



Published in final edited form as:

Exp Hematol. 2016 November ; 44(11): 1002–1012. doi:10.1016/j.exphem.2016.08.006.

Mechanisms of Heparanase Inhibitors in Cancer Therapy

Benjamin Heyman¹ and Yiping Yang^{1,2}

¹Division of Hematologic Malignancies and Cellular Therapy, Department of Medicine, Durham, North Carolina, USA

²Department of Immunology, Duke University, Durham, North Carolina, USA

Abstract

Heparanase is an endo- β -D-glucuronidase capable of cleaving heparan sulfate (HS) side chains contributing to break down of the extracellular matrix. Increased expression of heparanase has been found in numerous malignancies, and is associated with a poor prognosis. It has generated significant interest as a potential anti-neoplastic target because of the multiple roles it plays in tumor growth and metastasis. The pro-tumorigenic effects of heparanase are enhanced by the release of HS side chains, with subsequent increase in bioactive fragments and increased cytokine levels; both promoting tumor invasion, angiogenesis and metastasis. Preclinical experiments have shown heparanase inhibitors to substantially reduce tumor growth and metastasis leading to clinical trials with heparan sulfate mimetics. In this review we will examine heparanase's role in tumor biology, its interaction with heparan surface proteoglycans, specifically syndecan-1; as well as the mechanism of action for heparanase inhibitors developed as anti-neoplastic therapeutics.

Introduction

The extracellular matrix (ECM) is composed of different proteins that maintain cellular organization and architecture. It was initially felt to be inactive, but later appreciated as a dynamic entity, where significant cell signaling interactions occur.¹ The ECM contains heparan sulfate proteoglycans (HSPGs), collagen, fibronectin, laminin, and growth factors.¹ HSPGs are ubiquitous macromolecules that are integral parts of normal tissue architecture. They possess various functions including: cell attachment/adhesion, components of structural integrity, reservoirs for growth factors, and act as cofactors in signaling pathways.^{2,3} HSPGs are comprised of a core protein attached to one of several negatively charged polysaccharide chains of heparan sulfate glycosaminoglycans (GAGs). Heparan sulfate (HS) is composed of repeating units of glucosamine and glucuronic/iduronic acid residues.⁴

Address correspondence to: Dr. Yiping Yang, Department of Medicine, Duke University Medical Center, Box 103005, Durham, North Carolina 27710. Phone: (919) 668-0932; Fax: (919) 684-9594; yang0029@mc.duke.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflicts of interest disclosure: The authors have no conflicts of interest to disclose.

Heparanase is an endo- β -D-glucuronidase that cleaves HS side chains. This results in structural changes and the release of bioactive HS fragments from the ECM.⁵ Over the past two decades much work has been dedicated to examining the role of heparanase in cancer biology. Various methods of analysis have revealed that heparanase expression is augmented in numerous cancers, including hematologic malignancies, carcinomas and sarcomas.^{6–15} Furthermore, elevated heparanase levels are associated with reduced post-operative survival, increased angiogenesis, and metastasis.^{8,12,13,16} All of these factors have sparked the development of heparanase inhibitors as novel anti-cancer agents. In this article we will review the function of heparanase in cancer biology and focus on the development of heparanase inhibitors, their specific mechanism of action, and relevant clinical findings to date.

Heparanase and Heparan Sulfate/Syndecan-1 Axis

Mammalian cells express a single functional heparanase enzyme, heparanase-1.¹⁷ Heparanase-2, a heparanase homologue was cloned, but is incapable of performing HS degrading activity.^{18,19} It may however, regulate heparanase-1 activity.²⁰ The heparanase gene is located on chromosome 4q21.3 and is highly conserved throughout different species.²¹ It is first expressed as preproheparanase, with the N-terminal signal removed upon translocation to the endoplasmic reticulum, generating a 65 kDa proheparanase, it is then moved to the Golgi apparatus where it is encapsulated and secreted. Once secreted it interacts with extracellular components before being internalized and mobilized to the late endosome/lysosome where it undergoes post-translational proteolysis and alternative splicing to become active heparanase.^{22–25} The active form of heparanase consists of a heterodimer composed of an 8 and 50 kDa subunit that are non-covalently linked. The heparanase structure contains a TIM barrel fold, which incorporates the enzyme's active site; and a distinct C-terminus domain that has non-catalytic properties and is involved in heparanase's non-enzymatic signaling and secretory function.^{26–28} Recently, the human heparanase enzyme structure was solved, confirming the TIM barrel fold structure.²⁹

Heparanase expression is under tight regulation. In non-cancerous cells the heparanase promoter is constitutively inhibited secondary to promoter methylation and activity of wild type p53, which suppresses transcription of the heparanase gene by directly binding to its promoter.³⁰ Furthermore, additional regulation occurs during post-translational processing. Cathepsin L is necessary for post-translational activation of heparanase, and inhibitors of cathepsin L impede the formation of active heparanase.³¹ In non-pathologic states, heparanase expression is restricted primarily to platelets, activated white blood cells and the placenta with little or no expression in connective tissue or normal epithelium.⁵ Moreover, it is most active under acidic conditions (pH 5–6), during inflammation or within the tumor microenvironment.¹⁶

The syndecans (SDCs) are a family of four HSPGs that are either membrane bound or soluble. They have diverse functions including cell differentiation, cell adhesion, cytoskeletal organization, cell migration/invasion, and angiogenesis.^{32–35} Syndecan-1 (SDC-1) has been the most extensively studied and is found principally on epithelial cell surfaces. However, it is also present during different stages of lymphoid development,

specifically on pre-B cells and plasma cells.^{36,37} Loss of both syndecan-1 and E-cadherin from the cell surface is considered an integral step in neoplastic epithelial-mesenchymal cell transition.³⁸

The heparanase/SDC-1 axis is a key regulator of cell signaling within tumor cells and the microenvironment, especially in multiple myeloma.³⁹ Syndecan-1 is made of three domains: 1) an extracellular domain composed mostly of heparan sulfate GAGs; 2) a transmembrane domain; and 3) a highly conserved cytoplasmic domain.⁴⁰ Syndecan-1 can be shed and mobilized via proteolytic cleavage of the extracellular domain near the plasma membrane. This is primarily performed by shedases, frequently matrix metalloproteinases (MMP).⁴¹ Shed syndecan-1 contains bound HS chains within the ectodomain (which typically contain bound growth factor) and thus can become a paracrine signaler by transferring signaling proteins from one cell to another.⁴¹ In the case of malignancy, this is often from a cancer cell to a stromal cell.^{42,43} Syndecan-1 shedding is regulated by various extracellular mechanisms including: heparanase, growth factors (FGF-2), and chemokines.⁴⁴⁻⁴⁶

Heparanase increases syndecan-1 shedding, both in human myeloma and breast cancer cell lines, by augmenting expression of MMP-9 through upregulation of ERK phosphorylation.⁴⁷ Heparanase also reduces the length of HS chains attached to syndecan-1, enhancing the rate at which shedases cleave the core protein.⁴⁷ Syndecan-1 is also shed constitutively, which is accelerated in tumors, typically in response to growth factors, chemokines or other agonists.⁴⁸ Recently, it was found that chemotherapy stimulates syndecan-1 shedding in colorectal cancer, pancreatic cancer, and human myeloma cell lines, increasing the risk for relapse and chemotherapy resistance.^{49,50}

The heparanase/syndecan-1 axis regulates growth factor release, thus modulating cellular proliferation.⁵¹ Both hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) are regulated by the heparanase/syndecan-1 axis. HGF is a cytokine that enhances growth, motility, and angiogenesis of tumor cells.⁵² Heparanase has been demonstrated to increase expression of HGF in myeloma cell lines. Shed syndecan-1 binds to secreted HGF, facilitating a paracrine and autocrine signaling cascade via cell surface receptor c-Met.⁵² Similarly, heparanase enhances VEGF secretion from tumor cells. Secreted VEGF subsequently binds shed syndecan-1 in the ECM stimulating angiogenesis and endothelial invasion via the Erk pathway.⁴³ In breast cancer, shed syndecan-1 promotes angiogenesis and growth via activation of FGF-2.⁴² In multiple myeloma, shed syndecan-1 in the bone marrow ECM enhances growth, angiogenesis and metastasis of myeloma cells within the bone. Cell membrane syndecan-1 promotes myeloma cell adhesion and inhibits invasion. Conversely, heparanase facilitates invasion of myeloma by increasing the expression and shedding of syndecan-1.^{43,47,53}

Heparanase and syndecan-1 can also be transported to the nucleus to regulate gene expression. Shed syndecan-1 and the full syndecan-1 protein have been identified in the nucleus.⁵¹ Similarly, HS has also been identified in the nucleus, both as free chains and bound to syndecan-1. Syndecan-1 transports HS to the nucleus, as it does so for FGF2. In general, nuclear HS and syndecan-1 are anti-proliferative and decrease gene transcription. Specifically, highly sulfated nuclear HS chains are mostly inhibitory.^{51,54} This is in contrast

to extracellular shed syndecan-1, which promotes cell migration, angiogenesis, invasion and proliferation.⁵¹ Once in the nucleus HS can regulate gene expression by decreasing histone acetylation and inhibiting transcription factors.⁵⁵ Both syndecan-1 and HS can inhibit histone acetyl transferase enzyme (HAT), reducing gene expression and tumor growth.^{56,57} Conversely, heparanase augments gene expression in the nucleus and promotes growth.⁵⁸ In T-lymphocytes heparanase binds to euchromatin, altering gene transcription.⁵⁸ Heparanase increases DNA topoisomerase I activity in metastatic breast cancer.⁵⁹ Lastly, heparanase decreases nuclear syndecan-1 levels, increasing gene expression and promoting aggressive tumor phenotype secondary to augmented HAT expression.⁶⁰

Many studies have examined syndecan-1 expression as a prognostic tool in solid and hematologic malignancies. High levels of stromal expression of syndecan-1 are a negative prognostic factor in multiple malignancies. Low levels of epithelial syndecan-1 are generally an indicator of advanced disease and poor prognosis. It is believed that the loss of syndecan-1 represents cancer cells with high malignant and metastatic potential. Increased levels of soluble (shed) syndecan-1 also signify advanced disease and poor prognosis. However, this has not been consistent for all malignancies, as increased levels of soluble syndecan-1 have also been associated with improved prognosis.^{51,61-72} In both non-small cell lung cancer and multiple myeloma, loss of syndecan-1 decreases response to standard chemotherapy.^{73,74} Because soluble syndecan-1 can be detected in plasma, it serves as an attractive biomarker and therapeutic target.

The Role of Heparanase in Cancer Biology

As previously mentioned, increased heparanase expression within numerous malignancies is associated with poor prognosis. Early studies in murine T-lymphoma and melanoma cell lines demonstrated that cells that over-express heparanase transformed from a non-metastatic to a highly invasive metastatic phenotype.^{24,75} Heparanase's direct role in malignancy was confirmed when heparanase inhibition/silencing in cancer cell lines resulted in a significant reduction in the invasive phenotype of cells.⁷⁶⁻⁸⁰ Since then, there has been much work exploring the relationship between heparanase and tumor metastasis.

Heparanase's main enzymatic activity is the cleavage of HS side chains from HSPGs, releasing growth factors and cytokines that can then propagate cellular signaling pathways facilitating the remodeling of the extracellular matrix, particularly the subendothelial capillary basement membrane of endothelial cells (EC).⁸¹ This step is necessary prior to endothelial migration during angiogenesis.⁸¹ Heparanase can also release heparan sulfate bound proangiogenic growth factors, bFGF, HGF, PDGF and VEGF, from the extracellular matrix to indirectly promote endothelial cell migration and proliferation.^{82,83} Heparanase-induced HS fragments retain biological activity and can enhance growth factor activity.^{14,84} Heparanase is preferentially located at the sites of sprouting EC's, with little evidence of heparanase present on inactive blood vessels.⁸⁵ Moreover, tumors with elevated heparanase levels have significantly higher microvessel density than tumors with low heparanase expression; and inhibition of heparanase result in decreased microvessel density.^{14,27,85,86} Similarly, heparanase increases lymphatic vessel density and lymph node metastasis through increased expression of VEGF-C in head and neck carcinoma.⁸⁷

Heparanase also augments angiogenesis and tumor activity independent of its enzymatic activity at its C-terminus domain. Heparanase increases the expression of VEGF in heparanase-transfected cell lines via Src upregulation and p38 phosphorylation.⁸⁸ Heparanase overexpression in multiple cell lines augmented endothelial cell stimulation, migration and invasion secondary to Akt phosphorylation. Akt phosphorylation was found to be independent of heparanase activity, and increased twofold in the presence of heparin.^{89,90} In multiple myeloma, heparanase enhanced the activation of the insulin receptor signaling pathway, with subsequent stimulation of insulin receptor phosphorylation, increased protein kinase C (PKC) activity and augmented expression of insulin receptor substrate-1 (IRS-1). This results in increased ERK signaling and myeloma cell survival and growth.⁹¹ Lastly, over-expression of heparanase in head and neck cancers causes greater EGFR activation leading to enhanced cell proliferation and tumor growth independent of heparanase's enzymatic activity.⁹²

Heparanase has also been demonstrated to promote chemotherapy resistance in myeloma.^{93,94} Chemotherapy directed at the nuclear factor-kappa B (NF- κ B) pathway results in increased expression of heparanase in tumor cells.⁹⁴ The amplified heparanase expression in tumor cells results in activation of the NF- κ B pathway, chemotherapy resistance, and an aggressive tumor phenotype.⁹⁴ Chemotherapy also induces release of soluble heparanase by myeloma cells, where it can be taken up by macrophages or other tumor cells.⁹⁴ Soluble heparanase causes an increase in TNF- α production by macrophages, and induces expression of HGF, VEGF and MMP-9 in tumor cells.⁹⁴ It has also been demonstrated to activate the ERK and Akt signaling pathways in myeloma.⁹⁴ Ronaparstat, a heparanase inhibitor, curbs the effects of soluble heparin, resensitizes myeloma cells to chemotherapy, and had the potential to prevent growth of tumors after treatment with chemotherapy.⁹³

Heparanase has been shown in myeloma to augment the expression of mesenchymal markers.⁹⁵ Specifically, increased heparanase expression in both myeloma and endothelial cells correlates with the augmented expression of mesenchymal markers vimentin and fibronectin.⁹⁵ Mechanistically, the alteration in mesenchymal markers is thought to be promoted by the ERK signaling pathway.⁹⁵ These findings support that heparanase promotes angiogenesis and metastasis in multiple myeloma by supporting the mesenchymal transition of both tumor and endothelial cells.⁹⁵

Recently, Heparanase was found to have a critical role in modulating autophagy in tumor cells.⁹⁶ Lysosomal heparanase fuses with autophagosomes contributing to the cellular control of autophagy. Tumor cells that overexpress heparanase were found to have increased levels of autophagy, which promoted tumor growth and chemotherapy resistance.⁹⁶ Mechanistically, autophagy induction by heparanase occurs through the mammalian target of rapamycin complex 1 pathway (MTORC1). Decreased phosphorylation of RPS6KB/p70 S6-kinase, a MTORC1 substrate, was found in cells over expressing heparanase indicating increased autophagy. In mice deficient for heparanase, there was reduced phosphorylation RPS6KB, with a resulting decrease in autophagy.⁹⁶ Directly inhibiting autophagy with the lysosomal inhibitor chloroquine prevented chemotherapy resistance.⁹⁶ Lastly, tumor growth was further attenuated with the addition of heparanase inhibitor PG545 to chloroquine.^{96,97}

Furthermore, heparanase over-expression increases cell adhesion both in an HS-dependent and independent manner. HS dependent adhesion and clustering is mediated through syndecans and glypicans. HS side chains are linked to PKC α , Src and Rac1 activation.⁹⁸ HS independent adhesion and clustering are mediated through Akt, p38, and Src activation.⁹⁸ Recent studies have also demonstrated that heparanase may also act as a procoagulant. Heparanase over expression in multiple cancer cell lines is correlated with marked increase of tissue factor (TF) and factor Xa levels.^{99,100} Moreover, increased heparanase activity leads to greater expression of TF, which interacts with tissue factor pathway inhibitor (TFPI) on endothelial and tumor cell surfaces, causing release of TFPI and enhanced local coagulation activity.¹⁰¹ Recently, platelets over expressing heparanase were found to have stronger adhesion.¹⁰² Activated platelets were also found to have upregulated expression of heparanase and P-selectin.¹⁰²

Lastly, elevated heparanase expression was found by IHC in patients with JAK-2 positive myeloproliferative disorders or erythropoietin receptor-transfected glioma cells.¹⁰³ While inhibition with hydroxyurea or ruxolitinib lead to decreased levels. Thus, the epo receptor and JAK-2 may contribute to heparanase up-regulation in these cell lines.¹⁰³

Heparanase Inhibitors as Novel Cancer Therapeutics

Heparanase promotes tumor cell proliferation, growth and angiogenesis by its activity within the tumor cells, at the cell surface, and within the tumor microenvironment. Since heparanase is implicated in many features of tumor progression, it is an ideal therapeutic target. Additionally, since there is only one functional mammalian heparanase, there are no redundant enzymes able to act in its place. Lastly, since heparanase is typically not expressed in most normal tissue, side effects secondary to inhibition should be minimal.

Since the recognition of heparanase as a promotor of tumor progression, several heparanase inhibitors have been produced. Generations of a selective inhibitor had been limited due to lack of knowledge of the full 3D structure of mammalian heparanase, which has only recently been solved.²⁹ Heparin is a logical choice, as it is a close mimetic of HS. However, its use as a therapeutic anti-cancer agent is limited by its potent anticoagulant effects. LMWH is a possible alternative. The use of LMWH to improve survival of patients with cancer has been controversial. Some studies have demonstrated improved survival, while others yield no benefit.^{104–106} Mechanistically, heparin and LMWH are believed to alter tumor growth by both their anticoagulant properties, and anticoagulant-independent effects that inhibit cell adhesion, metastasis, and angiogenesis.^{107,108} In non-small cell lung cancer, enoxaparin decreased expression of both c-Myc and CD44, and cancer cell proliferation.¹⁰⁹ Additionally, Dalteparin, tinzaparin, and enoxaparin have been shown to decrease FGF-induced mitogenesis via ERK kinase inhibition in tumor-derived endothelial cells, augmenting tumor growth and angiogenesis.¹¹⁰ Tinzaparin and UFH decreased metastases in colon adenocarcinoma and melanoma cell lines secondary to inhibition of P and L selectin.¹¹¹ Tinzaparin also prevented lung metastasis in severe combined immunodeficiency mice inoculated with human breast cancer cells by inhibiting the interaction between CXCL12 and CXCR4.¹¹² However, most of the anti-neoplastic properties of LMWHs are due to the inhibition of growth factors and angiogenesis^{108,113} Tinzaparin, inhibited

endothelial tube formation, VEGF expression and angiogenesis secondary to TFPI release from endothelial cells.^{114,115}

In addition to heparin and LMWH, there are various strategies employed attempting to inhibit heparanase. Heparan sulfate mimetics, modified heparins and related polysulfated compounds have been the most studied.¹¹⁶ Heparan sulfate mimetics have lower anticoagulant activity and greater selectivity for heparanase than heparin, allowing for a higher therapeutic window. Most heparan sulfate mimetics are carbohydrate based.¹¹⁷ Here we focus on heparanase inhibitors investigated as cancer therapeutics and provide a more comprehensive list of agents in (Table 1)

PI-88 (Mupafostat) is a mixture of highly sulfonated mannan oligosaccharides, predominately penta and tetra-saccharides, isolated from the yeast species *pichia holstii*, NRRL Y-2448.¹¹⁸ PI-88 has demonstrated its anti-angiogenic and anti-metastatic effects principally by inhibiting heparanase; and blocking interactions of FGF-1, FGF-2, and VEGF, with their receptor HS.^{119–121} It also stimulates the release of TFPI, further potentiating its anti-angiogenic effects.¹²¹ In preclinical models PI-88 decreased the rate of invasive rat mammary adenocarcinoma cells and reduced metastasis.¹¹⁹ It also reduced leukemic cell burden in mouse models.¹²² Lastly, it has inhibited late stage tumor growth and early progenitor lesions in a pancreatic neuroendocrine mouse model. This was associated with a decrease in cell proliferation, angiogenesis and increased tumor cell death.¹²³

PI-88 is the most extensively studied heparan sulfate mimetic in clinical trials, having undergone multiple phase I and II trials.^{124–126} Most recently it was studied in a phase II trial as adjuvant therapy for patients with hepatocellular carcinoma (HCC) after attempted curative resection. It was found to be safe at a dose of 160 mg/day, with promising improvements in recurrence rates especially in subgroup analysis.¹²⁷ This led to a phase III trial as adjuvant therapy for patients with HCC after attempted curative resection, however this was recently stopped because at interim analysis the drug failed to reach primary objective. (NCT01402908)

PG545 is a synthetic, single molecular entity containing lipophilic modifications unlike PI-88, which is a mixture derived from heparin or fermentation products from yeast.¹²² Such modifications in PG545 allow for improved pharmacokinetic properties and reduced anticoagulant activity.¹²⁸ PG545 is a competitive inhibitor of heparanase and inhibits proangiogenic growth factors VEGF, FGF-1 and 2.¹²⁹ In pancreatic cell lines it inhibited Wnt/ β -catenin signaling decreasing the proliferation of tumor cells.¹³⁰ It has been studied in multiple preclinical models in various tumor subtypes demonstrating potent anti-tumor, anti-metastatic, and anti-angiogenic effects. This has included breast, hepatocellular, melanoma, ovarian and lymphoma.^{129,131–133} It works synergistically with standard chemotherapy in preclinical murine models.^{130,131,134}

Recently, PG545 was studied in ovarian cancer murine model and its impact on metabolism and tumor growth.¹³⁵ Sulfatase-1 (HSulf-1) deficiency promoted glycolysis, resulting in impaired mitochondrial function and a reduction in oxidation phosphorylation.¹³⁵ Mechanistically, HSulf-1 deficiency leads to increased levels of c-myc, via activation of p-

ERK by HB-EGF signaling.¹³⁵ PG545 decreased glycolysis through inhibition of p-ERK and c-Myc; indicating that it may have similar mode of action as HSulf-1 in altering tumor metabolism.¹³⁵

Importantly, PG545 is the only HS mimetic reported to have immunostimulatory activity against lymphoma resulting in significant anti-tumor activity.¹³⁴ In a murine model, mice were treated with placebo, single agent cyclophosphamide, single agent PG545, or a combination of PG545 and cyclophosphamide. The combination of PG545 and cyclophosphamide resulted in a complete response and 100% sixty day survival.¹³⁴ PG545 exerted its major anti-lymphoma effects through the activation of the innate immune system via natural killer (NK) cells. Mechanistically, NK cell activation occurred through the Toll like-receptor 9/MyD88 pathway. Specifically, PG545 enhances the accumulation of oligodeoxynucleotides (CpG DNA) in the lysosomes of dendritic cells (DC), leading to an increased production of pro-inflammatory cytokines IL-12, IL-6 and TNF- α . IL-12 production by dendritic cells was found to be critical for increased NK cell activation.¹³⁴ PG545 is currently undergoing investigation with phase 1 trials in patients with advanced solid tumors. (NCT02042781)

SST0001 (Roneparstat) is a modified heparin that is 100% N-acetylated and 25% glycol split. SST0001 inhibits heparanase enzymatic activity and exhibits a decreased ability to release extracellular matrix-bound FGF-2 as compared with unmodified heparin. N-acetylation causes heparin to lose its affinity for antithrombin, decreasing anticoagulant activity.¹³⁶ Recently, the pharmacokinetics of SST0001 demonstrated that the mechanism of heparanase inhibition was based on drug concentration, suggesting the existence of multiple protein–ligand interactions.¹³⁷ SST0001 in multiple myeloma cells lines inhibited heparanase, and the expression of HGF, VEGF, and MMP-9 resulting in decreased angiogenesis. It also decreased shedding of syndecan-1 and heparanase-mediated degradation of syndecan-1 HS chains, which promotes myeloma growth.¹³⁶ Recently, SST0001 in combination with standard chemotherapy diminished the growth of disseminated myeloma tumors *in vivo*. SST0001 also decreased regrowth of myeloma tumors *in vivo* after completion of chemotherapy.¹³⁸ It was also studied in pediatric sarcoma, metastatic lepatinib resistant breast cancer, and pancreatic preclinical models; demonstrating effective anti-tumor activity.^{139–141} Specifically, in pediatric sarcoma it demonstrated synergy with other anti-angiogenic agents (i.e. bevacizumab).¹³⁹ SST0001 is currently undergoing phase I clinical trials in patients with advanced multiple myeloma, and was found to be safe at a dose of 200mg/day.¹⁴² (NCT01764880)

M402 (Necuparanib) is a N-sulfate glycol-split modified heparin. It was specifically engineered to substantially reduce anticoagulant activity while retaining its heparan sulfate-like binding properties to multiple targets involved in tumor progression and metastasis. It inhibits heparanase and endothelial sprouting in response to FGF2, HB-EGF, and VEGF.¹⁴³ Furthermore, it has demonstrated both as single agent and in combination with standard chemotherapy potent anti-metastatic activity in preclinical models.¹⁴³ It is currently in a phase I/II trial in combination with nab-paclitaxel and gemcitabine for the treatment of metastatic pancreatic cancer. (NCT01621243)

Nucleic-acid based inhibitors have also been used to modulate the effect of heparanase. Defibrotide is an orally bioavailable polydisperse oligonucleotide. It decreases heparanase expression and tumor growth in multiple myeloma cell lines. Interestingly, defibrotide did not have any direct cytotoxic effects on myeloma cells, but rather enhanced combination chemotherapy likely by augmenting the myeloma microenvironment.¹⁴⁴ It was well tolerated and safe in a phase I/II clinical trial in patients with relapsed/refractory multiple myeloma in combination with multi-agent chemotherapy.¹⁴⁵

Small molecule inhibitors against heparanase have either been discontinued or are still in preclinical studies. This lack of progress in this area is likely secondary to the inability to solve the crystal structure until recently. Suramin, a synthetic polysulfonated naphthylurea, was one of the first small molecule studied which has potent heparanase inhibition. It has been found to inhibit heparanase activity in melanoma, cervical, hepatocellular and ovarian cancer cell lines.^{146–148} It has not transitioned into clinical trials because its side-effect profile is too toxic. Multiple others have been studied and none have reached clinical trials.⁴¹

Anti-heparanase antibodies are another novel therapeutic to inhibit heparanase. Initial studies were successful in targeting and inhibiting heparanase, but did not produce significant anti-tumor effects. Recently, in a preclinical lymphoma mouse model, two antibodies directed the heparanase enzyme were employed.¹⁴⁹ One of the antibodies was directed against the KKDC peptide, while the second targeted the full length protein.¹⁴⁹ The results demonstrated significant inhibition of heparanase extracellularly and prevented uptake of heparanase intracellularly; resulting in reduced cellular invasion and metastasis.¹⁴⁹ Furthermore, when used in combination there was a potent synergistic effect in lymphoma murine models, with significant reduction in tumor growth and metastasis.¹⁴⁹ Interestingly, the antibodies had no direct cytotoxic effect on the tumor cells, but exerted their effect on the tumor microenvironment.¹⁴⁹

Vaccination against heparanase is also being explored. Heparanase is an ideal target for vaccination because it typically not expressed in non-pathologic cells, and thus the risk for autoimmunity is low. Heparanase peptides have been demonstrated to elicit a strong cytotoxic T-Cell (CTL) response *in vitro*.¹⁵⁰ This lead to the development of heparanase vaccines. The most common strategy employed thus far is the multiple antigen peptide vaccine approach (MAP). In the MAP vaccine approach multiple copies of antigenic peptides are bound to a non-immunogenic lysine-based dendritic scaffold resulting in an increased recognition by immune cells, and initiation of a stronger immune response.¹⁵¹ Recently, a B-cell MAP demonstrated a marked decrease in heparanase activity and expression of VEGF and FGF2; resulting in decreased microvessel density and tumor volume in a hepatocellular carcinoma murine model.¹⁵² Lastly, a recent T-cell MAP induced a heparanase-specific CTL to lyse tumor cells with a resultant increase in CTL secretion of interferon- γ in multiple malignancies. Importantly, it did not lyse heparanase-expressing autologous lymphocytes and dendritic cells.¹⁵³

Conclusions and future directions

There has been significant progress in understanding heparanase function in cancer biology over the past decades. Specifically, the interactions that occur within the tumor microenvironment between heparanase, HSPGs, and growth factors that stimulate tumor growth and metastasis have resulted in the development of heparanase inhibitors. Most recently, it has been demonstrated to promote tumor autophagy and enhance chemoresistance which can be reversed by heparanase inhibitors.^{93,96} Much of the preclinical work has focused on heparanase's role in tumor growth and metastasis, however it has been reported to play role in other pathologic disease states such as inflammation, vascular disease, and kidney disease are currently being explored.^{4,154}

Despite the significant role of heparanase in tumor growth and metastasis, the optimal approach for heparanase inhibition is still unknown. There are four heparan sulfate mimetics that show significant anti-tumor and anti-metastatic activity in preclinical models that are currently undergoing clinic trials. Preclinical studies have demonstrated efficacy as a single agent, in combination with chemotherapy to decrease chemotherapy resistance, as well as a maintenance therapy after finishing chemotherapy. It is likely that heparanase inhibitors will need to be combined with other drug combinations to achieve a satisfactory response.

Now that the crystal structure of mammalian heparanase has been solved, substantial improvement in designing future heparanase inhibitors, including small molecule inhibitors, can occur. Further work is still needed on understanding the non-enzymatic function of heparanase, identifying other cellular targets, which would allow for successful development of inhibitors specifically for its non-enzymatic function. Lastly, heparanase is considered an optimal target for immune-based therapy, for which more therapies will hopefully be developed in the coming years.

References

1. Iozzo R, San Antonia JD. Heparan Sulfate Proteoglycans: Heavy Hitters in the angiogenesis arena. *J Clin Invest.* 2001; 108:349–355. [PubMed: 11489925]
2. Kim S-H, Turnbull J, Guimond S. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *J Endocrinol.* 2011; 209:139–151. [PubMed: 21307119]
3. Soares M, Teixeira FC, Fontes M, et al. Heparan Sulfate Proteoglycans May Promote or Inhibit Cancer Progression by Interacting with Integrins and Affecting Cell Migration. *Biomed Res Int.* 2015; 2015:453801. [PubMed: 26558271]
4. Vlodaysky I, Beckhove P, Lerner I, et al. Significance of Heparanase in Cancer and Inflammation. *Cancer Microenviron.* 2012; 5:115–132. [PubMed: 21811836]
5. Friedmann Y, Vlodaysky I, Aingorn H, et al. Expression of heparanase in normal, dysplastic, and neoplastic human colonic mucosa and stroma. Evidence for its role in colonic tumorigenesis. *Am J Pathol.* 2000; 157:1167–1175. [PubMed: 11021821]
6. Sato T, Yamaguchi A, Goi T, et al. Heparanase expression in human colorectal cancer and its relationship to tumor angiogenesis, hematogenous metastasis, and prognosis. *J Surg Oncol.* 2004; 87:174–181. [PubMed: 15334632]
7. Xu X, Quiros RM, Maxhimer JB, Gattuso P, Prinz RA, et al. Inverse correlation between heparan sulfate composition and heparanase-1 gene expression in thyroid papillary carcinomas: a potential role in tumor metastasis. *Clin Cancer Res.* 2003; 9:5968–5979. [PubMed: 14676122]

8. Rohloff J, Zinke J, Schoppmeyer K, et al. Heparanase expression is a prognostic indicator for postoperative survival in pancreatic adenocarcinoma. *Br J Cancer*. 2002; 86:1270–1275. [PubMed: 11953884]
9. Gohji K, Okamoto M, Kitazawa S, et al. Heparanase protein and gene expression in bladder cancer. *J Urol*. 2001; 166:1286–1290. [PubMed: 11547059]
10. Masola V, Maran C, Tassone E, et al. Heparanase activity in alveolar and embryonal rhabdomyosarcoma: implications for tumor invasion. *BMC Cancer Microenviron*. 2009; 28:304.
11. Maxhimer J, Quiros RM, Stewart R, et al. Heparanase-1 expression is associated with the metastatic potential of breast cancer. *Surgery*. 2002; 132:326–333. [PubMed: 12219030]
12. Takaoka M, Naomoto Y, Ohkawa T, et al. Heparanase expression correlates with invasion and poor prognosis in gastric cancers. *Lab Invest*. 2003; 83:613–622. [PubMed: 12746471]
13. Beckhove P, Helmke BM, Ziouta Y, et al. Heparanase expression at the invasion front of human head and neck cancers and correlation with poor prognosis. *Clin Cancer Res*. 2005; 11:2899–2906. [PubMed: 15837740]
14. Kelly T, Miao HQ, Yang Y, et al. High heparanase activity in multiple myeloma is associated with elevated microvessel density. *Cancer Res*. 2003; 63:8749–8756. [PubMed: 14695190]
15. Bitan M, Polliack A, Zecchina G, et al. Heparanase expression in human leukemias is restricted to acute myeloid leukemias. *Exp Hematol*. 2002; 30:34–41. [PubMed: 11823035]
16. Vreys V, David G. Mammalian heparanase: what is the message? *J Cell Mol Med*. 2007; 11:427–452. [PubMed: 17635638]
17. Ilan N, Elkin M, Vlodacsky I. Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis. *Int J Biochem & Cell Biol*. 2006; 38:2018–2039. [PubMed: 16901744]
18. McKenzie E, Tyson K, Stamps A, et al. Cloning and expression profiling of Hpa2, a novel mammalian heparanase family member. *Biochem Biophys Res Commun*. 2000; 276:1170–1177. [PubMed: 11027606]
19. Levy-Adam F, Feld S, Cohen-Kaplan V, et al. Heparanase 2 interacts with heparan sulfate with high affinity and inhibits heparanase activity. *J Biol Chem*. 2010; 285:28010–28019. [PubMed: 20576607]
20. Arvatz G, Shafat I, Levy-Adam F, et al. The heparanase system and tumor metastasis: is heparanase the seed and soil? *Cancer Metastasis Rev*. 2011; 30:253–268. [PubMed: 21308479]
21. Dong J, Kukula A, Toyoshima M, et al. Genomic organization and chromosome localization of the newly identified human heparanase gene. *Gene*. 2000; 253:171–178. [PubMed: 10940554]
22. Kussie P, Hulmes JD, Ludwig DL, et al. Cloning and Functional expression of a human heparanase gene. *Biochem Biophys Res Commun*. 1999; 261:183–187. [PubMed: 10405343]
23. Toyoshima M, Nakajima M. Human heparanase. Purification, characterization, cloning and expression. *J Biol Chem*. 1999; 274:24153–24160. [PubMed: 10446189]
24. Vlodavsky I, Friedman Y, Elkin M, et al. Mammalian heparanase: Gene cloning, expression and function in tumor progression and metastasis. *Nat Med*. 1999; 5:793–802. [PubMed: 10395325]
25. Zetser A, Levy-Adam F, Kaplan V, et al. Processing and activation of latent heparanase occurs in lysosomes. *J Cell Sci*. 2004; 117:2249–2258. [PubMed: 15126626]
26. Fux L, Ilan N, Sanderson RD, et al. Heparanase: busy at the cell surface. *Trends Biochem Sci*. 2009; 34:511–519. [PubMed: 19733083]
27. Zhou Z, Bates M, Madura JD. Structure modeling, ligand binding, and binding affinity calculation (LR-MM-PBSA) of human heparanase for inhibition and drug design. *Proteins*. 2006; 65:580–592. [PubMed: 16972282]
28. Levy-Adam F, Abboud-Jarrous G, Guerrini M, et al. Identification and characterization of heparin/heparan sulfate binding domains of the endoglycosidase heparanase. *J Biol Chem*. 2005; 280:20457–20466. [PubMed: 15760902]
29. Wu L, Viola CM, Brzozowski AM, et al. Structural characterization of human heparanase reveals insights into substrate recognition. *Nat Struct Mol Biol*. 2015; 22:1016–1022. [PubMed: 26575439]

30. Baraz L, Haupt Y, Elkin M, et al. Tumor suppressor p53 regulates heparanase gene expression. *Oncogene*. 2006; 25:3939–3947. [PubMed: 16474844]
31. Abboud-Jarrous G, Rangini-Guetta Z, Aingorn H, et al. Site-directed mutagenesis, proteolytic cleavage, and activation of human proheparanase. *J Biol Chem*. 2005; 280:13568–13575. [PubMed: 15659389]
32. Liu W, Litwack ED, Stanley MJ, et al. Heparan sulfate proteoglycans as adhesive and anti-invasive molecules. Syndecans and glypican have distinct functions. *J Biol Chem*. 1998; 273:22825–22832. [PubMed: 9712917]
33. Beauvais DM BB, Rapraeger AC. The syndecan-1 ectodomain regulates alphavbeta3 integrin activity in human mammary carcinoma cells. *J Cell Biol*. 2004; 167:171–181. [PubMed: 15479743]
34. Lee H, Kim Y, Choi Y, et al. Syndecan-2 cytoplasmic domain regulates colon cancer cell migration via interaction with syntenin-1. *Biochem Biophys Res Commun*. 2011; 409:148–153. [PubMed: 21569759]
35. Lee J, Park H, Chung H, et al. Syndecan-2 regulates the migratory potential of melanoma cells. *J Biol Chem*. 2009; 280:27167–27175.
36. Sanderson R, Lalor P, Bernfield M. B lymphocytes express and lose syndecan at specific stages of differentiation. *Cell Regul*. 1989; 1:27–35. [PubMed: 2519615]
37. Chilosi M, Adami F, Lestani M, et al. CD138/syndecan-1: a useful immunohistochemical marker of normal and neoplastic plasma cells on routine trephine bone marrow biopsies. *Mod Pathol*. 1999; 12:1101–1106. [PubMed: 10619261]
38. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009; 119:1420–1428. [PubMed: 19487818]
39. Ramani V, Purushothaman A, Stewart MD, et al. The heparanase/syndecan-1 axis in cancer: mechanisms and therapies. *FEBS J*. 2013; 280:2294–2306. [PubMed: 23374281]
40. Bernfield M, Götte M, Park PW, et al. Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem*. 1999; 68:729–777. [PubMed: 10872465]
41. Hammond E, Khurana A, Shridhar V, et al. The role of heparanase and sulfatases in the modification of heparan sulfate proteoglycans within the tumor microenvironment and opportunities for novel cancer therapeutics. *Front Oncol*. 2014; 24:195.
42. Su G, Blaine SA, Qiao D, et al. Shedding of syndecan-1 by stromal fibroblasts stimulates human breast cancer cell proliferation via FGF2 activation. *J Biol Chem*. 2007; 282:14906–14915. [PubMed: 17344212]
43. Purushothaman A, Uyama T, Kobayashi F, et al. Heparanase-enhanced shedding of syndecan-1 by myeloma cells promotes endothelial invasion and angiogenesis. *Blood*. 2010; 115:2449–2457. [PubMed: 20097882]
44. Ramani V, Pruett PS, Thompson CA, et al. Heparan sulfate chains of syndecan-1 regulate ectodomain shedding. *J Biol Chem*. 2012; 287:9952–9961. [PubMed: 22298773]
45. Ding K, Lopez-Burks M, Sánchez-Duran JA, et al. Growth factor-induced shedding of syndecan-1 confers glypican-1 dependence on mitogenic responses of cancer cells. *J Cell Biol*. 2005; 171:729–738. [PubMed: 16286510]
46. Bass MDM, Humphries MJ. Syndecans shed their reputation as inert molecules. *Sci Signal*. 2009; 2:pe18. [PubMed: 19336838]
47. Yang Y, Macleod V, Miao HQ, et al. Heparanase enhances syndecan-1 shedding: a novel mechanism for stimulation of tumor growth and metastasis. *J Biol Chem*. 2007; 282:13326–13333. [PubMed: 17347152]
48. Manon-Jensen T, Itoh Y, Couchman JR. Proteoglycans in health and disease: the multiple roles of syndecan shedding. *FEBS J*. 2010; 277:3876–3889. [PubMed: 20840585]
49. Ramani V, Sanderson RD. Chemotherapy stimulates syndecan-1 shedding: a potentially negative effect of treatment that may promote tumor relapse. *Matrix Biol*. 2014; 35:215–222. [PubMed: 24145151]
50. Ramani V, Vlodaysky I, Ng M, et al. Chemotherapy induces expression and release of heparanase leading to changes associated with an aggressive tumor phenotype. *Matrix Biol*. 2016 [epub ahead of print].

51. Szatmári T, Ötvös R, Hjerpe A, et al. Syndecan-1 in Cancer: Implications for Cell Signaling, Differentiation, and Prognostication. *Dis Markers*. 2015; 2015:796052. [PubMed: 26420915]
52. Ramani V, Yang Y, Ren Y, et al. Heparanase plays a dual role in driving hepatocyte growth factor (HGF) signaling by enhancing HGF expression and activity. *J Biol Chem*. 2011; 286:6490–6499. [PubMed: 21131364]
53. Yang Y, Yaccoby S, Liu W, et al. Soluble syndecan-1 promotes growth of myeloma tumors in vivo. *Blood*. 2002; 100:610–617. [PubMed: 12091355]
54. Cheng F, Petersson P, Arroyo-Yanguas Y, et al. Differences in the uptake and nuclear localization of anti-proliferative heparan sulfate between human lung fibroblasts and human lung carcinoma cells. *J Cell Biochem*. 2001; 83:597–606. [PubMed: 11746503]
55. Dudás J, Ramadori G, Knittel T, et al. Effect of heparin and liver heparan sulphate on interaction of HepG2-derived transcription factors and their cis-acting elements: altered potential of hepatocellular carcinoma heparan sulphate. *Biochem J*. 2000; 350:245–251. [PubMed: 10926850]
56. Buczek-Thomas J, Hsia E, Rich CB, et al. Inhibition of histone acetyltransferase by glycosaminoglycans. *J Cell Biochem*. 2008; 105:108–120. [PubMed: 18459114]
57. Stewart M, Ramani VC, Sanderson RD. Shed syndecan-1 translocates to the nucleus of cells delivering growth factors and inhibiting histone acetylation: a novel mechanism of tumor-host cross-talk. *J Biol Chem*. 2015; 290:941–949. [PubMed: 25404732]
58. He Y, Sutcliffe EL, Bunting KL, et al. The endoglycosidase heparanase enters the nucleus of T lymphocytes and modulates H3 methylation at actively transcribed genes via the interplay with key chromatin modifying enzymes. *Transcription*. 2012; 3:130–145. [PubMed: 22771948]
59. Zhang L, Sullivan P, Suyama J, et al. Epidermal growth factor-induced heparanase nucleolar localization augments DNA topoisomerase I activity in brain metastatic breast cancer. *Mol Cancer Res*. 2010; 8:278–290. [PubMed: 20164500]
60. Purushothaman A, Hurst DR, Pisano C, et al. Heparanase-mediated loss of nuclear syndecan-1 enhances histone acetyltransferase (HAT) activity to promote expression of genes that drive an aggressive tumor phenotype. *J Biol Chem*. 2011; 286:30377–30383. [PubMed: 21757697]
61. Götte M, Kersting C, Ruggiero M, et al. Predictive value of syndecan-1 expression for the response to neoadjuvant chemotherapy of primary breast cancer. *Anticancer Res*. 2006; 26:621–627. [PubMed: 16739330]
62. Nguyen T, Grizzle WE, Zhang K, et al. Syndecan-1 overexpression is associated with nonluminal subtypes and poor prognosis in advanced breast cancer. *Am J Clin Pathol*. 2013; 140:468–474. [PubMed: 24045542]
63. Juuti A, Nordling S, Lundin J, et al. Syndecan-1 expression--a novel prognostic marker in pancreatic cancer. *Oncology*. 2005; 68:97–106. [PubMed: 15886501]
64. Wiksten J, Lundin J, Nordling S, et al. Epithelial and stromal syndecan-1 expression as predictor of outcome in patients with gastric cancer. *Int J Cancer*. 2001; 95:1–6. [PubMed: 11241302]
65. Hasengaowa KJ, Kusumoto T, et al. Prognostic significance of syndecan-1 expression in human endometrial cancer. *Ann Oncol*. 2005; 16:1109–1115. [PubMed: 15851381]
66. Kusumoto T, Kodama J, Seki N, et al. Clinical significance of syndecan-1 and versican expression in human epithelial ovarian cancer. *Oncol Rep*. 2010; 23:917–925. [PubMed: 20204274]
67. Mundt F, Heidari-Hamedani G, Nilsson G, et al. Diagnostic and prognostic value of soluble syndecan-1 in pleural malignancies. *Biomed Res Int*. 2014; 2014:419853. [PubMed: 25147801]
68. Szarvas T, Reis H, Kramer G, et al. Enhanced stromal syndecan-1 expression is an independent risk factor for poor survival in bladder cancer. *Hum Pathol*. 2014; 45:674–682. [PubMed: 24656090]
69. Zellweger T, Ninck C, Mirlacher M, et al. Tissue microarray analysis reveals prognostic significance of syndecan-1 expression in prostate cancer. *Prostate*. 2003; 55:20–29. [PubMed: 12640657]
70. Seidel C, Sundan A, Hjorth M, et al. Serum syndecan-1: a new independent prognostic marker in multiple myeloma. *Blood*. 2000; 95:388–392. [PubMed: 10627439]
71. Bodoor K, Matalka I, Hayajneh R, et al. Evaluation of BCL-6, CD10, CD138 and MUM-1 expression in diffuse large B-cell lymphoma patients: CD138 is a marker of poor prognosis. *Asian Pac J Cancer Prev*. 2012; 13:3037–3046. [PubMed: 22994707]

72. R G. Advances in the molecular functions of syndecan-1 (SDC1/CD138) in the pathogenesis of malignancies. *Crit Rev Oncol Hematol*. 2015; 94:1–17. [PubMed: 25563413]
73. Anttonen A, Leppä S, Ruotsalainen T, et al. Pretreatment serum syndecan-1 levels and outcome in small cell lung cancer patients treated with platinum-based chemotherapy. *Lung Cancer*. 2003; 41:171–177. [PubMed: 12871780]
74. Kawano Y, Fujiwara S, Wada N, et al. Multiple myeloma cells expressing low levels of CD138 have an immature phenotype and reduced sensitivity to lenalidomide. *Int J Oncol*. 2012; 41:876–884. [PubMed: 22766978]
75. Nakajima M, Irimura T, Di Ferrante D, et al. Heparan sulfate degradation: relation to tumor invasive and metastatic properties of mouse B16 melanoma sublines. *Science*. 1983; 220:611–613. [PubMed: 6220468]
76. Uno F, Fujiwara T, Takata Y, et al. Antisense-mediated suppression of human heparanase gene expression inhibits pleural dissemination of human cancer cells. *Cancer Res*. 2001; 61:7855–7860. [PubMed: 11691803]
77. Lerner I, Baraz L, Pikarsky E, et al. Function of heparanase in prostate tumorigenesis: potential for therapy. *Clin Cancer Res*. 2008; 14:668–676. [PubMed: 18212251]
78. Roy M, Reiland J, Murry BP, et al. Antisense-mediated suppression of Heparanase gene inhibits melanoma cell invasion. *Neoplasia*. 2005; 7:253–262. [PubMed: 15799825]
79. Xiong Z, Lü MH, Fan YH, et al. Downregulation of heparanase by RNA interference inhibits invasion and tumorigenesis of hepatocellular cancer cells in vitro and in vivo. *Int J Oncol*. 2012; 40:1601–1609. [PubMed: 22267022]
80. Edovitsky E, Elkin M, Zcharia E, et al. Heparanase gene silencing, tumor invasiveness, angiogenesis, and metastasis. *J Natl Cancer Inst*. 2004; 96:1219–1230. [PubMed: 15316057]
81. Peterson S, Liu J. Multi-faceted substrate specificity of heparanase. *Matrix Biol*. 2013; 32:223–227. [PubMed: 23499529]
82. Myler H, West JL. Heparanase and platelet factor-4 induce smooth muscle cell proliferation and migration via bFGF release from the ECM. *J Biochem*. 2002; 131:913–922. [PubMed: 12038989]
83. Meirovitz A, Goldberg R, Binder A, et al. Heparanase in inflammation and inflammation-associated cancer. *FEBS J*. 2013; 280:2307–2319. [PubMed: 23398975]
84. Sanderson RDYY, Suva LJ, et al. Heparan sulfate proteoglycans and heparanase--partners in osteolytic tumor growth and metastasis. *Matrix Biol*. 2004; 23:341–352. [PubMed: 15533755]
85. Elkin M, Ilan N, Ishai-Michaeli R, et al. Heparanase as mediator of angiogenesis: mode of action. *FASEB J*. 2001; 15:1661–1663. [PubMed: 11427519]
86. Watanabe M, Aoki Y, Kase H, et al. Heparanase expression and angiogenesis in endometrial cancer. *Gynecol Obstet Invest*. 2003; 56:77–82. [PubMed: 12904690]
87. Cohen-Kaplan V, Naroditsky I, Zetser A, et al. Heparanase induces VEGF C and facilitates tumor lymphangiogenesis. *Int J Cancer*. 2008; 123:2566–2573. [PubMed: 18798279]
88. Zetser A, Bashenko Y, Edovitsky E, et al. Heparanase induces vascular endothelial growth factor expression: correlation with p38 phosphorylation levels and Src activation. *Cancer Res*. 2006; 66:1455–1463. [PubMed: 16452201]
89. Gingis-Velitski S, Zetser A, Flugelman MY, et al. Heparanase induces endothelial cell migration via protein kinase B/Akt activation. *J Biol Chem*. 2004; 279:23536–23541. [PubMed: 15044433]
90. Zetser A, Bashenko Y, Miao HQ, et al. Heparanase affects adhesive and tumorigenic potential of human glioma cells. *Cancer Res*. 2003; 63:7733–7741. [PubMed: 14633698]
91. Purushothaman A, Babitz SK, Sanderson RD. Heparanase enhances the insulin receptor signaling pathway to activate extracellular signal-regulated kinase in multiple myeloma. *J Biol Chem*. 2012; 287:41288–41296. [PubMed: 23048032]
92. Cohen-Kaplan V, Doweck I, Naroditsky I, et al. Heparanase augments epidermal growth factor receptor phosphorylation: correlation with head and neck tumor progression. *Cancer Res*. 2008; 68:10077–10085. [PubMed: 19074873]
93. Ramani VC, Zhan F, He J, et al. Targeting heparanase overcomes chemoresistance and diminishes relapse in myeloma. *Oncotarget*. 2016; 7:1598–1607. [PubMed: 26624982]

94. Ramani VC, Vlodaysky I, Ng M, et al. Chemotherapy induces expression and release of heparanase leading to changes associated with an aggressive tumor phenotype. *Matrix Biol.* 2016
95. Li J, Pan Q, Rowan PD, et al. Heparanase promotes myeloma progression by inducing mesenchymal features and motility of myeloma cells. *Oncotarget.* 2016; 7:11299–11309. [PubMed: 26849235]
96. Shteingauz A, Boyango I, Naroditsky I, et al. Heparanase Enhances Tumor Growth and Chemoresistance by Promoting Autophagy. *Cancer Res.* 2015; 75:3946–3957. [PubMed: 26249176]
97. Ilan N, Shteingauz A, Vlodaysky I. Function from within: Autophagy induction by HPSE/heparanase--new possibilities for intervention. *Autophagy.* 2015; 11:2387–2389. [PubMed: 26571129]
98. Levy-Adam F, Ilan N, Vlodaysky I. Tumorigenic and adhesive properties of heparanase. *Semin Cancer Biol.* 2010; 20:153–160. [PubMed: 20619346]
99. Nadir Y, Brenner B, Zetser A, et al. Heparanase induces tissue factor expression in vascular endothelial and cancer cells. *J Thromb Haemost.* 2006; 4:2443–2451. [PubMed: 16970801]
100. Nadir Y, Brenner B, Fux L, et al. Heparanase enhances the generation of activated factor X in the presence of tissue factor and activated factor VII. *Haematologica.* 2010; 95:1927–1934. [PubMed: 20634491]
101. Nadir Y, Brenner B, Gingis-Velitski S, et al. Heparanase induces tissue factor pathway inhibitor expression and extracellular accumulation in endothelial and tumor cells. *Thromb Haemost.* 2008; 99:133–141. [PubMed: 18217145]
102. Cui H, Tan YX, Osterholm C, et al. Heparanase expression upregulates platelet adhesion activity and thrombogenicity. *Oncotarget.* 2016 [Epub ahead of print].
103. Kogan I, Chap D, Hoffman R, et al. JAK-2 V617F mutation increases heparanase procoagulant activity. *Thromb Haemost.* 2016; 115:73–80. [PubMed: 26489695]
104. Klerk C, Smorenburg SM, Otten HM, et al. The effect of low molecular weight heparin on survival in patients with advanced malignancy. *J Clin Oncol.* 2005; 23:2130–2135. [PubMed: 15699479]
105. Zhang N, Lou W, Ji F, et al. Low molecular weight heparin and cancer survival: clinical trials and experimental mechanisms. *J Cancer Res Clin Oncol Rep.* 2016 [Epub ahead of print].
106. Sanford D, Naidu A, Alizadeh N, et al. The effect of low molecular weight heparin on survival in cancer patients: an updated systematic review and meta-analysis of randomized trials. *J Thromb Haemost.* 2014; 12:1076–1085. [PubMed: 24796727]
107. Balzarotti M, Fontana F, Marras C, et al. In vitro study of low molecular weight heparin effect on cell growth and cell invasion in primary cell cultures of high-grade gliomas. *Oncol Res.* 2006; 16:245–250. [PubMed: 17294805]
108. Franchini M, Mannucci PM. Low-molecular-weight heparins and cancer: focus on antitumoral effect. *Ann Med.* 2015; 47:116–121. [PubMed: 25766973]
109. Abu Arab W, Kotb R, Sirois M, et al. Concentration- and time-dependent effects of enoxaparin on human adenocarcinomic epithelial cell line A549 proliferation in vitro. *Can J Physiol Pharmacol.* 2011; 89:705–711. [PubMed: 21905823]
110. Smiley S, Henry DO, Wong MK. The mechanism of low molecular weight heparin (LMWH) inhibition of tumor growth. *J Clin Oncol.* 2006; 24:18S.
111. Stevenson J, Choi SH, Vark A. Differential metastasis inhibition by clinically relevant levels of heparins--correlation with selectin inhibition, not antithrombotic activity. *Clin Cancer Res.* 2005; 11:7003–7011. [PubMed: 16203794]
112. Harvey J, Mellor P, Eldaly H, et al. Inhibition of CXCR4-mediated breast cancer metastasis: a potential role for heparinoids? *Clin Cancer Res.* 2007; 13:1562–1570. [PubMed: 17332302]
113. Marchetti M, Vignoli A, Russo L, et al. Endothelial capillary tube formation and cell proliferation induced by tumor cells are affected by low molecular weight heparins and unfractionated heparin. *Thromb Res.* 2008; 121:637–645. [PubMed: 17692905]
114. Mousa S, Petersen LJ. Anti-cancer properties of low-molecular-weight heparin: preclinical evidence. *Thromb Haemost.* 2009; 102:258–267. [PubMed: 19652876]

115. Mousa S, Mohamed S. Inhibition of endothelial cell tube formation by the low molecular weight heparin, tinzaparin, is mediated by tissue factor pathway inhibitor. *Thromb Haemost.* 2004; 92:627–633. [PubMed: 15351861]
116. Pisano C, Vlodasky I, Ilan N, et al. The Potential of heparanase as a therapeutic target in cancer. *Biochem Pharmacol.* 2014; 89:12–19. [PubMed: 24565907]
117. Coombe D, Kett WC. Heparin mimetics. *Handb Exp Pharmacol.* 2012; 207:361–383.
118. Ferro V, Fewings K, Palermo MC, et al. Large-scale preparation of the oligosaccharide phosphate fraction of *Pichia holstii* NRRL Y-2448 phosphomannan for use in the manufacture of PI-88. *Carbohydr Res.* 2001; 332:183–189. [PubMed: 11434376]
119. Parish C, Freeman C, Brown KJ, et al. Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity. *Cancer Res.* 1999; 59:3433–3441. [PubMed: 10416607]
120. Cochran S, Li C, Fairweather JK, et al. Probing the interactions of phosphosulfomannans with angiogenic growth factors by surface plasmon resonance. *J Med Chem.* 2003; 46:4601–4608. [PubMed: 14521421]
121. Ferro V, Dredge K, Liu L, et al. PI-88 and novel heparan sulfate mimetics inhibit angiogenesis. *Semin Thromb Hemost.* 33:557–568. 200. [PubMed: 17629854]
122. Iversen P, Sorensen DR, Benestad HB. Inhibitors of angiogenesis selectively reduce the malignant cell load in rodent models of human myeloid leukemias. *Leukemia.* 2002; 16:376–381. [PubMed: 11896541]
123. Joyce J, Freeman C, Meyer-Morse N, et al. A functional heparan sulfate mimetic implicates both heparanase and heparan sulfate in tumor angiogenesis and invasion in a mouse model of multistage cancer. *Oncogene.* 2005; 24:4037–4051. [PubMed: 15806157]
124. Basche M, Gustafson DL, Holden SN, et al. A phase I biological and pharmacologic study of the heparanase inhibitor PI-88 in patients with advanced solid tumors. *Clin Cancer Res.* 2006; 12:5471–5480. [PubMed: 17000682]
125. Lewis K, Robinson WA, Millward MJ, et al. A phase II study of the heparanase inhibitor PI-88 in patients with advanced melanoma. *Invest New Drugs.* 2008; 26:89–94. [PubMed: 17891338]
126. Chow L, Gustafson DL, O'Bryant CL, et al. A phase I pharmacological and biological study of PI-88 and docetaxel in patients with advanced malignancies. *Cancer Chemother Pharmacol.* 2008; 63:65–74. [PubMed: 18320191]
127. Liu C, Chang J, Lee PH, et al. Adjuvant heparanase inhibitor PI-88 therapy for hepatocellular carcinoma recurrence. *World J Gastroenterol.* 2014; 20:11384–11393. [PubMed: 25170226]
128. Dredge K, Hammond E, Davis K, et al. The PG500 series: novel heparan sulfate mimetics as potent angiogenesis and heparanase inhibitors for cancer therapy. *Invest New Drugs.* 2010; 28:276–283. [PubMed: 19357810]
129. Dredge K, Hammond E, Handley P, et al. PG545, a dual heparanase and angiogenesis inhibitor, induces potent anti-tumor and anti-metastatic efficacy in preclinical models. *British Journal of Cancer.* 2011; 104:635–642. [PubMed: 21285983]
130. Jung D, Yun M, Kim EO, et al. The heparan sulfate mimetic PG545 interferes with Wnt/ β -catenin signaling and significantly suppresses pancreatic tumorigenesis alone and in combination with gemcitabine. *Oncotarget.* 2015; 6:4992–5004. [PubMed: 25669977]
131. Winterhoff B, Freyer L, Hammond E, et al. PG545 enhances anti-cancer activity of chemotherapy in ovarian models and increases surrogate biomarkers such as VEGF in preclinical and clinical plasma samples. *European Journal of Cancer.* 2015; 51:879–892. [PubMed: 25754234]
132. Ostapoff K, Awasthi N, Cenik B, et al. PG545, an Angiogenesis and Heparanase inhibitor, reduced primary tumor growth and metastasis in experimental pancreatic cancer. *Mol Cancer Ther.* 2013; 12:1190–1201. [PubMed: 23696215]
133. Hammond E, Brandt R, Dredge K. PG545, a Heparan Sulfate Mimetic, Reduces Heparanase expression In Vivo, blocks spontaneous metastases and enhances overall survival in the 4T1 Breast carcinoma model. *PLoS One.* 2012; 7:e52175. [PubMed: 23300607]
134. Brennan T, Lin L, Brandstadter J, et al. Heparan sulfate mimetic PG545-mediated antilymphoma effects require TLR9 dependent NK cell activation. *J Clin Invest.* 2016; 126:207–219. [PubMed: 26649979]

135. Mondal S, Roy D, Camacho-Pereira J, et al. HSulf-1 deficiency dictates a metabolic reprogramming of glycolysis and TCA cycle in ovarian cancer. *Oncotarget*. 2015; 6:33705–33719. [PubMed: 26378042]
136. Ritchie J, Ramani VC, Ren Y, et al. SST0001, a chemically modified heparin, inhibits myeloma growth and angiogenesis via disruption of the heparanase/syndecan-1 axis. *Clin Cancer Res*. 2011; 17:1382–1393. [PubMed: 21257720]
137. Pala D, Rivara S, Mor M, et al. Kinetic analysis and molecular modeling of the inhibition mechanism of roneparstat (SST0001) on human heparanase. *Glycobiology*. 2016; 26:640–654. [PubMed: 26762172]
138. Ramani V, Zhan F, He J, et al. Targeting heparanase overcomes chemoresistance and diminishes relapse in myeloma. *Oncotarget*. 2016; 7:1598–1607. [PubMed: 26624982]
139. Cassinelli G, Lanzi C, Tortoreto M, et al. Antitumor efficacy of the heparanase inhibitor SST0001 alone and in combination with antiangiogenic agents in the treatment of human pediatric sarcoma models. *Biochem Pharmacol*. 2013; 85:1424–1432. [PubMed: 23466421]
140. Meirovitz A, Hermano E, Lerner I, et al. Role of heparanase in radiation-enhanced invasiveness of pancreatic carcinoma. *Cancer Res*. 2011; 71:2772–2780. [PubMed: 21447736]
141. Zhang L, Ngo JA, Wetzel MD, et al. Heparanase mediates a novel mechanism in lapatinib-resistant brain metastatic breast cancer. *Neoplasia*. 2015; 17:101–113. [PubMed: 25622903]
142. Galli M, Magen H, Hermann E, et al. Roneparstat (SST0001), an Innovative Heparanase (HPSE) Inhibitor for Multiple Myeloma (MM) Therapy: First in Man Study. *Blood*. 2015; 126:S3246.
143. Zhou H, Roy S, Cochran E, et al. M402, a novel heparan sulfate mimetic, targets multiple pathways implicated in tumor progression and metastasis. *PLoS One*. 2011; 6:e21106. [PubMed: 21698156]
144. Mitsiades C, Rouleau C, Echart C, et al. Preclinical studies in support of defibrotide for the treatment of multiple myeloma and other neoplasias. *Clin Cancer Res*. 2009; 15:1210–1221. [PubMed: 19228727]
145. Palumbo A, Larocca A, Genuardi M, et al. Melphalan, prednisone, thalidomide and defibrotide in relapsed/refractory multiple myeloma: results of a multicenter phase I/II trial. *Haematologica*. 2011; 95:1144–1149.
146. Nakajima M, DeChavigny A, Johnson CE, et al. Suramin. A potent inhibitor of melanoma heparanase and invasion. *J Biol Chem*. 1991; 266:9661–9666. [PubMed: 2033058]
147. Li H, Li H, Qu H, et al. Suramin inhibits cell proliferation in ovarian and cervical cancer by downregulating heparanase expression. *Cancer Cell Int*. 2015; 15:52. [PubMed: 26052253]
148. Tayel A, Abd El Galil KH, Ebrahim MA, et al. Suramin inhibits hepatic tissue damage in hepatocellular carcinoma through deactivation of heparanase enzyme. *Eur J Pharmacol*. 2014; 728:151–160. [PubMed: 24530413]
149. Weissmann M, Arvatz G, Horowitz N, et al. Heparanase-neutralizing antibodies attenuate lymphoma tumor growth and metastasis. *Proc Natl Acad Sci U S A*. 2016; 113:704–709. [PubMed: 26729870]
150. Tang X, Liang GP, Li C, et al. Cytotoxic T lymphocyte epitopes from human heparanase can elicit a potent anti-tumor immune response in mice. *Cancer Immunol Immunother*. 2010; 59:1041–1047. [PubMed: 20182872]
151. JP T. Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. *Proc Natl Acad Sci U S A*. 1988; 85:5409–5413. [PubMed: 3399498]
152. Zhang J, Yang J, Cai Y, et al. Multiple antigenic polypeptide composed of heparanase B-cell epitopes shrinks human hepatocellular carcinoma in mice. *Oncol Rep*. 2015; 33:1248–1256. [PubMed: 25522727]
153. Tang X, Guo SL, Wang GZ, et al. In vitro and ex vivo evaluation of a multi-epitope heparinase vaccine for various malignancies. *Cancer Sci*. 2014; 105:9–17. [PubMed: 24152338]
154. Masola V, Zaza G, Onisto M, et al. Impact of heparanase on renal fibrosis. *J Transl Med*. 2015; 13:181. [PubMed: 26040666]
155. Tayel A, Ebrahim MA, Ibrahim AS, et al. Cytotoxic effects of suramin against HepG2 cells through activation of intrinsic apoptotic pathway. *J BUON*. 2014; 19:1048–1054. [PubMed: 25536615]

156. Li Y, Liu H, Huang YY, et al. Suppression of endoplasmic reticulum stress-induced invasion and migration of breast cancer cells through the downregulation of heparanase. *Int J Mol Med*. 2013; 31:1234–1242. [PubMed: 23467544]
157. Simmons S, Jämsä H, Silva D, et al. Anti-heparanase aptamers as potential diagnostic and therapeutic agents for oral cancer. *PLoS One*. 2014; 9:e96846. [PubMed: 25295847]
158. Dong W, Zhao H, Zhang C, et al. Gene silencing of heparanase results in suppression of invasion and migration of hepatoma cells. *World J Surg Oncol*. 2014; 12:85. [PubMed: 25185798]
159. Liu M, Zhang Y, Liao Y, et al. Evaluation of the Antitumor Efficacy of RNAi-Mediated Inhibition of CDC20 and Heparanase in an Orthotopic Liver Tumor Model. *Cancer Biother Radiopharm*. 2015; 30:233–239. [PubMed: 26132704]
160. Liu X, Tang QS, Chen HC, et al. Lentiviral miR30-based RNA interference against heparanase suppresses melanoma metastasis with lower liver and lung toxicity. *Int J Biol Sci*. 2013; 9:564–577. [PubMed: 23847439]

Highlights

- Review the role of heparanase and heparanase inhibitors in cancer biology and therapy.
- Heparanase promotes invasion, angiogenesis, and tumor metastasis in preclinical models.
- Inhibition of heparanase results in decreased tumor growth and metastasis *in vivo*.
- Several classes of heparanase inhibitors are presently being investigated.

Table 1

Heparanase Inhibitors Currently in Development

Drug Name	Drug Category	Mechanism of Action	Clinical Trial	Note	References
PL-88 (Mupafostat)	Heparan Sulfate Mimetic	Inhibits heparanase; ↓FGF-1, FGF-2, and VEGF	Phase III in HCC (NCT01402908)	Also releases TFPI; ↓angiogenesis.	118,121
PG545	Heparan Sulfate Mimetic	Inhibits heparanase; ↓FGF-1, FGF-2, and VEGF.	Phase I in advanced solid tumors. (NCT02042781)	In lymphoma exerts major anti-tumor effects by ↑ NK cell activity.	129,134
SST0001 (Roneparstat)	Heparan Sulfate Mimetic	Inhibits heparanase; ↓HGF, VEGF, and MMP-9. ↓shedding of syndecan-1	Phase I in R/R multiple myeloma. (NCT01764880)	Decreased regrowth of myeloma tumors <i>in vivo</i> after completion of chemotherapy.	136,138
M402 (Necuparamib)	Heparan Sulfate Mimetic	Inhibits heparanase; ↓ EC sprouting, FGF2, HB-EGF, and VEGF	Phase I/II trial for the treatment of metastatic pancreatic cancer. (NCT01621243)		143
Suramin	Small Molecule Inhibitor	Inhibits heparanase; ↓FGF-2 and Caspase-3.	None	↑HSPG's, ↓fibrosis and hepatic tissue breakdown in HCC murine model.	148,155
OGT2115	Small Molecule Inhibitor	Inhibits heparanase	None	Suppresses metastasis induced by endoplasmic reticulum stress from chemotherapy in breast cancer cells.	156
9E8 & H1023	Anti-heparin Antibodies	Inhibition of heparanase; ↓cellular invasion and metastasis.	None	Potent synergism when combination of both antibodies used in myeloma and lymphoma murine models.	149

Drug Name	Drug Category	Mechanism of Action	Clinical Trial	Note	References
B-Cell MAP	Vaccine	Inhibits heparanase; ↓expression of VEGF and bFGF.	None	↓Microvessel density and tumor volume in HCC murine model.	152
T-Cell MAP	Vaccine	CTL dependent lysis of tumor cells, ↑IFN- γ .	None	No activity against autologous lymphocytes and dendritic cells.	146
Defibrotide	Nucleic-acid based inhibitors	↓heparanase expression and tumor growth	Phase I/II trial in R/R myeloma with combination chemotherapy. (NCT00406978)	No direct cytotoxic effect on myeloma.	144,145
Single Strand DNA aptamers	Nucleic-acid based inhibitors	Decreased expression of heparanase; ↓tissue invasion of tumor cells	None	No direct cytotoxic effects on oral cancer cells. Stable, with ↓complex formation.	157
RNAi (siRNA/shRNA)	Nucleic-acid based inhibitors	Decreased expression of heparanase; ↓invasion and migration of tumor cells	None	Found to work in multiple cells lines including: HCC and melanoma.	158-160

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript