with the tumor cells and mimic the bone marrow microenvironment [281]. This allows evaluation of therapeutics in a microenvironment, which is somewhat closer to the actual disease situation. However, several important aspects such as vascularization, role of immune system, and perfusion are still lacking in these 3D culture models. Organ-on-a-chip technology is another advanced tool to study *in vitro*, which overcomes the disadvantage of lack of perfusion [282]. Nevertheless, in order to establish

circulation, biodistribution, safety and efficacy profiles *in vivo*, animal models are required before first-in-human trial of nanomedicines. Good animal models are essential for pre-clinical testing in order to obtain predictable and translational results. Tumor models in general essentially lack the predictive power that is required to translate preclinical efficacy into clinical activity [283]. Hence, animal models used to study drug delivery to HM should preferably be orthotopic, accurate and clinically more relevant making translation of results towards patients more reliable. To improve animal models to study drug delivery and therapeutic efficacy in HM a number of factors should be considered. Firstly, use of a patient-derived xenograft model, rather than a cell line-derived xenograft or murine models, prevents the clonal selection introduced by *in vitro* culturing of tumor cells [283] and additionally allows studying patient-specific sensitivity to certain drugs ('personalized medicine'). Secondly, animal species used for *in vivo* testing models are physiologically and anatomically very different from humans. Introducing tumors by bulk inoculation or implantation of exogenous tumor cells also deviates in various ways drastically from the normal pathobiology of cancer. Therefore it is realistic that growth and development of these tumors in animal models is also different than in humans. EPR effect is the gold standard of nanomedicine delivery to the tumor tissues [284]. However, there appears a clear difference in the magnitude of the EPR effect between animal models and the human disease. This makes translational studies based on this effect more challenging [285]. Recent reports highlighted the variable significance of EPR phenomena in patients [286]. The EPR effect in patients varies greatly between cancer types, between tumors of same cancer type, and even within a tumor itself [254] [285]. On the other hand, even a small EPR effect might offer considerable increased accumulation of a nanomedicine formulation over the conventional drug. Also there are advantages beyond the EPR effect such as tailored pharmacokinetics-pharmacodynamic profiles, improving cellular uptake, balancing off- and on-target accumulation, and local release kinetics of the active compound. These should also be considered in the activity of nanomedicines [285] [95]. Nevertheless, spontaneous tumor models and humanized models carrying human genes, cells and/or tissues, likely simulate the conditions in humans during cancer development far closer [165,287]. Lastly, to allow xenograft models (using either cell lines or patient cells) to grow in mice, immunodeficient strains are used (possibly in combination with sublethal irradiation). Dependent on the mouse strain, these mice lack all or parts of their (innate) immune system. This likely introduces critical deviations compared to patients when studying nanomedicines in these models, such as drastically altered pharmacokinetics and pharmacodynamics [288]. It is important to understand the 'translational value' of used models towards cancer in humans. For instance, in recent debate regarding development and testing of nanomedicines, the actuality of the EPR effect in the clinic and the translational value of animal models in (solid) cancer research has been discussed [254,289,290]. Better models provide more accurate information, which is crucial for screening of potential new drugs and nanomedicines, not only to make it to the clinic faster but also to be able to dismiss unsuccessful formulations in a much earlier stage of development.

## 8. Conclusions and future perspective

Several approaches are employed in the clinic for the treatment of HM, including radiotherapy, stem cell transplantation, immunotherapy and chemotherapy. Many (chemo)therapeutic drugs have been developed to inhibit various cellular and/or molecular pathways. Combination of two or more drugs is often used to attack the tumor cells by hampering multiple oncogenic pathways. This frequently results in improved overall survival, and in some cases higher cytogenetic and molecular responses. However, (chemo)therapeutic drugs are associated with various limitations. First, most of these drugs are (semi) synthetic chemical entities which have short half-lives and rapid

clearance from the circulations, resulting in a poor pharmacokinetic profile and reduced bioavailability. Lower bioavailability requires high dosing schedules, which cause dose-related and thus dose-limiting toxicities. Second, lack of specificity of these drugs towards the malignant organ/tissue affects healthy tissues leading to off-target toxicities. Third, resistance to drugs is often seen. This acquired resistance to one or more (multi-drug resistance or MDR) (chemo)therapeutic drugs may occur due to several micro-environmental and cellular phenomena [291,292]. The latter includes, but is not limited to: induction of cell survival pathways, inability to induce apoptosis, overexpression of certain membrane-embedded drug efflux pumps, such as P-glycoproteins and MDR proteins. Resistant malignant cells express elevated amounts of these proteins on their membranes [293–295].

Nanomedicines such as liposomes and polymeric micelles will increase the statistical chances of active compounds to be accumulated at the malignant sight, by changing pharmacokinetic properties of drug molecules leading to enhanced retention in the circulation. The net effect is higher local drug concentrations at the site of interest while healthy organs remain minimally exposed. The fact that hematological malignancies are also characterized by increased microvessel density as a result of angiogenesis, provides a rationale to use these nanocarrier systems for this class of cancer as well. Lessons learned from the success of nanoformulations such as Doxil® and VYXEOS® have proven the therapeutic value of nanomedicines in HM where liposomal encapsulation improved safety and efficacy of existing chemotherapeutics.

Moreover, nanomedicines may partly, if not completely, overcome MDR. Since transport of free small drug molecules into malignant cells is mainly by passive diffusion through the cell membrane, they encounter membrane proteins including drug efflux pumps that potentially reduce their net concentration in the cytoplasm, consequently leading to suboptimal concentrations in the target cell, resulting in MDR. Nanomedicines, on the other hand, due to their large size, are generally taken up by endocytosis. Hence, they bypass this mechanism of drug efflux and the concentration within the cell is much higher as compared to free drug [291,294,295]. Although, results from *in vitro* experiments showed only a slight improvement in overcoming MDR by using classical nanocarrier systems [294]. This data should be interpreted carefully, as *in vitro* models lack several important aspects of tumor, for example angiogenesis, EPR effect, complex tumor micro-environment etc.

Despite a reasonable understanding of pathology, molecular mechanisms behind disease progression and resistance, and extensive research in the field, only a few of formulations could successfully reach to clinic for treatment of HM (as is true for the treatment of solid tumors). The explanation lies in the reality that even though nanoparticle systems show substantial benefits in preclinical settings, their clinical translation still remains difficult. Loading drug molecules into nanoparticles, stability of nanoparticle formulations, and *in vivo* release of encapsulated drugs at the target site are some challenges met in development of successful nanomedicine formulations. Moreover, lack of appropriate animal models that mimic the actual clinical situation (etiology/pathology/progression) in HM makes it difficult to predict favorable outcomes for clinical trials. Nevertheless, in last few decades nanomedicines have been appreciated and proven as potential drug delivery systems. Several formulations are successfully developed and clinically approved not only for treatment of many types of malignancies but also for chronic inflammatory diseases. More research in the field could in the future lead to novel nanoparticulate formulations for treatment of various types of HM in patients.

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