Anti-Endosialin Antibody Drug Conjugate: Potential in Sarcoma and Other Malignancies

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Abstract

Endosialin/TEM1/CD248 is a cell surface protein expressed at high levels by the malignant cells of about 50% of sarcomas and neuroblastomas. The antibody-drug conjugate (ADC) anti-endosialin-MC-VC-PABC-MMAE was selectively cytotoxic to endosialin-positive cells in vitro and achieved profound and durable antitumor efficacy in preclinical human tumor xenograft models of endosialin-positive disease. MC-VC-PABC-MMAE was conjugated with anti-endosialin with 3-4 MMAE molecules per ADC. The anti-endosialin-MC-VC-PABC-MMAE conjugate was tested for activity in four human cell lines with varied endosialin levels. The HT-1080 fibrosarcoma cells do not express endosialin, A-673 Ewing sarcoma cells and SK-N-AS neuroblastoma cells are moderate expressers of endosialin, and SJSA-1 osteosarcoma cells express very high levels of endosialin. To determine whether endosialin expression was maintained in vivo, A-673 Ewing sarcoma, SK-N-AS neuroblastoma and SJSA-1 osteosarcoma cells were grown as xenograft tumors in nude mice. The SK-N-AS neuroblastoma and the A-673 Ewing’s sarcoma lines were selected for in vivo efficacy testing of the anti-endosialin-MC-VC-PABC-MMAE conjugate. The treatment groups included a vehicle control, unconjugated anti-endosialin, an admix control consisting of anti-endosialin and a dose of free MMAE equivalent to the dose administered as the ADC, and the anti-endosialin-MC-VC-PABC-MMAE conjugate. The unconjugated anti-endosialin had no antitumor activity and resulted in similar tumor growth as the vehicle control. The admix control produced a modest tumor growth delay. Administration of the anti-endosialin-MC-VC-PABC-MMAE conjugate resulted in a marked prolonged tumor response of both xenografts. These proof-of-concept results break new ground and open a promising drug discovery approach to these rare and neglected tumors.
INTRODUCTION

Endosialin/CD248/TEM1, a transmembrane glycoprotein expressed on pericytes and fibroblasts during tissue development and present in the adult in several mesenchymal cell types, is associated with tumor neovascularization and inflammation and has emerged as a molecular marker and therapeutic target for sarcoma (1-12). First recognized as the antigen of an antibody raised in mice against human fetal fibroblasts (FB5), endosialin was found to be expressed by human solid tumor vasculature (13) and was detected in a subset of cells enriched for endothelium via selection with P1H12, an anti-CD146 antibody, from a colorectal tumor specimen (14). Endosialin expression in tumor vasculature occurs mainly in pericytes and stromal fibroblasts (15-17).

In mouse embryos, endosialin/TEM1-lacZ co-localizes with most vimentin-positive cells and a large portion of CD31- or desmin-positive cells. In the mouse, endosialin is expressed throughout embryonic and adult development in mesenchymal cells related to blood vessels (18). Endosialin−/− mice have no defect in pericyte recruitment, suggesting a role for endosialin in pericyte/endothelial cell cooperation during vascular patterning (3). Endosialin−/− mice have higher than normal bone mass due to increased osteoblast-mediated bone formation (1). Growth of syngeneic tumors was reduced in CD248CyD/CyD mice, which lack the endosialin cytoplasmic domain. CD248CyD/CyD fibroblasts have impaired PDGF-BB-induced migration, decreased matrix metalloproteinase (MMP)-9 secretion and higher transcript levels of the tumor suppressors transgelin (SM22a), Hes and Hey1 (6).
Endosialin is expressed by human, but not mouse, CD8+ naive T cells, specifically CD8+ CCR7+ CD11a low naive T cells, and on CD8+ T cells in the thymus. Endosialin knock-down in naive CD8+ T cells increased cell proliferation; thus, endosialin has opposing functions on hematopoietic (CD8+) and stromal cells (5).

The function and mechanisms of regulation of endosialin are still incompletely understood. Recently, several new anti-endosialin monoclonal antibodies became available, two recognize the C-type lectin-like domain-Sushi/SCR/CCP and four recognize the sialomucin domain. In addition a yeast-derived anti-endosialin biobody-78 was developed (4, 19).

The earliest indication that endosialin may be expressed by malignant cells was in the 1992 publication by Rettig et al. who reported immunoreactivity of FB5 in several neuroblastoma cell lines and mentioned FB5+ malignant cells in a subset of sarcomas (13). Further evidence for endosialin expression by tumor cells came in 2005 with immunostaining of malignant fibrous histiocytoma and liposarcoma showing tumor cell immunoreactivity (20).

Further, endosialin expression was assessed in 86 formalin-fixed, paraffin-embedded human clinical sarcoma specimens. Immunoreactive tissue components were malignant cells, stromal cells and vasculature. Seventy (81%) were positive for endosialin, with 44 (51%) reaching at least 50% coverage of immunoreactive tissue components. Staining intensity was scored on the scale 0, 1+, 2+, 3+. All nine sarcoma subtypes tested included specimens with at least 50% immunoreactive tissue components positive with a minimum of 2+ staining intensity, indicating the high prevalence of endosialin in sarcomas (12). A retrospective analysis of diagnostic reports showed that endosialin can be detected in high-grade disease and metastasis. In disseminated human sarcoma xenografts, endosialin protein expression was maintained at different anatomic sites (7-9). An anti-endosialin MORAb-004, which is a humanized FB5 antibody, has completed
phase 1 clinical trial and is currently in phase 2 clinical trials in endometrial cancer, ovarian cancer, melanoma, neuroectodermal tumors and sarcoma (21, 22).

The requirements for a cell surface molecule to be suitable as an antibody-drug conjugate (ADC) target are well-established (23-30). The optimal ADC has antigen recognition that is not different from the unconjugated antibody. ADCs usually include 2–4 highly potent anticancer agent small molecule drugs. The covalent linker that tethers the antibody to the small molecule drug must be stable in plasma and labile when internalized by the target cell.

The drugs often used in ADCs, maytansines and dolastatins, target microtubules. The dynamic flux of microtubules is a key target of anticancer therapies. Although principally recognized in mitotic function for their role in separating the duplicate set of chromosomes during cell division, microtubules are an essential cytoskeleton component and are critical in directional transport of proteins and organelles, maintenance of cell motility, cell shape and scaffolding, intracellular transport, secretion, neurotransmission and relay of signaling between cell surface receptors and the nucleus (31, 32). The biologic function of microtubules relies on the assembly and disassembly dynamics of tubulin polymerization (33, 34).

An anti-endosialin-MC-VC-PABC-monomethyl auristatin E ADC was prepared and assessed in cell culture and in two human tumor xenograft models, demonstrating high specificity and profound, durable antitumor efficacy.
MATERIALS AND METHODS

Anti-endosialin-MC-VC-PABC-monomethylauristatin E. The fully human anti-endosialin antibody was generated through a partnership with Kyowa Hakko Kirin Co., Ltd, Takasaki, Japan, as described previously (12). Synthetic procedures for the conjugation of the linker-functionalized monomethylauristatin E (MMAE) to anti-endosialin were performed as detailed in the following published procedure reports (35, 36). Briefly, anti-endosialin-MC-VC-PABC-MMAE was prepared by partial reduction of the antibody interchain disulfides with 3.3 molar equivalents of tris-(2-carboxyethyl)-phosphine hydrochloride in sodium borate buffer pH = 8.0 for 2 hr at 37°C. After cooling on ice, 4.8 molar equivalents of maleimide-linker-MMAE derivative in DMSO were added and the reaction was allowed to proceed for 30 min at 4°C. Excess small molecule was removed using QuadraPure™ DET (Sigma-Aldrich Co., St. Louis, MO) polystyrene scavenging beads. Yields were 85–95% based upon protein recovery.

The drug/antibody ratio (DAR) was determined by C8 reversed-phase LC/MS following published procedures (35, 36). The ADC was deglycosylated with PNGase F overnight at 37°C, dialyzed to remove salt, and reduced to smaller fragments (light and heavy chains) using 20 mM dithiothreitol (DTT) for 30 min at 37°C. Aliquots were injected in the LC/MS instrument and electrospray ionization mass spectra of light and heavy chains were recorded and deconvoluted, revealing conjugation profiles that included free, mono-, di-, and tri-conjugated species. The integrated peak areas were then used to determine the weighted average ratios for light and heavy chains. The DAR was determined by doubling and adding the light and heavy chains ratios. The DAR for the ADC was in the range of 3–5. Aggregation was determined by size exclusion HPLC.
and the conjugate was > 98% monomeric. Purity was determined by C18 RP-HPLC and there was < 0.5% unconjugated linker-functionalized MMAE derivative in each sample.

**Cells.** The human sarcoma cell lines HT-1080 (fibrosarcoma), A-673 (Ewing’s sarcoma), SJSA-1 (osteosarcoma) and the SK-N-AS neuroblastoma cell line were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and used over the next 6 months. The SK-N-AS cells underwent cytogenetic analysis and MYCN FISH analysis and the A673 cells underwent cytogenetic analysis prior to use in in vivo studies (12). All cells were propagated in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA).

**Flow Cytometry.** Analysis of endosialin expression in live cells by flow cytometry was conducted as described previously using a fully human monoclonal antibody raised against human endosialin, anti-endosialin-MC-VC-PABC-MMAE and a fully human isotype control antibody raised against dinitrophenol (DNP) (12). Sample acquisition was conducted on a FACS Calibur instrument (Becton Dickinson Labware, Franklin Lakes, NJ) and analysis was conducted with Flow Jo (Tree Star Inc., Ashland, OR).

**Growth Inhibition.** The cells were detached using trypsin-EDTA (Invitrogen, Carlsbad, CA) and washed once with RPMI 1640 medium supplemented with 5% FBS. The cells (2 x 10^3) were plated in a 96-well plate in RPMI supplemented with 5% FBS. After 24 hrs, the cells were exposed to MMAE-conjugated or unconjugated anti-endosialin for 96 hrs at 37°C. After 96 hrs, cell number was determined with the CellTiter-Glo reagent (Promega, Madison, WI) using a calibration curve. Luminescence was measured with a Bio-Tek (Highland Park, Winooski, VT) Synergy HT plate reader utilizing the associated Kineticalc software, Version #3.4. Luminescence data were converted to growth fraction by comparison to the luminescence for the
untreated control for each cell line, and IC$_{50}$ values were determined from the graphical data. Each cell line was tested in at least two independent experiments.

**Immunohistochemistry.** Immunohistochemistry was performed as described previously (12).

**In vivo xenografts.** All procedures were conducted according to a protocol approved by the Institutional Animal Care and Use Committee in accordance with the Federal Animal Welfare Act (9 CFR, 1992) in an AAALAC-accredited facility. For subcutaneous models, SK-N-AS neuroblastoma cells and A-673 Ewing’s sarcoma cells grown in culture were implanted subcutaneously (1x10$^6$) in the flanks of nude mice (Harlan Laboratories, Inc., Indianapolis, IN). The gender of the mice matched that of the cells. Animals were euthanized by CO$_2$ asphyxiation when weight loss reached 10% of body weight or when experiencing any sign of pain or distress.

The efficacy of anti-endosialin-MC-VC-PABC-MMAE was compared to that of unconjugated anti-endosialin, to a mixture of anti-endosialin and free MMAE providing the same dose of MMAE as in the conjugate (admix), and to the vehicle (PBS). Treatments were initiated when tumors reached 200 mm$^3$. Animals were randomized by tumor size and assigned to treatment or control groups ($n = 9$–10). Anti-endosialin-MC-VC-PABC-MMAE was tested on two schedules: alternate days (M, W, F) for 2 weeks (total 6 doses) and once weekly for 4 weeks. Tumor volumes were calculated using the formula ($w^2 x l$) x 0.52. Mouse weight and tumor dimensions were measured twice weekly. The data are presented as mean tumor volume +/- SD. The antitumor activity of the compounds was determined by calculating tumor growth delay (T-C) in days at a tumor volume of 1500 mm$^3$. Increase-in-lifespan was determined as a secondary endpoint with removal from study due to tumor size. Fold increase-in-lifespan was calculated from median survival in days for the treated versus control groups. Kaplan-Meier survival curves
were prepared using the GraphPad Prism software to determine the median survival times for each treatment group.
RESULTS

Monomethylauristatin E (MMAE) was reacted with maleimidocaproyl-valine-citrulline with a \( p \)-aminobenzylcarbamate spacer to produce MC-VC-PABC-MMAE, which was ready for conjugation with anti-endosialin. The inter-chain sulfhydryl groups of the antibody were reduced to allow reaction with MC-VC-PABC-MMAE to produce the ADC in a manner analogous to the preparation of brentuximab vedotin (3740). The reaction conditions were optimized to produce ADCs with a mean number of drug molecules in the range 3–5 (Figure 1). High performance liquid chromatography was used to determine the antibody:drug ratio and measure the percentage of protein that was unconjugated or highly conjugated. Liquid chromatography coupled with mass spectroscopy allowed determination of the number of drug molecules present on the antibody light chain and heavy chain (Figure 1).

In a group of 42 human sarcoma cell lines grown in monolayer culture, SJSA-1 osteosarcoma cells expressed the highest endosialin levels by flow cytometry (7-9). Live SJSA-1 cells were stained with naked anti-endosialin, anti-endosialin-MC-VC-PABC-MMAE (3.8 MMAE/Mab) or an isotype control-MC-VC-PABC-MMAE (4.9 MMAE/Mab) (Figure 2). The flow cytometry histograms show that there is no binding of the isotype control antibody to the SJSA-1 cells while the binding of the unconjugated endosialin and that of anti-endosialin-MC-VC-PABC-MMAE to the SJSA-1 cells is similar, indicating that conjugating the drug plus linker to the antibody protein did not alter the ability of the antibody to bind to the cell surface antigen.

The anti-endosialin-MC-VC-PABC-MMAE conjugate was tested for activity in a 96-hr growth inhibition assay in four human cell lines expressing varied endosialin levels (Figure 3). The HT-1080 fibrosarcoma cells do not express endosialin, A-673 Ewing’s sarcoma cells and
SK-N-AS neuroblastoma cells are moderate expressers of endosialin, and SJSA-1 osteosarcoma cells express very high levels of endosialin. HT-1080 cells are not sensitive to the anti-endosialin-MC-VC-PABC-MMAE conjugate even at the highest concentration tested. A-673 and SK-N-AS cells are similarly sensitive to the anti-endosialin-MC-VC-PABC-MMAE conjugate with IC50s of 0.5 and 0.3 μg/ml Mab, respectively (Figure 3B, C), and an IC50 of 1.5 μg/ml Mab was reached in SJSA-1 cells (Figure 3D).

To determine whether endosialin expression was maintained in vivo, A-673 Ewing’s sarcoma, SK-N-AS neuroblastoma and SJSA-1 osteosarcoma cells were grown as xenograft tumors in nude mice. Tumors were collected when they reached approximately 400 mm3 in volume, then formalin fixed and paraffin embedded. The tumor specimens were analyzed for endosialin expression by immunohistochemistry and scored for staining intensity by two pathologists (Figure 4). Although A-673 cells and SK-N-AS cells in culture expressed very similar endosialin levels, when grown in vivo A-673 tumors expressed endosialin with 1+ intensity while SK-N-AS tumors expressed endosialin with 2+ intensity. SJSA-1 tumors had a markedly heterogeneous endosialin expression pattern. Some regions of the tumor expressed endosialin with 3+ intensity and some regions expressed no endosialin.

The SK-N-AS neuroblastoma and the A-673 Ewing’s sarcoma lines were selected for in vivo efficacy testing of the anti-endosialin-MC-VC-PABC-MMAE conjugate. The anti-endosialin antibody does not cross-react with the mouse homolog of endosialin, therefore the only endosialin-expressing cell in the study were the human tumor cells. For the SK-N-AS experiment, treatment was initiated when the tumors reached 200 mm3 in volume. All treatments were administered by intravenous injection into the tail vein on alternate days for 2 weeks for a total of 6 injections. The treatment groups included a vehicle control, unconjugated anti-
endosialin (20 mg/kg), an admix control consisting of anti-endosialin and a dose of free MMAE equivalent to the dose administered as the ADC (20 mg/kg), and the anti-endosialin-MC-VC-PABC-MMAE conjugate (20 mg/kg) (**Figure 5A**). None of the treatments produced body weight loss in the mice (**Supplemental Figure 1**). The unconjugated anti-endosialin had no antitumor activity and resulted in similar tumor growth as the vehicle control. The admix control produced a tumor growth delay of 10 days. Administration of the anti-endosialin-MC-VC-PABC-MMAE conjugate resulted in a marked prolonged tumor response. Two of nine mice were lost to tumor growth, one on day 54 and one on day 81. The experiment was terminated on day 97. Survival for the mice is shown in **Figure 5B**. Administration of the unconjugated anti-endosialin did not alter the survival of the mice compared to the vehicle control. Treatment with the unconjugated anti-endosialin along with the MMAE free small molecule (admix control) produced an increased median survival of 11 days, while treatment with the anti-endosialin-MC-VC-PABC-MMAE conjugate resulted in an extended survival that did not reach the median by day 97 when the experiment was terminated.

The in vivo efficacy experiment with A-673 Ewing’s sarcoma examined the effects of a once weekly schedule for four doses and a dose-response range of ADC doses. The treatment groups included a vehicle control, unconjugated anti-endosialin (15 mg/kg), an admix control in which anti-endosialin was administered with MMAE free small molecule (15 mg/kg), and three dose levels of the anti-endosialin-MC-VC-PABC-MMAE conjugate (1, 5 and 15 mg/kg) (**Figure 6A**). As in the SK-N-AS efficacy study, the unconjugated anti-endosialin had no effect on the growth of A-673 Ewing’s sarcoma tumors. The admix control produced a modest tumor growth delay. A clear dose response was observed with the anti-endosialin-MC-VC-PABC-MMAE conjugate. The dose of 1 mg/kg had a small effect while the dose of 5 mg/kg had a moderate
effect on 50% of the mice and a marked antitumor effect on 50% of the mice (Figure 6B). The
dose of 15 mg/kg produced a marked and prolonged antitumor effect such that 80% of the mice
survived until the experiment was terminated on day 150.
DISCUSSION

Bone and soft tissue sarcoma are currently treated with conventional cytotoxic agents, including vincristine, dacarbazine, doxorubicin, cyclophosphamide, cisplatin, gemcitabine and docetaxel (41). High-risk neuroblastoma is currently treated with anthracyclines, alkylators, platinum compounds, topoisomerase II inhibitors, radiotherapy and myeloablative therapy (42). New therapies for these rare and neglected tumors are urgently needed. ADCs are now a clinically established therapeutic modality, most recently exemplified in the approval of trastuzumab emtansine (T-DM1; Kadcyla), in which the monoclonal anti-HER2 antibody trastuzumab is covalently linked to the maytansine derivative DM1, for HER2-positive metastatic breast cancer. The accessibility of endosialin, as a cell surface antigen, to antibodies and its high level of expression in sarcomas and neuroblastomas makes it a potentially suitable ADC target (43). A humanized monoclonal anti-endosialin, ontuxizumab (MORAb-004), is currently in Phase 1 and Phase 2 clinical trials. Ontuxizumab binds to endosialin on tumor vascular pericytes, tumor stromal cells and directly on a subset of malignant cells. Ontuxizumab is being investigated as a monoclonal antibody for the treatment of several types of cancer in adults and children and has received orphan drug designation for sarcoma. A Phase 1 study in pediatric patients with recurrent or refractory solid tumors or lymphoma was reported recently (44). A Phase 1 study of ontuxizumab in Japanese adults with solid tumors has been completed (45). A Phase 2 sarcoma study, which utilizes and adaptive design to identify subgroup populations during study is underway (46).
The function of endosialin remains largely unknown and it appears to be expressed at relatively high levels by about 50% of sarcomas. The implication is that endosialin is not essential for cell viability or proliferation. The requirements for an ADC target include being expressed on the cell surface and that an antibody to the extracellular domain of the target protein be internalized into the cell along with the target protein to enable delivery of the cytotoxic drug pay-load. Some ADC targets such as CD30 occur at high levels on the cell surface of specific diseases, anaplastic large cell lymphoma and Hodgkin lymphoma with sufficient frequency that diagnostic testing for the target is not essential. Brentuximab vedotin (SGN-35) reached FDA approval in 2011 for treatment of refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma without the requirement for testing tumor cells for the CD30 (47). Given the variability of endosialin expression across tumors and within tumors (7-9, 12), detection of endosialin protein using the immunohistochemical assay previously described (12) and used herein should be a key component of any endosialin-directed therapy as a companion diagnostic to select potential responders.

This present proof-of-concept study demonstrates that endosialin-positive tumors can be specifically and effectively targeted by a monoclonal anti-endosialin antibody conjugated to the potent cytotoxic small molecule MMAE. The response is complete and durable, warranting extensive preclinical evaluation of endosialin-directed ADCs in endosialin-positive disease. Anti-endosialin-MMAE brings the promise of personalized medicine to sarcoma and neuroblastoma patients.
REFERENCES


FIGURE LEGENDS


FIGURE 2. Endosialin protein expression by live SJSA-1 osteosarcoma cells in culture by flow cytometry as determined by unconjugated anti-endosialin (red), anti-endosialin-MC-VC-PABC-MMAE (green) and an antibody isotype control-MC-VC-PABC-MMAE (gray).

FIGURE 3. Determination of endosialin protein expression by flow cytometry and cellular growth inhibition by anti-endosialin-MC-VC-PABC-MMAE and by an antibody isotype control-MC-VC-PABC-MMAE upon 96 hrs of exposure to the agent for: A. human HT-1080 fibrosarcoma cells, B. human A-673 Ewing sarcoma cells, C. human SK-N-AS neuroblastoma cells and D. human SJSA-1 osteosarcoma cells. Experiments were repeated three times independently; bars are SEM.

FIGURE 4. Expression of endosialin by immunohistochemistry in three human tumor xenograft specimens. Scoring of immunohistochemical endosialin staining intensity is shown.

FIGURE 5. A. Growth of subcutaneously implanted human SK-N-AS neuroblastoma xenografts in female nude mice treated with vehicle (Control, ■), unconjugated anti-endosialin (20 mg/kg, iv, QODx3, 2 cycles) (□), anti-endosialin + MMAE (20 mg/kg, iv, QODx3, 2 cycles) (ADMIX,◊), or anti-endosialin-MC-VC-PABC-MMAE (20 mg/kg, iv, QODx3, 2 cycles) (▲). Treatments were administered by intravenous injection 3-times per week for 2 weeks. The data are the means +/- SD for groups of 9 mice. B. Survival of female nude mice bearing
subcutaneously implanted human SK-N-AS neuroblastoma xenografts after treatment with vehicle (Control, ■), unconjugated anti-endosialin (20 mg/kg, iv, QODx3, 2 cycles) (□), anti-endosialin + MMAE (20 mg/kg, iv, QODx3, 2 cycles) (ADMIX, ◊), or anti-endosialin-MC-VC-PABC-MMAE (20 mg/kg, iv, QODx3, 2 cycles) (▲). Treatments were administered by intravenous injection 3-times per week for 2 weeks. The data are the means +/- SD for groups of 9 mice.

**FIGURE 6 A.** Growth of subcutaneously implanted human A-673 Ewing’s sarcoma xenografts in female nude mice treated with vehicle (Control, ■), unconjugated (naked) anti-endosialin (15 mg/kg, iv, once weekly x 4) (□), anti-endosialin + MMAE (15 mg/kg, iv, once weekly x 4) (ADMIX, ◊), anti-endosialin-MC-VC-PABC-MMAE (1 mg/kg, iv, once weekly x 4) (▲), anti-endosialin-MC-VC-PABC-MMAE (5 mg/kg, iv, once weekly x 4) (▲), or anti-endosialin-MC-VC-PABC-MMAE (15 mg/kg, iv, once weekly x 4) (▲). The data are the means +/- SD for groups of 10 mice. **B.** Survival of female nude mice bearing subcutaneously implanted human A-673 Ewing’s sarcoma xenografts after treatment with vehicle (Control, ■), unconjugated (naked) anti-endosialin (15 mg/kg, iv, once weekly x 4) (□), anti-endosialin + MMAE (15 mg/kg, iv, once weekly x 4) (ADMIX, ◊), or anti-endosialin-MC-VC-PABC-MMAE (1 mg/kg, iv, once weekly x 4) (▲), anti-endosialin-MC-VC-PABC-MMAE (5 mg/kg, iv, once weekly x 4) (▲), or anti-endosialin-MC-VC-PABC-MMAE (15 mg/kg, iv, once weekly x 4) (▲). The data are the means +/- SD for groups of 10 mice.
Figure 1.

Anti-endosialin-MC-VC-PABC-monomethylauristatin E
Flow cytometry of live human SJSA-1 osteosarcoma cells

- Unconjugated anti-endosialin
- Anti-endosialin-MC-VC-PABC-MMAE (3.8 MMAE/Ab)
- Isotype control-MC-VC-PABC-MMAE (4.9 MMAE/Ab)

Figure 2.
Figure 3.

A. HT-1080 fibrosarcoma

IC$_{50}$ >3 µg/ml

B. A-673 Ewing sarcoma

IC$_{50}$ 0.5 µg/ml

C. SK-N-AS neuroblastoma

IC$_{50}$ 0.3 µg/ml

D. SJSA-1 osteosarcoma

IC$_{50}$ 1.5 µg/ml

- Anti-endosialin-VC-MC-PABC-MMAE (3.8)
- Isotype control-VC-MC-PABC-MMAE (4.9)
A-673 human Ewing sarcoma 1+ endosialin IHC staining

SK-N-AS human neuroblastoma 2+ endosialin IHC staining

SJSA-1 human osteosarcoma 3+ endosialin IHC staining

Figure 4.
Figure 5.
Molecular Cancer Therapeutics

Anti-Endosialin Antibody Drug Conjugate: Potential in Sarcoma and Other Malignancies


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