SGN–LIV1A: A Novel Antibody–Drug Conjugate Targeting LIV-1 for the Treatment of Metastatic Breast Cancer

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Abstract

In this article, we describe a novel antibody–drug conjugate (ADC; SGN–LIV1A), targeting the zinc transporter LIV-1 (SLC39A6) for the treatment of metastatic breast cancer. LIV-1 was previously known to be expressed by estrogen receptor–positive breast cancers. In this study, we show that LIV-1 expression is maintained after hormonal therapy in primary and metastatic sites and is also upregulated in triple-negative breast cancers. In addition to breast cancer, other indications showing LIV-1 expression include melanoma, prostate, ovarian, and uterine cancer. SGN–LIV1A consists of a humanized antibody conjugated through a proteolytically cleavable linker to monomethyl auristatin E, a potent microtubule-disrupting agent. When bound to surface-expressed LIV-1 on immortalized cell lines, this ADC is internalized and traffics to the lysosome. SGN–LIV1A displays specific in vitro cytotoxic activity against LIV-1–expressing cancer cells. In vitro results are recapitulated in vivo where antitumor activity is demonstrated in tumor models of breast and cervical cancer lineages. These results support the clinical evaluation of SGN–LIV1A as a novel therapeutic agent for patients with LIV-1–expressing cancer.

Introduction

In the United States, nearly 300,000 women are diagnosed with breast cancer each year and it is the second leading cause of cancer-related mortality in women. Surgery, radiation, hormone therapy, and chemotherapy are effective treatments for many, but over 40,000 patients succumb to the disease annually. Breast cancers are classified on the basis of three protein expression markers: estrogen receptor (ER), progesterone receptor (PgR), and the overexpression of the growth factor receptor HER2/neu. Hormonal therapies, including tamoxifen and aromatase inhibitors, can be effective in treating tumors that express the hormone receptors ER and PgR. HER2-directed therapies are useful for tumors that express HER2/neu; these tumors are the only class of breast cancer that is currently eligible for immunotherapy. For these patients, unconjugated antibodies (Herceptin, Perjeta) are generally used in combination with chemotherapy. The treatment options for triple-negative breast tumors, those that do not express ER, PgR, or HER2/neu, are restricted to chemotherapy, radiation, and surgery. In addition, there are limited effective treatment options available to patients with advanced-stage disease with relatively poor survival rates of stage III patients (52%) and significantly worse for stage IV patients (15%). There is clearly a significant need for effective treatments for late-stage breast cancer.

Antibody–drug conjugates (ADC) are a relatively new treatment modality that takes advantage of the exquisite specificity of monoclonal antibodies by using them to deliver a highly potent cytotoxic agent. The ADC described here is an anti–LIV-1 antibody linked via a cleavable dipeptide linker to monomethyl auristatin E (MMAE), the cytotoxic agent. Although there are at least 21 ADCs in clinical development (nine auristatin-based; ref. 1), only one is approved for use in breast cancer (Kadcyla for HER2⁺ patient populations).

LIV-1 is a member of the solute carrier family 39; a multi-span transmembrane protein with putative zinc transporter and metalloprotease activity (2, 3). It was first identified as an estrogen-induced gene in the breast cancer cell line ZR-75-1 (4). LIV-1 expression has been linked to epidermal-to-mesenchymal transition (EMT) in both normal vertebrate embryo development (5) and preclinal models (6–8) leading to malignant progression and metastasis. There is evidence of LIV-1 interacting with the transcription factors STAT3 and Snail to downregulate expression of E-cadherin to promote EMT (9, 10). Expression is also associated with lymph node involvement in breast cancer (11). In addition to
breast cancer, it has been detected in other neoplastic tissue types, including pancreatic, prostate, breast, melanoma, cervical, and uterine (8, 12, 13). We evaluated LIV-1 expression in a number of indications using immunohistochemical analysis on tissue biopsies. In addition, we performed quantitative flow cytometry to determine expression of LIV-1 on a panel of cell lines derived from various cancer types. Using a humanized antibody specific for LIV-1 (hLIV22) conjugated to MMAE (14), we demonstrated ADC internalization, in vitro cytotoxicity, and antitumor activity in in vivo breast and cervical cancer models.

Materials and Methods

**Cell lines and culture**

MCF-7 cells were obtained from three different sources: MCF-7 (ATCC HB-22) from the American Type Culture Collection (ATCC), MCF-7 DSMZ from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), and MCF-7 NCI from NCI-Frederick Cancer Cell Line Repository (NCI-Frederick, MD). Other cell lines were obtained from the ATCC with the exception of PC-3 and HupT3 cells which were obtained from DSMZ. All cell lines were received before 2010 and cultured according to the supplier’s recommendations; cell lines used for in vitro cytotoxicity (MCF-7 ATCC) and in vivo efficacy studies (MCF-7 NCI and HELA) were authenticated using the Cell Check service provided by IDEXX BioResearch [identity confirmation by short tandem repeat (STR)-based DNA profiling and multiplex PCR]. A Chinese Hamster Ovary (CHO) cell line expressing human LIV-1 was generated by transfecting a CHO DG44 cell line with a plasmid coding for the intact LIV-1 gene. DHFR selection was used to identify positive clones.

**Antibody humanization**

The murine antibody mLIV22 specifically binds an epitope in the extracellular N-terminus (residues 1–329) of LIV-1. Complementarity-determining region (CDR) grafting was used to generate the humanized anti–LIV-1 antibody hLIV22. First, the mLIV22 VL CDRs [as defined by Kabat (15)] were grafted on to the framework regions of human germline exons IGKV2-30 and JK4 obtained from NCBI and fused to human kappa constant domain. Likewise, the CDRs of mLIV22 VH were obtained from NCBI and fused to human IgG1 constant domain. Framework mutations, F36Y and S76N, and inhibitory concentrations (IC50) by fitting the data to a sigmoidal dose–response curve with variable slope.

**Competition binding**

LIV-1–expressing 293F cells (1 × 10⁵) in PBS were aliquotted in each well of 96-well v-bottom plates on ice. The cells were incubated for 1 hour with 5 nmol/L Alexa Fluor 647–labeled hLIV22 and increasing concentrations (from 0.03 to 500 nmol/L) of unlabeled mLIV22, hLIV22, or SGN–LIV1A. Cells were pelleted and washed two times with PBS. The cells were then pelleted and resuspended in 125 μL of PBS/FBS. Fluorescence was analyzed by flow cytometry and specific antigen density was calculated on the basis of a standard curve of log geometric mean fluorescence intensity versus log antigen-binding capacity.

**Conjugation of antibodies**

The hLIV22–vcMMAE ADC was prepared by partial reduction of antibody interchain disulfide bonds with tris (2-carboxyethyl)-phosphine (TCEP) followed by conjugation to maleimidocaproylvaline-citrulline-p-aminobenzoylloxycarbonyl-MMAE (vcMMAE) as described previously.
(16) with the following modifications. Partial reduction of the interchain disulfide bonds, to an average of two reduced disulfide bonds or four reactive thiols per antibody, was achieved by incubating antibody solutions with 2.5 mol/L equivalents of TCEP at 37°C in the presence of 1 mmol/L diethylthiotetraminepentaacetic acid for 1.5 hours. Final drug loading was determined by reverse-phase high-performance liquid chromatography under reducing conditions and by hydrophobic interaction chromatography (16).

**Fluorescence microscopy**

**ADC internalization images.** MCF7 cells were plated on fibronectin-coated 8-well chamber slides (BD Biosciences) and allowed to grow for 2 days at 37°C in appropriate media. Cells were then incubated with 1 μg/mL SGN–LIV1A with or without 10 μmol/L chloroquine for 0, 4, or 24 hours at 37°C. Cells were fixed and permeabilized using Cytofix/Cytoperm solution (BD Biosciences). ADC was detected with goat anti-human IgG Alexa Fluor 488 (Invitrogen). LAMP1, a lysosomal marker, was detected with biotylated mouse anti-human CD107a (BD Biosciences), followed by Alexa Fluor 594– conjugated streptavidin (Invitrogen). Cells were mounted in ProLong Gold Antifade with DAPI (Invitrogen). Images were acquired with a 63× oil objective on an Axiovert 200 M inverted fluorescence microscope. Microtubule Network Images. MCF7 cells were plated on D-lys–coated 8-well chamber slides (BD Biosciences) and allowed to grow for 2 days at 37°C in appropriate media. Cells were then incubated with 1 μg/mL or 10 ng/mL SGN-LIV1A for 0 or 24 hours at 37°C. Cells were fixed as described above. Tubulin was detected with mouse anti-α-tubulin Alexa Fluor 488 (Invitrogen). Cells were mounted in SlowFade with DAPI (Invitrogen). Images were acquired with a 63× oil objective on an Axiovert 200 M inverted fluorescence microscope.

**Cytotoxicity assay**

Tumor cells were incubated with SGN–LIV1A or hLIV22 for 96 hours at 37°C. Cell viability was measured by CellTiter-Glo (Promega Corporation) according to the manufacturer’s instructions. Cells were incubated for 25 minutes at room temperature with the CellTiter-Glo reagents and luminescence was measured on a Fusion HT fluorescent plate reader (PerkinElmer). Results are reported as EC50, the concentration of compound needed to yield a 50% reduction in viability compared with vehicle-treated cells (control = 100%).

**PK methods**

Six BALB/c mice were dosed i.v. with 3 mg/kg of SGN–LIV1A. Blood samples were drawn from the saphenous vein from alternating subgroups of 3 mice at 5 minutes, 6 and 24 hours, 2, 4, 7, 10, and 14 days after dose and processed to plasma. Samples were analyzed in a plate-based assay as follows: wells were coated overnight with a solution (0.5 μg/mL in 0.05 mol/L carbonic acid buffer, pH 9.6 of anti-human IgG kappa antibody (Antibody solutions; #A575-P). After washing with PBS-T, wells were blocked with 1% BSA for 1 hour at room temperature. After washing blocked plates with PBS-T, wells were incubated with samples at room temperature. After 1 hour, plates were washed with PBS-T and incubated for an additional hour with HRP-F(ab’)2 goat anti-human IgG Fc-gamma–specific (The Jackson Laboratory; #109-036-098). Following a final wash step, TMB substrate was added and incubated for 10 minutes before quenching with 1 N HCL, A450 was read and used to calculate serum antibody concentration.

**In vivo activity studies**

Tumor volume was calculated using the formula, \((A \times B^2)/2\), where \(A\) and \(B\) are the largest and second largest perpendicular tumor dimensions, respectively. Mean tumor volume and weight of mice were monitored and mice terminated when the tumor volume reached 1,000 mm³.

For MCF7-NCl and BR0555 studies, NOD.Cg-Pkrdcscid Il2rgtm1Wjl/SzJ mice (NSG; The Jackson Laboratory) were implanted subcutaneously with 17β-estradiol 90-day time release tablets ( Innovative Research of America). Studies were allowed 2 to 6 days recovery time from tablet implant before receiving cell or tissue implant. 17β-Estradiol tablets were implanted every 90 days thereafter.

MCF7-NCl mice were implanted at 5 × 10⁶/200 μL Matrigel HC25% (BD Biosciences). Once tumors reached a mean tumor volume of 100 mm³, mice were treated by intraperitoneal injection every 4 days for a total of four doses with either SGN–LIV1A (1 or 3 mg/kg) or human IgG/MMAE (hlg-vcMMAE) as a nonbinding control. An additional group of tumor-bearing mice (\(n = 5\)) was left untreated as a control.

BR0555 is a subcutaneous model derived from a patient with primary breast cancer (The Jackson Laboratory). NSG mice bearing tumors between 500 and 750 mm³ were sacrificed and the tumors were removed using an aseptic technique. Tumors were sectioned into small fragments approximately 3 to 5 mm³ and loaded into 14-gauge trocars. Mice were implanted subcutaneously in the right lateral flank and returned to a clean home box. Implanted mice were monitored once a week and started on study when their tumor reached approximately 250 mm³. In this model, mice enrolled to the study in a patient accrual fashion; days 58 through 78, and dosing started. At each evaluation, the available cohort of mice was distributed to study groups in an equal fashion with an \(n = 10\) per group.

Mice were treated by intraperitoneal injection every 4 days for a total of four doses with either SGN–LIV1A (1 or 3 mg/kg) or hlg-vcMMAE as nonbinding control. An additional group of tumor-bearing mice was left untreated as a control.

In the HeLa (ATCC CLL-2) in vivo study, female Athymic Nude-Foxn1nu (Harlan) were implanted with tissue...
fragments of tumors maintained in serial passage. Tumors were sectioned into small fragments approximately 3 to 5 mm³ and loaded into 14-gauge trocars. Mice were implanted subcutaneously in the right lateral flank and returned to a clean home box. On day 10 after implant, the mice were evaluated and randomly placed into study groups ($n = 8$) with a mean tumor size of approximately 100 mm³ and dosing was started. Mice were treated by intraperitoneal injection every 4 days for a total of four doses with either SGN–LIV1A (1 or 3 mg/kg) or hlgvcMMAE as nonbinding control. An additional group of tumor-bearing mice was left untreated as a control.

Results

LIV-1 is highly expressed in solid tumors of different origins

LIV-1 expression was evaluated in human normal tissue and tumor microarrays and in sets of larger tissue sections. LIV-1 is frequently expressed in breast, prostate, and melanoma, even in patients previously treated with hormonal therapies (Table 1 and Fig. 1). In contrast, ovarian, uterine, and lung cancers have measurable, but less frequent, LIV-1 expression. An extensive panel of normal human tissues were also examined and showed limited LIV-1 expression (Table 2 and Fig. 1). The normal tissues that stain positive for LIV-1 expression in IHC have variable expression. In breast tissue, 0% to 50% of the cells stain with an intensity of 1 to 2 on the same scale used for the neoplastic tissue. In prostate tissue, 50% to 100% of the cells stain with an intensity of 2 to 4. In testicular tissue, about 50% of the cells stain with an intensity up to 1. The broad expression of LIV-1 in breast, prostate, and melanoma tumors, coupled with the restricted normal tissue expression (breast, prostate, and testis), demonstrate that LIV-1 is a target well suited for an ADC therapeutic. The expression is most prominent in breast cancer, the focus of this study.

LIV-1 is highly expressed in post–hormone-treated primary and metastatic breast tumors

To determine the expression of LIV-1 in breast carcinomas, a murine anti–LIV-1 mAb was used for the detection in FFPE samples by IHC. We found that large sections of tumor samples provided better measure of LIV-1 expression in the tumor samples analyzed compared with commercially available tumor microarrays.

Because there is known positive correlation between LIV-1 expression and ER, we also evaluated breast tumor samples in which the patients had previously received hormone therapy for their ER⁺ cancers. We analyzed expression in breast cancer biopsies in patients having received hormonal therapy (tamoxifen or aromatase inhibitors). A total of 82 post–hormone therapy biopsies were studied and 88% of these expressed LIV-1 (at any level of intensity or %positive). As illustrated in Fig. 1A, 92% of primary site post–hormone-treated tumor biopsies expressed LIV-1 with intensity of the staining ranging from weak (1–2⁺) to strong (3–4⁺). About 50% of the cases had ≥50% to 100% of tumor cells expressing LIV-1. Good concordance was observed with the reactivity of another anti–LIV-1 mAb in formalin-fixed tissues (data not shown). The immunostaining pattern was characterized as both membranous and cytoplasmic. The staining was LIV-1 specific, based on concordant reactivity between the two anti–LIV-1 mAbs used and the absence of staining with an isotype-matched negative control antibody. We also studied post–hormonal therapy metastatic breast tumor biopsies. Eighteen out of 23 cases (78%) expressed LIV-1, with approximately 75% of cases staining ≥50% to 100% of the tumor cells (Fig. 1B).

Expression of LIV-1 in triple-negative (ER⁻, PgR⁻, Her-2-unamplified) primary breast tumors was also evaluated. We observed 65% LIV-1⁻ in a set of 20 cases, with 40% (8 of 20) showing ≥50% to 100% of tumor cells positive, albeit with lower intensity of LIV-1 expression (Fig. 1C) compared with ER⁺ cases. Representative images of staining intensity are shown in Fig. 1D.

Quantitative flow cytometric analysis of LIV-1 expression on human cancer cell lines

Cell surface expression of LIV-1 in human tumor cell lines was evaluated using quantitative flow cytometry. The panel of cell lines included breast, cervical, head and neck, hepatocellular, kidney, ovarian,

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Table 1. LIV-1 expression in multiple cancer types

<table>
<thead>
<tr>
<th>Neoplastic tissue</th>
<th>Number of positive samples</th>
<th>Number of samples examined</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>88</td>
<td>95</td>
<td>93</td>
</tr>
<tr>
<td>Melanoma</td>
<td>42</td>
<td>51</td>
<td>82</td>
</tr>
<tr>
<td>Prostate</td>
<td>36</td>
<td>50</td>
<td>72</td>
</tr>
<tr>
<td>Ovary</td>
<td>10</td>
<td>21</td>
<td>48</td>
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<tr>
<td>Uterus</td>
<td>6</td>
<td>20</td>
<td>30⁻</td>
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<tr>
<td>Lung</td>
<td>3</td>
<td>30</td>
<td>10⁻</td>
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pancreatic, prostate, and melanomas. The highest level of LIV-1 expression was observed in the MCF-7 breast cancer cell line from the ATCC (175,000 sites/cell) while ZR-75-1 (ATCC CRL-1500) had about 80,000 sites per cell (Table 3). Other cell lines (ovarian, pancreatic, head and neck, and melanomas) showed moderate to low level expression of LIV-1 by qFACS.

Figure 1. A–D, LIV-1 is expressed in primary and metastatic post–hormone-treated breast cancer cases. Tissue sections were preserved in formalin, expression was detected using Fast Red as a substrate for alkaline phosphatase. A, 54 of 59 (92%) of posttreatment, primary site breast cancer biopsies express LIV-1. B, 18 of 23 (78%) of posttreatment, metastatic breast cancer biopsies express LIV-1. C, 13 of 20 (65%) of primary site triple-negative breast cancer biopsies express LIV-1. D, examples of tissues with LIV-1–specific staining intensity of 1–4. E, examples of normal tissue staining, clockwise from top left: lung, colon, prostate, and breast.
Humanized LIV-22 affinity

Parental murine LIV22 (mLIV22) was humanized to hLIV22 using the CDR grafting method. To ensure that neither humanization nor conjugation minimal effects on binding to LIV-1, the binding affinity of mLIV22 was compared with hLIV22 and SGN–LIV1A in a competition binding assay. The IC₅₀ determined for mLIV22, hLIV22, and SGN–LIV1A were 3.5, 4.6, and 5.6 nmol/L, respectively. These data suggest that humanization did not significantly impact the binding affinity of hLIV22 to LIV-1.

Anti–LIV-1 ADCs are potent inhibitors of cell proliferation of MCF-7 breast carcinoma cells

The humanized LIV-1 antibody (hLIV-22) was conjugated to the antitubulin drug, vcMMAE, on reduced cysteines usually involved in interchain disulfide bonds with a mean stoichiometry of four drugs/antibody (16). The resulting ADC, SGN–LIV1A, has a potent cytotoxic activity against MCF-7 cell line (ATCC) with an EC₅₀ value of 6.3 ng/mL in a CellTiter-Glo (Promega) cytotoxicity assay (Fig. 2B). In contrast, neither unconjugated parental antibody (Fig. 2) nor an ADC control hIgG-vcMMAE showed substantial cytotoxic activity (EC₅₀ > 10,000 ng/mL; data not shown). A control nonlysosomal ADC internalized, but did not converge on the lysosome with 10^(-5) mol/L chloroquine treatment, suggesting the SGN–LIV1A lysosomal localization is not simply a collapse of intracellular vesicles (data not shown). In summary, SGN–LIV1A internalizes throughout a 24-hour treatment period and traffics to the lysosome in which proteolytic release of the cytotoxic payload occurs.

Disruption of microtubules

Treatment of LIV-1–expressing MCF-7 cells with SGN-LIV1A at doses as low as 10 ng/mL for 24 hours induced disruption of the microtubule network (Fig. 3B). At 24 hours, 49% (N = 150; Fig. 2) of cells treated with 1 µg/mL SGN-LIV1a or 16% (N = 100) of cells treated with 10 ng/mL SGN-LIV1a displayed condensed chromosomes and abnormal spindles. At 24 hours, 0% (N = 300) of cells treated with nonbinding control displayed this phenotype. These data are consistent with the proposed mechanism of action leading to mitotic arrest.

PK and in vivo ADC activity study using MCF-7, BR0555, and HeLa tumors

To measure the pharmacokinetic (PK) properties of SGN–LIV1A, a single 3 mg/kg dose was administered i.v. to BALB/C mice; blood samples were taken out to 14 days. The PK properties of the total antibody appear consistent with a two compartment model (Fig. 4A). The terminal half-life, calculated using nonlinear regression, is 6.8 days.

### Table 2. LIV-1 expression in normal tissue

<table>
<thead>
<tr>
<th>Normal tissues negative for anti–LIV-1 staining</th>
<th>Kidney</th>
<th>Skin</th>
<th>Normal tissues positive for anti–LIV-1 staining</th>
<th>Testis</th>
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<td>Adrenal gland</td>
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The antitumor activity of SGN–LIV1A was evaluated in xenograft models of breast, and cervical lineage. Two different breast cancer models were explored, one using MCF-7 cells and the other using a patient-derived tissue model. In the MCF-7 breast cancer cell xenograft model, tumor regressions were achieved with four 3 mg/kg doses of SGN–LIV1A given every 4 days (Fig. 4B), well below the mean-tolerated dose of 10 mg/kg in rodents. Although tumor growth delay was seen when dosing with the nontargeted ADC, when comparing it with the 3 mg/kg SGN–LIV1A dose group, \( P \) values compute to <0.05, meeting the threshold of statistical significance difference and indicating both a dose response and immunologic specificity of the targeted therapeutic. ADC activity in the absence of target has been documented previously on a number of antibody backbones and chemotype combinations (17–19). Noncancer antigen-dependent activity is a continuing topic of study and has been attributed to a combination of factors, including the enhanced permeability and retention effect of a tumor, ADC and drug linker stability, cellular susceptibility to the delivered cytotoxin, and relative cell permeability of the released drug.

The second breast cancer model (BR0555) was derived from the ER\(^+\), PR\(^+\), and HER2-infiltrating breast ductal carcinoma tumor from an 86-year-old Caucasian patient before any therapeutic intervention. Treatment of BR0555 tumor-bearing mice with SGN–LIV1A on a every 4 days schedule showed antitumor activity, resulting in pronounced tumor regressions (Fig. 4C).

SGN–LIV1A activity was also examined in the HeLa (cervical cancer–derived) xenograft model. Treatment with four 3 mg/kg doses of SGN–LIV1A given every 4 days resulted in significant tumor shrinkage compared with a nonbinding control ADC (Fig. 4D).

### Discussion

ADCs directed toward tumor-specific antigens are clinically proven as effective treatments of both solid and liquid tumors. We have shown in this study that LIV-1, an integral cell surface membrane protein, is a promising candidate for ADC therapy due to its broad expression in a number of cancer indications and limited normal tissue expression. LIV-1 is expressed across an array of cell lines from various lineages with surface copy numbers ranging.
from >170,000 to >5,000. Furthermore, inspection of archived biopsies showed a high percentage of primary, metastatic, and triple-negative breast cancer tissues that expressed the antigen. On the basis of these encouraging findings, we engineered a humanized anti–LIV-1 antibody that binds specifically to the extracellular domain of LIV-1, internalizes after antigen binding, and traffics to the lysosome. Using this antibody, we designed and generated SGN–LIV1A that leverages the specificity of the antibody and the activity of a potent microtubule-disrupting agent (MMAE) to produce a LIV-1–directed cytotoxic agent. SGN–LIV1A shows in vitro and in vivo potency and specificity when treating LIV-1–expressing cell lines and tumors.

In vitro assays showed that high LIV-1–expressing cell line was insensitive to treatment with the naked antibody alone. Cell lines that have low LIV-1 cell surface copy number are resistant to SGN–LIV1A and only show growth inhibition at concentrations >1,000 ng/mL.

We have shown that SGN–LIV1A is effective in in vivo xenograft models of different origin, including models of breast, prostate, and cervical lineage, delaying tumor growth at a relatively low dose (1 mg/kg/dose). Consistent with the proposed mechanism of ADC action, SGN–LIV1A was most effective in vivo on xenograft models with the highest expression of LIV-1, showing pronounced tumor regressions at doses of 3 mg/kg, while the antibody alone did not inhibit tumor growth at doses as high as 30 mg/kg. Cell surface LIV-1 copy number is difficult to accurately ascertain for tissues. However, IHC staining of xenograft sections indicates homogeneous LIV-1 levels (100% of the cells) at an intensity of 2 to 3, which is equivalent or less than the staining seen in over 50% of the metastatic breast biopsy sections.

Dependent upon the tumor receptor expression profile, there are now several targeted therapeutic approaches that can be used, including HER2 and hormone-directed regimens. However, regardless of their classification, patients who have relapsed with distant-stage metastatic
breast cancer have no curative therapeutic options open and face a 5-year survival rate of 24% (20). Current systemic treatments of these patients aim to prolong survival, control disease progression, alleviate symptoms, and enhance patient quality of life. In this study, we have shown that LIV-1 is expressed in all subtypes of breast cancer (including triple-negative) and that SGN–LIV1A is active as a single agent in preclinical models. These data in combination with the recent successes of ADCs support pursuing SGN–LIV1A as a new therapeutic modality for refractory metastatic breast cancer and other LIV-1–positive indications.

Disclosure of Potential Conflicts of Interest
D. Sussman, L.M. Smith, J.B. Miyamoto, A. Nesterova, L. Westendorf, and D.R. Benjamin have ownership interest (including patents) in Seattle Genetics, Inc. No potential conflicts of interest were disclosed by the other authors.

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Other (preparation of reagents): J.H. Hunter

Acknowledgments
The authors thank Jonathan Drachman for his careful review and assistance in completing this article.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 21, 2013; revised August 20, 2014; accepted September 14, 2014; published OnlineFirst September 24, 2014.
References

Molecular Cancer Therapeutics

SGN–LIV1A: A Novel Antibody–Drug Conjugate Targeting LIV-1 for the Treatment of Metastatic Breast Cancer


Mol Cancer Ther Published OnlineFirst September 24, 2014.

Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-13-0896

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