**Abstract:**

Heparan sulfate proteoglycans (HSPGs) play important roles in tumorigenesis by mediating tumor-stromal interactions through the presentation of growth factors, cytokines, and chemokines critical for tumor progression, survival and metastasis. M402 is a rationally engineered, non-cytotoxic HSPG mimetic, designed to disrupt tumor-host interactions. M402 binds and inhibits multiple factors including VEGF, FGF2, SDF-1α, and Platelet. A single 10 mg/kg subcutaneous (s.c.) dose of M402 effectively reduced seeding of B16F10 murine melanoma cells to the lung in a syngeneic experimental metastasis model. Chronic administration of M402, alone or in combination with cisplatin or docetaxel, inhibited spontaneous metastasis of orthotopically implanted 4T1 murine mammary carcinoma in this model. M402 treatment also normalized circulating levels of GR-1+ immature myeloid cells and platelet counts in 4T1 metastatic tumor bearing mice. Fluorescently-labeled M402 exhibited selective accumulation in the primary tumor. Immunohistochemical analyses of primary tumor presented a decrease in microvessel density in M402-treated animals, suggesting antiangiogenic may be one of the mechanisms involved in vivo. Importantly, M402, as monotherapy or in combination with chemotherapeutics, also revealed significant survival benefits in this aggressive tumor model. These data demonstrate that targeting HSPG biology may provide a useful approach to attenuate multiple pathways involved in tumor progression and metastasis.

**Methods and Results**

**Figure 1.** M402 has greatly reduced anticoagulant activity while retaining binding to many heparin binding proteins and inhibited tumor colonization in the murine B16F10 experimental metastasis model. (A) whole body bioluminescence quantified as photons/second over time. (B) Kaplan-Meier survival curves showing median survival of animals in different groups. (C) Immature myeloid cells. (D) Tumor non-infiltrated vessels in lung sections of animals treated with saline (n=10) or M402 (n=10) as described in the Methods section. (E) Lung histology and microvessel density of animals treated with saline or M402 (n=10) as described in the Methods section. (F) Lung histology and microvessel density of saline control mice and M402 treated mice. (G) Lung histology and microvessel density of saline control mice and M402 treated mice. (H) Lung histology and microvessel density of saline control mice and M402 treated mice. (I) Lung histology and microvessel density of saline control mice and M402 treated mice. (J) Lung histology and microvessel density of saline control mice and M402 treated mice. (K) Lung histology and microvessel density of saline control mice and M402 treated mice. (L) Lung histology and microvessel density of saline control mice and M402 treated mice. (M) Lung histology and microvessel density of saline control mice and M402 treated mice. (N) Lung histology and microvessel density of saline control mice and M402 treated mice. (O) Lung histology and microvessel density of saline control mice and M402 treated mice. (P) Lung histology and microvessel density of saline control mice and M402 treated mice. (Q) Lung histology and microvessel density of saline control mice and M402 treated mice. (R) Lung histology and microvessel density of saline control mice and M402 treated mice. (S) Lung histology and microvessel density of saline control mice and M402 treated mice. (T) Lung histology and microvessel density of saline control mice and M402 treated mice. (U) Lung histology and microvessel density of saline control mice and M402 treated mice. (V) Lung histology and microvessel density of saline control mice and M402 treated mice. (W) Lung histology and microvessel density of saline control mice and M402 treated mice. (X) Lung histology and microvessel density of saline control mice and M402 treated mice. (Y) Lung histology and microvessel density of saline control mice and M402 treated mice. (Z) Lung histology and microvessel density of saline control mice and M402 treated mice.

**Figure 2.** M402 inhibits tumor metastasis, circulating endothelial progenitor cells (MEPCs) and microvesSEL density in primary tumor. (A) Groups of female BALB/c mice (n=16) were inoculated orthotopically with 5x10⁴ 4T1-luc2-1A4 cells in the 4th mammary fat pad on day 0. M402 treatment was delivered by sc implanted osmotic pumps at 40mg/kg/day started on day 1. Primary tumors were removed on day 30 by surgery, weighed and fixed for histological analyses. (B) Hematoxylin and eosin (H&E) stained sections of primary tumor. (C) Microvessel density was assessed by immunohistochemistry. (D) Quantification of microvesSEL density in number of vessels 60x field. (E) Kaplan-Meier survival curves showing tumor control and survival benefit in 4T1 primary tumor model.

**Figure 3.** M402 inhibited tumor metastasis, circulating endothelial progenitor cells (MEPCs) and microvesSEL density in primary tumor. (A) Groups of female BALB/c mice (n=16) were inoculated orthotopically with 5x10⁴ 4T1-luc2-1A4 cells in the 4th mammary fat pad on day 0. M402 treatment was delivered by sc implanted osmotic pumps at 40mg/kg/day started on day 1. Primary tumors were removed on day 30 by surgery, weighed and fixed for histological analyses. (B) Hematoxylin and eosin (H&E) stained sections of primary tumor. (C) Microvessel density was assessed by immunohistochemistry. (D) Quantification of microvesSEL density in number of vessels 60x field. (E) Kaplan-Meier survival curves showing tumor control and survival benefit in 4T1 primary tumor model.

**Conclusions**

- M402 is a HSPG mimic with low anti-coagulant activity that retains binding to key factors crucial for tumor growth and metastasis.
- M402 inhibited tumor colonization to the lung in murine B16F10 melanoma experimental metastasis model.
- Chronic treatment with M402 in monotherapy or in combination with chemotherapeutic agents cisplatin or docetaxel inhibited spontaneous metastasis and prolonged survival in orthotopic murine 4T1 breast carcinoma model.
- Bioinformatics studies indicated that M402 accumulated and persisted in 4T1 primary tumors.
- M402 treatment normalizes MDSC levels, and inhibits microvesSEL density in 4T1 model suggesting possible interference with tumor-host interactions by M402.