Screening of a growth inhibitor library of sarcoma cell lines to identify potent anti-cancer drugs

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SUMMARY

There is a need for novel drugs for sarcoma treatment. In the present study, to identify inhibitors with potential therapeutic utility in sarcomas, we screened the growth inhibitory effects of 361 inhibitors, including experimental reagents and anti-cancer drugs approved for use in non-sarcoma malignancies and those under clinical trials. The inhibitors were initially tested using 10 osteosarcoma cell lines. The half-maximal inhibitory concentration (IC₅₀) of leptomycin B, actinomycin D, chetomin, and staurosporine was <100 nM in all the cell lines. As the promiscuous effects of staurosporine on kinases make it unsuitable for clinical applications, the other three inhibitors were tested in an additional 15 sarcoma cell lines derived from synovial sarcoma, fibrosarcoma, liposarcoma, rhabdomyosarcoma, malignant peripheral nerve sheath tumor, leiomyosarcoma, and Ewing’s sarcoma. The IC₅₀ of leptomycin B and actinomycin D was <100 nM in all cell lines and that of chetomin was <100 nM in all but three synovial sarcoma cell lines. Although the clinical development of leptomycin B, a chromosomal region maintenance (CRM1)/exportin (XPO1) inhibitor, was discontinued because of toxicity, a previous clinical trial revealed that other CRM1/XPO1 inhibitors, such as selinexor, have anti-tumor effects in sarcomas. Actinomycin D has proven clinical utility in the treatment of sarcomas. Chetomin disrupts the interaction of hypoxia-inducible factor-1 with the transcriptional coactivator p300 and its clinical utility has not been established in sarcomas. Chetomin exhibited growth inhibitory effects on sarcoma cells with different histological subtypes. Library screening is a powerful approach to detect the potential utility of anti-cancer drugs in sarcoma treatment.

Key words: actinomycin D, chetomin, drug library screening, leptomycin B, sarcoma, selinexor

INTRODUCTION

Sarcomas are tumors of mesenchymal tissues, such as connective tissue and bone. They are rare tumors, accounting for approximately 1% of all human malignancies. However, in children and young adults, sarcomas are common and account for 15% of all pediatric cancers. Sarcomas have diverse histology with >50 histological subtypes, which might exhibit distinct molecular and clinical features. Unique genetic aberrations, such as chromosomal translocations, are known to occur in one third of all sarcomas; however, the common molecular backgrounds have not been identified in the rest of them. Partly because of their low incidence, novel therapeutic approaches have not been developed for sarcoma treatment. For example, chemotherapy was introduced for the treatment of osteosarcoma in the 1970s. It has improved the cure rate in patients with localized osteosarcoma from 15%–20% achieved by surgery alone to approximately 70%.[7,8] However, the prognosis for osteosarcoma has not significantly improved since then. Moreover, the availability of molecular targeted drugs for sarcomas is still limited, and their optimized indications are widely discussed. Thus, there is a strong interest in the development of novel anti-cancer drugs for sarcoma treatment.

Among the various drug development approaches, re-purposing is a strategy used to expand the indications of approved drugs and to identify novel indications for failed ones[10]. The advantage of re-purposing over de novo drug discovery is that it can considerably reduce the traditional timeline and cost of drug development by bypassing the discovery and early clinical phases of drug development[11]. Re-purposing is a crucial approach for the development of novel therapeutics for rare cancers, such as sarcomas. The
development of novel drugs might require hundreds of millions of dollars and over a decade to perform clinical trials. Considering the small market size and low number of candidate patients for the clinical trials, it is not feasible to develop novel drugs de novo for rare cancers. As the therapeutic options are generally limited for rare cancers, re-purposing might offer a solution for the urgent need for sarcoma treatment options.

In the present study, we aimed to identify inhibitors that exhibit considerable inhibitory effects in different cell lines, because the number of individual sarcoma subtypes is limited and the development of anti-cancer drugs with versatile indications for sarcomas is of high priority. We screened 361 inhibitors in 10 osteosarcoma cell lines. The inhibitory effects of the three most potent inhibitors on cell proliferation were assessed in an additional 15 sarcoma cell lines with different histological origins. Our findings suggest that the screening of existing growth inhibitors is a promising approach to develop novel therapeutic strategies for the treatment of sarcoma. We also aimed to identify issues that can be improved in this strategy and discuss these issues from different perspectives.

MATERIALS AND METHODS

Sarcoma cell lines

In the present study, we included 25 sarcoma cell lines with different histological backgrounds. The cell lines were derived from osteosarcoma, synovial sarcoma, fibrosarcoma, liposarcoma, rhabdomyosarcoma, malignant peripheral nerve sheath tumor, leiomyosarcoma, and Ewing’s sarcoma. The cell lines used and their tissue culture conditions are summarized in Table 1.

Small compounds with possible anti-cancer activity

We included 361 inhibitors from the following libraries: SCADS Inhibitor Kit I ver3.1, II ver1.5, III ver1.5, and IV ver2.2 (Tokyo, Japan). The inhibitors used are listed in Supplementary Table 1.

High throughput screening

The cells were seeded in 96-well plates at the densities presented in Table 1. Twenty-four hours after seeding, the inhibitors were added to the wells. The final concentration of inhibitors in the assay ranged from 0 to 10,000 nM. The cells were incubated for 72 h and the viability of treated cells was measured using CCK-8 reagent (Dojindo Molecular Technologies Inc., Kumamoto, Japan). After incubation with the CCK8 reagent for 3 h, the absorbance of the solution was measured at a wavelength of 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). The titration response data were plotted and modeled using a four-parameter logistic curve (GraphPad Prism 7, GraphPad Software Inc., LaJolla, CA, USA). The half maximal inhibitory concentration (IC50) values were calculated for all 361 inhibitors. The initial screening of 361 inhibitors was performed in triplicate three times and the validation of selected inhibitors was performed in triplicate two times.

Table 1. Sarcoma cell lines used in this study.

| Histological origin | Name of cell lines | Tissue culture medium | Number of cells/well |
|---------------------|--------------------|-----------------------|----------------------|
| Osteosarcoma        | 143B               | DMEM/10%FBS           | 2000                 |
| Osteosarcoma        | MG-63              | DMEM/10%FBS           | 2000                 |
| Osteosarcoma        | MNNG/HOS           | DMEM/10%FBS           | 2000                 |
| Osteosarcoma        | HS-Os-1            | DMEM/10%FBS           | 1000                 |
| Osteosarcoma        | NOS-10             | DMEM/10%FBS           | 1000                 |
| Osteosarcoma        | U2OS               | DMEM/10%FBS           | 4000                 |
| Osteosarcoma        | HOS (TE85)         | DMEM/10%FBS           | 4000                 |
| Osteosarcoma        | SJSA-1             | DMEM/10%FBS           | 3000                 |
| Osteosarcoma        | NY                 | DMEM/10%FBS           | 4000                 |
| Osteosarcoma        | HuO 3N1            | RPMI1640/10%FBS       | 6000                 |
| Osteosarcoma        | HuO 9N2            | RPMI1640/10%FBS       | 5000                 |
| Ewing’s sarcoma     | A-673              | DMEM/10%FBS           | 2000–4000            |
| Fibrosarcoma        | HT 1080            | DMEM/10%FBS           | 1000                 |
| Fibrosarcoma        | Hs 633T            | DMEM/10%FBS           | 2000                 |
| Leiomysarcoma        | SK-LMS-1           | DMEM/10%FBS           | 3000                 |
| MPNST                | sNF96.2            | DMEM/10%FBS           | 3000                 |
| MPNST                | HS-Sch-2           | DMEM/10%FBS           | 3000                 |
| Liposarcoma          | 94T778             | RPMI1640/10%FBS       | 5000                 |
| Liposarcoma          | 93T449             | RPMI1640/10%FBS       | 5500                 |
| Rhabdomyosarcoma     | RD                 | DMEM/10%FBS           | 2000                 |
| Rhabdomyosarcoma     | SJCRH30            | RPMI1640/10%FBS       | 6000                 |
| Synovial sarcoma     | HS-SYII            | DMEM/10%FBS glucose (1 g/L) | 10000          |
| Synovial sarcoma     | SYO-1              | DMEM/10%FBS glucose (1 g/L) | 5000              |
| Synovial sarcoma     | YaFuSS             | DMEM/10%FBS glucose (1 g/L) | 10000         |
| Synovial sarcoma     | 1273/99            | DMEM/10%FBS glucose (1 g/L) | 5000              |
RESULTS AND DISCUSSION

First, the individual and average IC_{50} values of all 361 inhibitors were determined in 10 osteosarcoma cell lines (Supplementary Table 2). The heat-map showed that for the inhibitors whose average IC_{50} in the 10 osteosarcoma cell lines ranged between 100 and 10,000 nM, the IC_{50} values varied widely between the different osteosarcoma cell lines (Fig. 1A). The inhibitors with an average IC_{50} value of <100 nM were leptomycin B, actinomycin D, chetomin, and staurosporine, with 1.3, 52, 61, and 65 nM, respectively (Fig. 1B, Supplementary Table 2). The IC50 values of the examined inhibitors are summarized in Supplementary Tables 2–4.

Staurosporine strongly binds to the ATP-binding site of kinase and inhibits kinases with negligible selectivity^{12}. Because of its substantially promiscuous inhibitory effects on kinases, the clinical use of staurosporine is not feasible. Therefore, we did not examine the inhibitory activity of staurosporine further.

We examined the inhibitory activity of leptomycin B, actinomycin D, and chetomin in an additional 15 sarcoma cell lines with varied histological backgrounds (Table 1). The tumor inhibitory effects of leptomycin B were consistent among the 15 sarcoma cell lines examined (Fig. 2). The average IC_{50} of leptomycin B was 4.23 nM, ranging from 0.97 to 8.97 nM. The average IC_{50} in these additional 15 sarcoma cell lines was close to that in the 10 osteosarcoma cell lines. In four sarcoma cell lines, HT1080 (fibrosarcoma), 9T1449 (liposarcoma), HS-Sch2 (MPNST), and sNF96.2 (MPNST),

Fig. 1. IC50 values of the 361 inhibitors and the growth inhibition curve of four inhibitors in the 10 osteosarcoma cell lines.

A. The heat map shows the IC50 values if the inhibitors in individual osteosarcoma cell lines. Note that leptomycin B, actinomycin D, chetomin, and staurosporine exhibited consistently low IC50 values across the 10 osteosarcoma cell lines. B. Growth inhibition curve of four inhibitors, leptomycin B, actinomycin D, chetomin, and staurosporine, in 10 osteosarcoma cell lines. Each osteosarcoma cell line is indicated by a different color and the names of the cell lines are presented in the key provided. The error bars represent the standard deviation from experiments with two independent cultures.
among the cell lines. The lowest IC\textsubscript{50} values were observed in SJSA1, HT1080, HS-Sch2, sNF96.2, and SK-LMS-1, ranging from 52.96 to 91.64 nM. As selinexor had a significant inhibitory effect in a limited number of sarcoma cell lines, it might not have wide-spectrum anti-sarcoma effects, necessitating the selection of patients with sarcoma for selinexor treatment. For selinexor to be clinically feasible, a predictive biomarker has to be identified. The cell lines along with the various IC\textsubscript{50} values will be useful resources for the development of such predictive biomarkers.

The effects of actinomycin D on cell viability were consistent across all 15 sarcoma cell lines examined, with an average IC\textsubscript{50} value of 56 nM, ranging from 50 to 84 nM (Fig. 2). In the 10 osteosarcoma cell lines, the average IC\textsubscript{50} of actinomycin D was 52 nM, ranging from 50 to 66 nM. Thus, showing similar growth inhibitory activity in all 25 sarcoma cell lines examined.

Actinomycin D is isolated from soil bacteria belonging to the genus \textit{Streptomyces}, and has been used in the treatment of several types of malignancies, including sarcomas. Actinomycin D binds to the DNA duplex and prevents the elongation of the RNA chain by RNA polymerase, and interferes with DNA replication\textsuperscript{21}. Our \textit{in vitro} observations suggested the therapeutic utility of actinomycin D, which is consistent with the present use of actinomycin D in the clinical management of sarcoma.

The inhibitory effects of chetomin on cell viability were consistent among the 12 sarcoma cell lines; the IC\textsubscript{50} values were as low as those in osteosarcoma cells in all, but three synovial sarcoma cell lines—1273/99, HS-SYII, and YaFuSS. In the 12 sarcoma cell lines, the average IC\textsubscript{50} of chetomin was 53 nM, ranging from 50 to 56 nM. In contrast, the three synovial sarcoma cell lines, including 1273/99, HS-SYII, and YaFuSS, were resistant to the treatment with chetomin, and the average IC\textsubscript{50} of chetomin was 506 nM, ranging from 417 to 727 nM. Thus, showing similar growth inhibitory activity in all 25 sarcoma cell lines examined.

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Chetomin is a metabolite complex produced by fungi. It was identified as a disrupter of binding of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha to p300 through high-throughput screening of a natural and synthetic compound library of >600,000 substances. Consequently, chetomin can reduce hypoxia-inducible transcription, attenuate the HIF pathway within tumors, and inhibit tumor growth.

Chetomin was used in the treatment of patients with myeloma. The IC50 of chetomin in myeloma cell lines was between 2.3 and 6.8 nM, which were lower than those in the sarcoma cell lines used in the present study (Supplementary Table 3). Although the type of malignancy was different, if this difference in IC50 values reflects the in vivo response of tumors to chetomin, it might be challenging to use chetomin for the treatment of sarcomas.

Recently, Teicher et al. screened 100 FDA-approved oncology agents and 345 investigational agents in 63 sarcoma cell lines. However, leptomycin B, actinomycin D, chetomin, and staurosporine were not identified as effective inhibitors of sarcoma cell growth in their study. The difference between their results and those of the present study can be attributed to two reasons. First, their study did not include leptomycin B, chetomin, and staurosporine. They focused on approved molecular targeted drugs, but we examined both approved and investigational inhibitors. Second, the cell lines examined differed between the two studies. For example, although both studies examined 10 osteosarcoma cell lines, only three cell lines, U2OS, HOS, and SJSA-1, were common between these studies. They studied 21 Ewing’s sarcoma cell lines, but we examined only one (i.e., A-673). The nine rhabdomyosarcoma cell lines examined in their study included both rhabdomyosarcoma cell lines that were screened in the present study (RD and RJCRH30). We examined two different MPNST cell lines. They examined the cell lines of rhabdoid tumor, dedifferentiated liposarcoma, uterine sarcoma, chondrosarcoma, alveolar soft part sarcoma, giant cell sarcoma, and epithelioid sarcoma, whereas, we did not. Considering the diversity of sarcomas, a meta-analysis of the efficacy of drugs among multiple studies should yield more conclusive results.

Although our findings support the potential utility of inhibitor screening, certain limitations need to be discussed. First, for the initial screening, we used osteosarcoma cell lines because the cell lines from patients with osteosarcoma provided the largest selection in the public cell bank. In contrast, we examined only a few cell lines for the other sarcoma subtypes because multiple cell lines are not publicly available for most sarcomas. To facilitate in vitro screening, a larger selection of sarcoma cell lines is needed. However, as publicly available sarcoma cell lines are limited, additional patient-derived cancer models need to be established. Second, anti-tumor effects were evaluated only by measuring cell viability. Additional phenotype changes, such as spheroid formation, colony formation, migration, and invasion, should be assessed to evaluate the in vivo efficacy of tumor inhibitors. Third, in vitro inhibitory effects do not always indicate clinical utility and drugs with clinical utility might not always show in vitro inhibitory effects. For example, we did not identify pazopanib as a potent inhibitor of sarcoma cells in the present study. However, in a previous phase III trial in patients with metastatic sarcomas, pazopanib treatment resulted in statistically significant improvement in progression-free survival over a placebo control. It was subsequently approved for the treatment of advanced sarcomas, but its efficacy in the treatment of osteosarcoma was not established in this phase III study.

Osteosarcoma patients frequently develop metastatic lesions, and pazopanib treatment resulted in statistically significant improvement in progression-free survival over a placebo control. It was subsequently approved for the treatment of advanced sarcomas, but its efficacy in the treatment of osteosarcoma was not established in this phase III study. Therefore, it is worth investigating the anti-tumor effects of pazopanib in osteosarcoma cells using a patient-derived xenograft model. Despite the negative results for pazopanib, our screening did identify actinomycin D, which has been used clinically in the treatment of sarcomas, as a potent growth inhibitor of sarcoma cells in vitro. Therefore, the utility of screening inhibitor libraries for drug development is worth further consideration. Fourth, we selected inhibitors with consistently low IC50 values across multiple osteosarcoma cell lines. However, because of heterogeneous molecular backgrounds of osteosarcoma, this criterion may be too strict, and the inhibitors with the inconsistently low IC50 values might also be worth investigating in subsequent studies.

Our results indicate that screening of compound libraries might be an effective approach to identify potent inhibitors for sarcoma treatment. However, some limitations of such screening methods should be considered along with the methods to overcome them. Before initiating screening of an inhibitor library, previous reports and background information regarding the inhibitors should be considered. In the present study, although staurosporine exhibited an extremely low IC50, we did not investigate it further because of its non-specific effect on a variety of kinases, which could result in potential adverse effects. Similarly, because of its reported adverse effects, leptomycin B cannot be clinically used as a potent anti-tumor drug for sarcoma treatment.
The therapeutic utility of chetomin has been evaluated in clinical trials, and according to the reported IC₅₀p, the sarcoma cells tested in the present study might be more resistant to chetomin treatments than the malignancies included in the clinical trials. These observations suggest that the inhibitors whose limitations or adverse effects have been established in previous studies might have to be eliminated from the screening. Consistently high activity in histologically varied cells may be important while considering drug development for the treatment of sarcoma. Because of the low incidence might exhibit considerable clinical benefits in sarcoma patients.

CONCLUSIONS

The screening of the effects of growth inhibitors on the viability of sarcoma cells is an effective approach to identify potent drugs for the treatment of sarcoma. The cell lines used for screening, evaluation methods, and post in vitro screening strategy are key factors for the clinical application of viable candidates.

ABBREVIATIONS

CRM1, chromosomal region maintenance; HIF, hypoxia inducible factor; XPO1, exportin 1; IC₅₀p, inhibitory concentration.

REFERENCES

1) Fletcher CDM, Bridge JA, Hogendoorn P, Mertens F. WHO classification of tumours of soft tissue and bone. Fourth Edition ed. Geneva: WHO Press; 2013.
2) Osuna D, de Alava E. Molecular pathology of sarcomas. Rev Recent Clin Trials. 2009;4:12–26.
3) Thornton K. Chemotherapeutic management of soft tissue sarcoma. Surg Clin North Am. 2008;88:647–660, viii.
4) Taylor BS, Barretina J, Maki RG, Antonescu CR, Singer S, Ladanyi M. Advances in sarcoma genomics and new therapeutic targets. Nat Rev Cancer. 2011;11:541–557.
5) Nielsen TO, West RB. Translating gene expression into clinical care: sarcomas as a paradigm. J Clin Oncol. 2010;28:1796–1805.
6) Bovee JV, Hogendoorn PC. Molecular pathology of sarcomas: concepts and clinical implications. Virchows Arch. 2010;456:193–199.
7) Provisor AJ, Ettinger LJ, Nachman JB, et al. Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children’s Cancer Group. J Clin Oncol. 1997;15:76.
8) Kager L, Tamamyan G, Biełack S. Novel insights and therapeutic interventions for pediatric osteosarcoma. Future Oncol. 2016.
9) Sborov D, Chen JL. Targeted therapy in sarcomas other than GIST tumors. J Surg Oncol. 2015;111:632–640.
10) Bertolini F, Sukhatme VP, Bouche G. Drug repurposing in oncology—patient and health systems opportunities. Nat Rev Clin Oncol. 2015;12:732–742.
11) Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov. 2004;3:673–683.
12) Karaman MW, Herrgard S, Treiber DK, et al. A quantitative analysis of kinase inhibitor selectivity. Nat Biotechnol. 2008;26:127–132.
13) Hamamoto T, Gunji S, Tsuji H, Beppu T. Leptomycins A and B, new antifungal antibiotics. I. Taxonomy of the producing strain and their fermentation, purification and characterization. J Antibiot. 1983;36:639–645.
14) Hamamoto T, Seto H, Beppu T. Leptomycins A and B, new antifungal antibiotics. II. Structure elucidation. J Antibiot. 1983;36:646–650.
15) Hamamoto T, Uozumi T, Beppu T. Leptomycins A and B, new antifungal antibiotics. III. Mode of action of leptomycin B on Schizosaccharomyces pombe. J Antibiot. 1985;38:1573–1580.
16) Kudo N, Wolf B, Sekimoto T, et al. Leptomycin B inhibition of signal-mediated nuclear export by direct binding to CRM1. Exp Cell Res. 1998;242:540–547.
17) Mahipal A, Malafa M. Importins and exportins as therapeutic targets in cancer. PT. 2016;164:135–143.
18) Dickmanns E, Monecke T, Ficner R. Structural basis of targeting the exportin CRM1 in cancer. Cells. 2015;4:538–568.
19) Newlands ES, Rustin GJ, Brampton MH. Phase I trial of elactocin. Br J Cancer. 1996;74:648–649.
20) Abdul Razak AR, Mau-Soerensen M, Gabriyl NY, et al. First-in-class, first-in-human phase I study of selinexor, a selective inhibitor of nuclear export, in patients with advanced solid tumors. J Clin Oncol. 2016.
21) Sobell HM. Actinomycin and DNA transcription. Proc Natl Acad Sci U S A. 1985;82:5328–5331.
22) Brewer D, Duncan JM, Jerram WA, et al. Oxivine ill-thrift in Nova Scotia. 5. The production and toxicology of chetomycin, a metabolite of chaetomium spp. Can J Microbiol. 1972;18:1129–1137.
23) Sekita S, Yoshihira K, Natori S, et al. Mycotoxin production by Chaetomium spp. and related fungi. Can J Microbiol. 1981;27:766–772.
24) Kung AL, Zabludoff SD, France DS, et al. Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. Cancer Cell. 2004;6:33–43.
25) Viziteu E, Grandmougin C, Goldschmidt H, et al. Chetomin, targeting HIF-1alpha/p300 complex, exhibits antitumour activity in multiple myeloma. Br J Cancer. 2016;114:519–523.
26) Teicher BA, Polley E, Kunkel M, et al. Sarcoma cell line screen of oncology drugs and investigational agents identifies patterns associated with gene and microRNA expression. Mol Cancer Ther. 2015;14:2452–2462.
27) Pan X, Yoshida A, Kawai A, Kondo T. Current status of publicly available sarcoma cell lines for use in proteomic studies. Expert Rev Proteomics. 2016;13:227–240.
28) van der Graaf WT, Blay JY, Chawla SP, et al. Pazopanib for...
metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2012;379:1879–1886.

29) Safwat A, Boysen A, Lucke A, Rossen P. Pazopanib in metastatic osteosarcoma: significant clinical response in three consecutive patients. Acta Oncol. 2014;53:1451–1454.

30) Outani H, Tanaka T, Wakamatsu T, et al. Establishment of a novel clear cell sarcoma cell line (Hewga-CCS), and investigation of the antitumor effects of pazopanib on Hewga-CCS. BMC Cancer. 2014;14:455.

31) Hosaka S, Horiuchi K, Yoda M, et al. A novel multi-kinase inhibitor pazopanib suppresses growth of synovial sarcoma cells through inhibition of the PI3K-AKT pathway. J Orthop Res. 2012;30:1493–1498.

32) Kim ST, Jang HL, Lee SJ, et al. Pazopanib, a novel multi-targeted kinase inhibitor, shows potent in vitro antitumor activity in gastric cancer cell lines with FGFR2 amplification. Mol Cancer Ther. 2014;13:2527–2536.

33) Ranieri G, Mammi M, Donato Di Paola E, et al. Pazopanib a tyrosine kinase inhibitor with strong anti-angiogenetic activity: a new treatment for metastatic soft tissue sarcoma. Crit Rev Oncol Hematol. 2014;89:322–329.

34) Podar K, Tonon G, Sattler M, et al. The small-molecule VEGF receptor inhibitor pazopanib (GW786034B) targets both tumor and endothelial cells in multiple myeloma. Proc Natl Acad Sci U S A. 2006;103:19478–19483.