Enfortumab Vedotin Antibody-Drug Conjugate Targeting Nectin-4 Is a Highly Potent Therapeutic Agent in Multiple Preclinical Cancer Models


Abstract

The identification of optimal target antigens on tumor cells is central to the advancement of new antibody-based cancer therapies. We performed suppression subtractive hybridization and identified nectin-4 (PVRL4), a type I transmembrane protein and member of a family of related immunoglobulin-like adhesion molecules, as a potential target in epithelial cancers. We conducted immunohistochemical analysis of 2,394 patient specimens from bladder, breast, lung, pancreatic, ovarian, head/neck, and esophageal tumors and found that 69% of all specimens stained positive for nectin-4. Moderate to strong staining was especially observed in 60% of bladder and 53% of breast tumor specimens, whereas the expression of nectin-4 in normal tissue was more limited. We generated a novel antibody–drug conjugate (ADC) enfortumab vedotin comprising the human anti-nectin-4 antibody conjugated to the highly potent microtubule-disrupting agent MMAE. Hybridoma (AGS-22ME) and CHO (ASG-22CE) versions of enfortumab vedotin (also known as ASG-22ME) ADC were able to bind to cell surface–expressed nectin-4 with high affinity and induced cell death in vitro in a dose-dependent manner. Treatment of mouse xenograft models of human breast, bladder, pancreatic, and lung cancers with enfortumab vedotin significantly inhibited the growth of all four tumor types and resulted in tumor regression of breast and bladder xenografts. Overall, these findings validate nectin-4 as an attractive therapeutic target in multiple solid tumors and support further clinical development, investigation, and application of nectin-4–targeting ADCs. Cancer Res; 76(10); 3003–13. ©2016 AACR.

Introduction

Cancers of epithelial origin represent a significant global medical challenge that impacts patients, their families, and the health care system. Transitional cell carcinoma (TCC) of the bladder, a commonly diagnosed epithelial cancer, has an incidence rate estimated at >72,000 cases per year in the United States (1). Approximately 15,000 patients die annually from the metastatic form of the disease. Treatment options for metastatic TCC are quite limited and most were developed decades ago (1, 2). Recent advances in cancer genomics, such as identification of driver mutations and immune modulators that play a role in TCC, have motivated the development of targeted therapies that inhibit driver rearrangements (12). On the basis of the differential expression of nectin-4 in bladder cancers, we developed a novel ADC, enfortumab vedotin, comprising fusion between a fully human
and rehydration, tissue sections were treated for antigen retrieval with EDTA for 30 minutes and incubated with the primary antibody M22-244b3 or isotype control antibody mouse IgG1 for 1 hour. Expression was then detected with the BioGenex Super Sensitive Polymer-HRP IHC Detection Kit (BioGenex Laboratories Inc). The intensity and extent of nectin-4 expression was determined microscopically using the histochemical scoring system (H-score), defined as the sum of the products of the staining intensity (score of 0–3) multiplied by the percentage of cells (0–100) stained at a given intensity. Specimens were then classified as negative (H-score 0–14), weak (H-score 15–99), moderate (H-score 100–199), and strong (H-score 200–300).

Flow cytometry
Adherent cell monolayers were detached by treatment with 0.5 mmol/L EDTA in PBS, washed, and incubated with anti-nectin-4 antibodies for 1 hour at 4°C. The cells were then washed and stained with phycoerythrin-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories Inc) and analyzed by flow cytometry.

Antibody and ADC generation
The fully human mAb AGS-22M6 targeting the ECD of human nectin-4 was generated using the Xenomouse platform developed by Amgen (formerly Abgenix; ref. 14). Briefly, human IgG1κ antibody–producing Xenomice were immunized with the purified ECD of human nectin-4. B cells from lymph nodes and spleens were fused with the SP2/0 myeloma fusion partner. Hybridoma screening to identify antibodies specific for nectin-4 was performed using ELISA and FACS assays. For the recombinant expression of AGS-22M6 antibody in mammalian cells, the AGS-22M6 variable heavy chain and κ light chains were sequenced from the corresponding hybridoma and cloned into the pEEl2.4 expression vector (Lonza), and the resulting plasmid was transfected into CHO host cells (CHOK1SV, Lonza). Transfected cells were subjected to selection in the absence of glutamine to generate a stable antibody–producing cell line. The CHOκ1SV-derived antibody is referred to as AGS-22C. ADCs referred to as AGS-22M6E (also known as AGS-22ME) and AGS-22CE were generated via conjugation of hybridoma-produced AGS-22M6 and CHO-derived AGS-22C antibodies, respectively, to the small-molecule microtubule-disrupting agent MMAE via the cleavable drug linker maleimidoacrylicvaline-citrulline-p-aminobenzoyloxycarbonyl using technology licensed from Seattle Genetics, Inc as previously published (15). The antibodies AGS-22M6 and AGS-22C, as well as isotype control antibody, were conjugated to MMAE following partial reduction of interchain disulfide bonds with tris (2-carboxyethyl)-phosphine as described previously (16). The drug-to-antibody ratio was approximately 4:1.

Cytotoxicity assay
PC-3 cells engineered to express nectin-4 of human, monkey, or rat origin or T-47D breast carcinoma cells were incubated with serially diluted AGS-22M6E or isotype control ADC at 37°C in 5% CO2. Cell viability was measured after 5 days using AlamarBlue (Life Technologies). Percent survival was calculated as the number of live cells in treated wells divided by the number of live cells in control wells. The IC50 was derived from the survival curve using the sigmoid Emax nonlinear regression analysis function in GraphPad Prism (GraphPad).
**In vivo studies**

All experimental protocols were approved by Agensys’ Institutional Animal Care and Use Committee. The bladder cancer AG-B1 xenograft model was established at Agensys by serially passaging a patient specimen of bladder cancer in SCID mice. The AG-Br7 xenograft model was derived from a specimen of a patient with triple-negative breast cancer. AG-Panc4 was established from a patient with moderately differentiated adenocarcinoma of the pancreas (17). NCI-H322M lung adenocarcinoma cell line was used as a lung cancer model. Five- to 6-week-old ICR-SCID mice were obtained from Taconic BioSciences. For experiments with subcutaneous models of AG-B1, AG-Br7, and AG-Panc4, xenograft tumor fragments (5–6 pieces, 1 mm³ each) were implanted subcutaneously into the flank of each mouse. For experiments using the orthotopic breast tumor model AG-Br7, tumor pieces were enzymatically digested to single-cell suspension using Liberase Blendzyme (Roche Applied Science), and 3 × 10⁶ cells were injected into the mammary fat pad of individual female SCID mice. For experiments with NCI-H322M lung adenocarcinoma–derived xenografts, mice were injected with 2.5 × 10⁶ tumor cells subcutaneously into the flank of the mice. Number of mice used for each experiment is indicated in Fig. 6. Treatment by intravenous administration was initiated when tumors reached approximately 200 mm³ in size. Tumor length (L) and width (W) were measured with a caliper, and tumor volume was calculated using the formula \( V = \frac{L \times W^2}{2} \). Percent tumor regression was calculated as the tumor volume in a mouse treated with AGS-22M6E relative to the tumor volume in mice treated with control ADC or vehicle control. Percent tumor regression at the end of the study was calculated as the tumor volume in each mouse one day before sacrifice relative to the tumor volume in the same mouse on day 1 of the study. A statistical analysis of the tumor volumes at the start of treatment and one day before animal sacrifice was performed using the nonparametric Kruskal–Wallis test. Pairwise comparisons were made using the Tukey–Kramer method (two sided) on the ranks of the data to protect experiment-wise error rate.

**Results**

**Nectin-4 expression in normal tissues and tumor specimens**

In a series of discovery experiments using suppression subtractive hybridization as described previously (18), we identified nectin-4 (also known as PVRL4) as a gene markedly upregulated in bladder cancer. Nectin-4 is a type I transmembrane polypeptide member of the nectin family of adhesion molecules and was first identified through a bioinformatics search by Reymond and colleagues (19). We generated a mouse antibody, referred to as M22-244b3, directed against the ECD of human nectin-4 expressed in *E. coli*. M22-244b3 is specific for nectin-4 and does not crossreact with other nectin family members (data not shown). Immunohistochemical analysis using M22-244b3 of a panel of 294 normal tissue specimens representing 36 human organs showed homogenous weak to moderate staining mainly in human keratinocytes, skin appendages (sweat glands and hair follicles), transitional epithelium of bladder, salivary gland (ducts), esophagus, breast, and stomach. Representative specimens are shown in Fig. 1. Weak staining was detected in samples of larynx, pituitary, placenta, testis, ureter, and uterus.

Next, we conducted an extensive immunohistochemical analysis of nectin-4 expression in a total of 2,394 cases of human cancers from 34 tumor TMAs representing 7 different...
Expression of nectin-4 in human tumor specimens

<table>
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<tr>
<th>Cancer type</th>
<th>Strong N (%)</th>
<th>Moderate N (%)</th>
<th>Low N (%)</th>
<th>Negative N (%)</th>
<th>Overall positive (%)</th>
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<td><strong>Bladder</strong></td>
<td>162 (31)</td>
<td>154 (29)</td>
<td>118 (23)</td>
<td>90 (17)</td>
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<td>170 (26)</td>
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<td>54 (40)</td>
<td>56 (41)</td>
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<td>57 (20)</td>
<td>55 (30)</td>
<td>82 (45)</td>
<td>55</td>
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<td><strong>Total</strong> (N = 2,394)</td>
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<td>562 (24)</td>
<td>672 (28)</td>
<td>747 (31)</td>
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*aIntensity of staining: strong, H-score = 200–300; moderate, H-score = 100–199; low, H-score = 15–99; negative, H-score = 0–14.*

Table 1. Expression of nectin-4 in human tumor specimens

Antibody and ADC characterization

A fully human mAb, AGS-22M6, was generated by immunizing human IgG1–producing strain of XenoMouse with the human nectin-4 ECD. AGS-22M6 was selected among a panel of >50 distinct anti-nectin-4 mAb produced in the XenoMouse. This antibody binds to transfected human, monkey, or rat nectin-4 expressed on the cell surface of PC-3 cells (Fig. 3A) and endogenous nectin-4 expressed on the surface of NCI-H322M and T-47D cells (Fig. 3B). The PC-3 cell line was selected for transfection as it does not express endogenous nectin-4. The apparent affinity-binding constant ($K_a$) value for nectin-4 expressed on T-47D breast cancer cells was 0.01 nmol/L (Fig. 3C). We also found a similar $K_a$ for AGS-22M6E, the ADC produced by conjugation to vcMMAE (Fig. 3C). Thus, the conjugation process did not alter binding characteristics of parental antibody AGS-22M6 to nectin-4 on the surface of cancer cell lines.

To further characterize AGS-22M6, we constructed deletion mutants consisting of the membrane-distal (V) or membrane-proximal (C’C”) domains of nectin-4 fused to its transmembrane and intracellular domains. We expressed the domain-deleted proteins on the surface of Rat1(E) fibroblasts and conducted binding experiments with AGS-22M6 or another anti-nectin-4 antibody, AGS-22M104, which had been found to bind nectin-4 within its C’C” and not the V region. Notably, AGS-22M6 recognized an epitope located in the V-domain with the same apparent affinity as to the full-length nectin-4 expressed in the same host cells (Fig. 4A). AGS-22M6 did not bind to the C’C” domain. Proof that lack of binding of AGS-22M6 was not due to lack of expression of the C’C” domain mutant was demonstrated by the ability of AGS-22M104 to exhibit binding in the assay.

The V-domains of nectin molecules were previously demonstrated to mediate homo- and heterodimerization of nectins in cis- and trans- configurations, and nectin-4 was shown to interact with nectin-1 with nanomolar affinity (20). Therefore, we evaluated the ability of AGS-22M6 to block the interaction of nectin-1 with nectin-4 expressed on the surface of Rat1(E) fibroblasts. As shown in Fig. 4B, a biotinylated ECD of nectin-1 expressed as an Fc fusion protein was able to bind to cell surface nectin-4, and this interaction was effectively disrupted by the addition of either AGS-22M6 or the AGS-22M6E ADC.
In vitro cytotoxicity

The ability of the AGS-22M6E ADC to inhibit cell survival was evaluated in the PC-3 cell lines engineered to express human, monkey, or rat nectin-4, as well as in the T-47D human breast carcinoma cells that endogenously express nectin-4. Serial dilutions of AGS-22M6, AGS-22M6E ADC, and an isotype control ADC were added to cells, and cell viability was measured after 5 days. As shown in Fig. 5, AGS-22M6E ADC, but not unconjugated AGS-22M6 or control ADC, induced dose-dependent inhibition of cell viability both in T-47D cells and in PC-3 cells transfected with human, monkey, or rat nectin-4. AGS-22M6E ADC had no effect on mock-transfected PC-3 cell controls. We did not observe any significant cytotoxic activity using an isotype control ADC on the various PC-3 cell lines.

In vivo efficacy

To evaluate the therapeutic potential of AGS-22M6E ADC, we performed a set of in vivo efficacy studies in murine models of xenografted human cancers. Both cell line–derived and proprietary patient tumor–derived xenografts were selected on the basis of the expression of human nectin-4. A total of 13 xenograft models were evaluated across 4 cancer indications (bladder, breast, pancreas, and lung). Tumor size ranged from 100 to 200 mm³ at the start of the treatment in all models, and ADC was administered in all studies by intravenous injection. Overall, AGS-22M6E ADC induced tumor regression in 5 of the 13 models and resulted in significant inhibition of established tumor growth in 12 of 13 models examined. Representative studies using an AG-B1 bladder cancer model, two AG-Br7...
breast cancer models, and an AG-Panc4 pancreatic cancer model are described below. Nectin-4 expression in these xenograft tumors is shown in Fig. 6A. In all these in vivo antitumor efficacy studies, the ADCs were well tolerated. No reduction in body weight or signs of distress was observed in any of the treatment groups (data not shown).

Treatment of AG-B1 xenograft tumors with single 4 mg/kg doses of AGS-22M6E or two other anti-nectin-4 ADCs caused regression of established tumors (Fig. 6B). Immunohistochemical evaluation of the tumor site at the end of the study showed complete tumor eradication in 5 of the 6 mice treated with AGS-22M6E. Depending on the treatment regimen, AGS-22M6E was able to eradicate or significantly inhibit tumor growth in this bladder cancer model. As shown in Fig. 6C, AGS-22M6E at 0.8 mg/kg dosed every 4 days for a total of 5 doses significantly inhibited growth of established AG-B1 tumor xenografts when compared with control ADC at 0.8 mg/kg (P < 0.0001), whereas AGS-22M6E given at the lower dose of 0.4 mg/kg did not show significant antitumor activity when compared with any of the controls (P > 0.05). IHC analysis of xenografts from treated animals showed strong AGS-22M6E ADC localization to the xenograft tumors within 6 hours of treatment, whereas the control ADC is trapped in the stroma (Supplementary Fig. S1). Localization of ADC peaks at 24 hours and starts diffusing at 72 hours (Supplementary Fig. S1).

We next evaluated potency of AGS-22M6E against breast cancer using the AG-Br7 subcutaneous xenograft model. As shown in Fig. 6D, AGS-22M6E at 3 mg/kg had a potent tumor inhibitory effect when compared with the control ADC, the unconjugated antibody AGS-22M6 or the vehicle control: 91.3% inhibition versus control ADC (P < 0.0001); 94.2% inhibition versus AGS22M6 (P < 0.0001); and 93.9% inhibition versus vehicle (P < 0.0001). In addition, treatment with 3 mg/kg AGS-22M6E resulted in regression of the tumor by 62.9% at the end of the study relative to the starting tumor size (P < 0.0001). AGS-22M6E given at the lower dose of 1 mg/kg also showed significant effect when compared with either control ADC at the equivalent dose (P = 0.0371) or vehicle control (P = 0.0003), resulting in 44.5% and 34.6% tumor inhibition, respectively. A statistically significant difference (P = 0.0147) was detected when the two doses of AGS-22M6E were compared, indicating a dose-dependent effect of AGS-22M6E. The efficacy of AGS-22M6E was also examined against an orthotopic xenograft, in which AG-Br7 tumors were established in mammary fat pads of SCID mice. As shown in Fig. 6E, AGS-22M6E
Figure 4.
Identification of the AGS-22M6–binding domain. A, Rat1(E) cells recombinantly expressing either full-length nectin-4, or domain-deleted mutants containing only the V domain or C'-C'' domains were stained with AGS-22M6 or AGS-22M104 at 10 μg/mL for 1 hour at 4°C, followed by binding of phycoerythrin (PE)-conjugated anti-human IgG antibody. The cells were then analyzed by flow cytometry and histogram results (open histogram) were overlaid against Rat1(E) mock-transfected cells (solid histogram). B, a fusion of the Fc of human IgG1 with the ECD of recombinant nectin-1 (nectin-1-Fc) or a control-Fc protein were biotinylated and incubated with nectin-4–expressing Rat1(E) cells at 300 ng/mL in the absence or presence of increasing concentrations of AGS-22M6 or AGS-22M6E for 1 hour. Cells were washed, stained with streptavidin-PE (Jackson ImmunoResearch Laboratories), and analyzed by flow cytometry. Histogram of nectin-1-Fc compared with control-Fc binding in the absence of antibody is shown in the insert.
administered either as a single 10 mg/kg dose or as two 5 mg/kg doses resulted in complete eradication of established tumors. Lower doses were not explored in this model.

In the AG-Panc4 model, AGS-22M6E at 3 mg/kg led to tumor stasis, with significant tumor growth inhibition compared with isotype control ADC at equivalent dosage (Fig. 6F). In the NCI-H322M lung adenocarcinoma xenograft model, AGS-22M6E at 3 mg/kg resulted in 83.6% tumor regression compared with isotype control ADC at the same dose (P < 0.0001, Fig. 6G). In addition, treatment with AGS-22M6E at 3 mg/kg resulted in 20.5% tumor regression (P = 0.03461). AGS-22M6E given at a lower dose of 1.0 mg/kg did not show a significant effect when compared with control ADC at the equivalent dose (P = 0.1455).

In support of the clinical development of an ADC targeting nectin-4, the AGS-22M6 antibody produced in the hybridoma system was converted into a human recombinant product expressed in CHO cells as detailed in Materials and Methods. A comparability analysis between the hybridoma-derived AGS-22M6E ADC and the CHO-derived ASG-22CE ADC was performed in vitro and in vivo. Both ADCs showed identical in vitro characteristics: binding constants for nectin-4 were 0.057 and 0.060 nmol/L for AGS-22M6E and ASG-22CE, respectively, and IC50 of 1.523 and 1.674 nmol/L, respectively. When injected in mice, both AGS-22M6E and ASG-22CE showed comparable pharmacokinetic profile up to 10 days (Supplementary Fig. S2; Table 1). ADC and total IgG exhibited similar exposure and serum elimination half-life ranging from 1.5 to 2 days. They also exhibited equivalent dose-dependent tumor growth inhibition in the breast cancer AG-Br7 xenograft model (Fig. 6H).

**Discussion**

Nectin-4 was originally cloned from human trachea and described as an antigen with a restricted pattern of expression in normal tissues (19). More recently, nectin-4 was identified as an entry point for measles and other viruses and was proposed as a target for oncolytic viral therapy (21, 22). Although several groups reported the detection of nectin-4 expression in breast, ovarian, and lung cancers (23–26), no comprehensive analysis has been reported to date. Using suppression subtractive hybridization, we identified nectin-4 as one of the genes dramatically upregulated in bladder cancer specimens. Our RNA expression analysis of a larger cancer sample panel and of normal tissues also indicated that nectin-4 transcript is present at low levels in various normal tissues and is upregulated in a subset of breast, pancreatic, and lung cancer specimens (Agenysys, unpublished data). In this report, we used IHC to demonstrate nectin-4 protein expression in normal tissue specimens and in TMAs from various types of cancer. Our results showed broader expression in normal tissues than was previously reported. Nectin-4 expression was detected in the epithelia of bladder, breast, stomach, esophagus, salivary gland (ducts), and skin (epidermis and sweat glands). Epithelial components of other organs showed weaker and less consistent staining for nectin-4. Investigation of normal tissue antigen expression is essential for the clinical progression of an ADC. Prohibitive on-target toxicities have been observed for several ADCs, such as skin toxicities (27). The extensive expression profiling of nectin-4 described herein allowed us to identify normal tissues that may raise the potential risk of eliciting nectin-4 on-target toxicities. These have been investigated in two cross-reactive species (rat and cynomolgus monkey), as well as in the clinical setting. Preliminarily, the findings suggest a favorable therapeutic window, but details will be reported as part of a complete analysis of phase 1 results.

In cancer patient specimens, >50% samples tested positive for nectin-4 protein expression, and the level of expression, based on IHC staining intensity, was highest in bladder cancer...
Figure 6. Nectin-4–targeted ADC shows significant tumor growth inhibition in multiple xenograft models. A, examples of nectin-4 staining in the in vivo xenograft models of AG-B1, AG-Br7, AG-Panc4, and NCI-H322M, as well as mouse IgG1 isotype staining of AG-B1 as background staining control. Tumor growth over time for AG-B1 subcutaneous xenografts treated with three different ADC at 4 mg/kg (B); AG-B1 subcutaneous xenografts treated with AGS-22M6E (or controls) at 0.8 and 0.4 mg/kg (C); AG-Br7 subcutaneous xenografts treated with AGS-22M6E (or controls) at 1 and 3 mg/kg (D); AG-Br7 orthotopic xenografts treated with AGS-22M6E (or controls) at 1 and 3 mg/kg (E); and NCI-H322M subcutaneous xenografts treated with AGS-22M6E (or controls) at 1 and 3 mg/kg (F). H, tumor growth inhibition by AGS-22M6E ADC and AGS-22CE ADC in the breast cancer AG-Br7 xenograft model. Treatment was initiated on day 0 as indicated by arrows. Mean tumor volume data for each group were plotted over time with SE bars.
and breast cancer. In general, good correlation between protein expression based on IHC and previously observed transcript levels was observed. Overall, IHC analyses demonstrated moderate to strong expression (H-score > 100) of nectin-4 in 60% of bladder cancer and 53% of breast cancer specimens. In addition, 37% and 27% of pancreatic and lung cancer specimens, respectively, demonstrated moderate to strong expression. Generally, we observed similar overall frequency of nectin-4 expression in ovarian cancer as previously published by Derycke and colleagues (26).

Soluble form of Nectin-4 in patient serum and in ascites in case of ovarian cancer has been reported (24–26). Although the levels varied between reports, all investigators show an association between higher detected levels of soluble nectin-4 and disease progression. Many other membrane-anchored proteins release their ECD through proteolytic cleavage, such as HER2 and EGFR (28, 29). However, clinical significance of such form has been difficult to establish, and effect on response to targeted therapy has not been fully elucidated (30–32).

AGS-22M6, a fully human antibody targeting nectin-4, was selected from a panel of >50 antibodies for further evaluation as a drug conjugate based on its high affinity, broad species cross-reactivity, and ability to mediate very potent cell death both in vitro and in vivo. The ADC AGS-22M6E, composed of AGS-22M6E conjugated to MMAE, exhibited dose-dependent antitumor activity in vivo. At lower doses (≤1 mg/kg), the ADC inhibited growth of bladder and breast cancer xenografts. At higher doses (≥3 mg/kg), AGS-22M6E induced tumor regression of the subcutaneous bladder cancer xenograft, and the orthotopic breast cancer xenograft. In addition, AGS-22M6E inhibited the growth of both the pancreatic cancer xenograft and the lung adenocarcinoma xenograft, with associated modest tumor regression in the lung tumor model. The in vivo efficacy of AGS-22M6E generally correlated well with the expression level of nectin-4 in xenografts and cell lines. In most of the studies described herein, the parental unconjugated antibody AGS-22M6 was included as a control for efficacy. We concluded that binding of unconjugated antibody to nectin-4 does not result in antitumor activity in either in vitro or in vivo preclinical models.

Our data also demonstrated that under the conditions of an in vitro binding assay, AGS-22M6 and AGS-22M6E blocked the interaction of nectin-4 with nectin-1, its putative binding partner. The functional significance of this observation is presently unknown, as the role of nectin-4 in normal epithelial tissue structure and morphogenesis is poorly understood. We and others hypothesize that nectin-4 plays a role in the establishment and maintenance of adherens junctions, which in normal epithelium define cell polarity, a characteristic often lost during cancer progression (12). We have attempted, but not yet succeeded, in identifying an anti-nectin-4 antibody that intrinsically modulates tumor growth. In a recent publication, Pavlova and colleagues (33) demonstrated that nectin-4 promotes anchorage-independent growth in breast cancer cells by activation of the matrix-independent integrin β4/SHP-2/c-Src and that the transformation of breast cancer cells to anchorage independence is dependent on nectin-4. The authors also reported modest inhibition of growth of a breast cancer xenograft by a mAb targeted against human nectin-4. The mechanism of action of such antibody will need to be further investigated.

On the basis of its ability to induce tumor regression in human breast and bladder cancer specimens, as well its favorable toxicity profile in various species (Agensys, unpublished data), AGS-22M6E, the hydridoma-derived ADC, was investigated in early-phase clinical studies for safety and pharmacokinetics in patients with solid tumors that express nectin-4 (ClinicalTrials.gov identifier NCT01409135). Because pivotal registration trials will require an antibody produced at high titers using mammalian cells, we have subsequently developed a comparable CHO-derived anti-nectin-4 antibody, ASG-22CE. Our preclinical studies with ASG-22CE ADC showed that it has equivalent binding and potency as the hybridoma product. ASG-22CE entered the clinic in 2014 as monotherapy in patients with metastatic urothelial cancer, as well as other solid tumors that express nectin-4 (ClinicalTrials.gov identifier NCT02091999), and is currently under clinical development as enfrotumab vedotin.

Overall, the data presented herein indicate that nectin-4 is a viable target for ADC therapy in carcinomas expressing nectin-4 and support the evaluation of such ADCs in the clinical setting. The preclinical data demonstrate growth inhibition across multiple human xenograft tumor models and tumor regression in bladder and breast cancer models. Our finding of nectin-4 overexpression in human tumor specimens, particularly those from patients with breast, bladder, pancreatic, or lung cancer, suggests that a significant proportion of patients may benefit from nectin-4 targeted delivery of a potent cytotoxic agent to the tumor site.

Disclosure of Potential Conflicts of Interest

Enfortumab Vedotin is being co-developed in conjunction with Agensys’ partner, Seattle Genetics, Inc. No potential conflicts of interest were disclosed.

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