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Initial Testing (Stage 1) of Glembatumumab Vedotin (CDX-011) by the Pediatric Preclinical Testing Program

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Abstract

Background—Glembatumumab vedotin is an antibody-auristatin conjugate that targets cells expressing the transmembrane glycoprotein NMB (GPNMB, also known as osteoactivin). It has entered clinical evaluation for adult cancers that express GPNMB, including melanoma and breast cancer.

Procedures—Glembatumumab vedotin was administered intravenously at a dose of 2.5 mg/kg using a weekly x 3 schedule, and its antitumor activity was evaluated against selected Pediatric Preclinical Testing Program (PPTP) solid tumor xenografts using standard PPTP response metrics.

Results—Among PPTP xenografts, GPNMB was primarily expressed on the osteosarcoma xenografts, all of which expressed GPNMB at the RNA level, although at varying levels. Protein expression assessed by immunohistochemistry (IHC) showed variation across the osteosarcoma xenografts with one model showing no tumor cell expression. Glembatumumab vedotin induced statistically significant differences ($p < 0.05$) in event-free survival (EFS) distribution compared to control in each of the 6 osteosarcoma models studied. Three of 6 osteosarcoma xenografts demonstrated a maintained complete response (MCR). Two other xenografts showed progressive disease with growth delay, while the final xenograft showed progressive disease with no growth delay. Two of the osteosarcoma xenografts with MCRs showed the highest GPNMB expression at the RNA level. Conversely, the xenograft with the lowest GPNMB mRNA expression had the poorest response to glembatumumab vedotin. Two rhabdomyosarcoma xenografts that did not express GPNMB showed limited responses to glembatumumab vedotin.

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Conclusions—Glembatumumab vedotin yielded high-level activity against 3 of 6 osteosarcoma xenografts, with evidence for response being related to GPNMB expression levels.

Keywords

Preclinical Testing; Developmental Therapeutics; antibody-drug conjugate; osteosarcoma

INTRODUCTION

Glycoprotein NMB (GPNMB), or osteoactivin, is a transmembrane glycoprotein primarily expressed in intracellular compartments (e.g., lysosomes and melanosomes) in non-malignant cell such as melanocytes, osteoclasts, and osteoblasts [1-3]. GPNMB is also expressed on monocytes and dendritic cells, and its expression on the latter has been proposed to play a role in the inhibition of T-cell activation by antigen-presenting cells (APC) [4-6]. In hepatocellular carcinoma [7], breast cancer [8,9], glioblastoma [10], and melanoma [2,11], membrane GPNMP is overexpressed, making it a reasonable candidate for targeted therapeutics.

Glembatumumab vedotin is an antibody-drug conjugate (ADC) that combines an anti-GPNMB antibody with the anti-mitotic agent monomethyl auristatin E (vedotin) [11]. When internalized, vedotin is released and results in cell cycle arrest and cell death [12]. Glembatumumab vedotin showed in vitro cytotoxicity that was related to GPNMB expression, and it induced complete regressions in GPNMB-expressing melanoma and breast cancer xenografts [9,11,13].

Three clinical trials of glembatumumab vedotin (NCT00412828, NCT00704158, NCT01156753) have completed enrollment. In a phase 1/2 study for patients with melanoma, among 34 patients treated at the maximum tolerated dose (MTD), 1.88 mg/kg every 3 weeks, 56% showed tumor shrinkage with 15% achieving an objective response [14]. In a phase 1/2 study for patients with breast cancer, among 42 patients treated at the MTD, 12% showed an objective response, with 29% of those with confirmed GPNMB expression showing an objective response [15]. In the phase 2 EMERGE study, 122 patients with refractory metastatic breast cancer whose tumors were positive for GPNMB were randomly assigned to glembatumumab vedotin or “investigator's choice” (IC) [16]. The overall response rate for patients receiving glembatumumab vedotin was 19%, with a response rate for those with high GPNMB expression (defined by expression in ≥25% of tumor cells) being 32% (versus 13% for patients receiving IC) [16]. In the EMERGE trial, patients receiving glembatumumab vedotin experienced less hematologic toxicity than those receiving IC, with the primary glembatumumab vedotin associated adverse events being rash and neuropathy [16]. Considering the results from all three clinical trials, the initial glembatumumab vedotin experience suggests that GPNMB expression may correlate with response [15].

We report the results of glembatumumab vedotin evaluated in vivo by the NCI- supported Pediatric Preclinical Testing Program (PPTP). Given the specificity of glembatumumab vedotin for transmembrane GPNMB, testing was restricted to six osteosarcoma xenografts

known to express GPNMB and to two non-GPNMB expressing rhabdomyosarcoma xenografts.

MATERIALS AND METHODS

In vivo tumor growth inhibition studies

CB17SC *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted sarcomas (osteosarcoma, rhabdomyosarcoma), [17]. Female mice were used irrespective of the patient gender from which the original tumor was derived. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Eight to ten mice were used in each control or treatment group. Tumor volumes (cm³) were determined and responses were determined using three activity measures as previously described [17]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drugs and Formulation

Glembatumumab vedotin was provided to the Pediatric Preclinical Testing Program by Celldex Therapeutics Inc., through the Cancer Therapy Evaluation Program (NCI). It was provided as a 5 mg/ml solution formulated in sucrose (10%), histidine (0.01 M), histidine hydrochloride (0.01 M), and Polysorbate 20 (0.02%) at pH of 6.0 ± 0.5. Glembatumumab vedotin was diluted in sterile saline to prepare a 0.5 mg/ml working solution and stored for up to 7 days at 4°C, protected from light. Glembatumumab vedotin was administered IV at 2.5 mg/kg to mice using a q 7 days × 3 schedule with an additional 3 weeks of observation. Glembatumumab vedotin was provided to each consortium investigator in coded vials for blinded testing.

GPNMB Immunohistochemistry

Xenograft sections were stained for GPNMB expression at Clariant Diagnostic Services, Inc. (Aliso Viejo, CA). Staining parameters were optimized (antibody titer, antigen retrieval, incubation time), using known positive and negative cell lines and expression was confirmed using a commercial array containing 80 tissue cores from osteosarcoma specimens (US Biomax). Briefly formalin-fixed paraffin embedded slides were deparaffinized, washed and antigen retrieval was performed with citrate buffer for 10 min. at 99 °C (pH 6.0). Sections were incubated with polyclonal goat anti-GPNMB (R&D Systems) an antibody has weak reactivity with mouse GPNMB, overnight@4°C, and GPNMB was detected with donkey anti-goat HRP (Jackson Labs), followed by visualization with substrate, diaminobenzidine (Vector Labs) and counterstaining with hematoxylin for 5 minutes. This staining procedure showed a 3+ intensity of cytoplasm and membrane staining GPNMB in known positive cell

line MCF7 breast carcinoma and breast tissue with no non-specific background staining in known negative cell line TK-10 renal cell carcinoma. Stained slides were manually evaluated by a Clariant pathologist using a standard bright field microscope. Intensity of staining was graded on the following scale: 1+ (weak), 2+ (moderate), 3+ (strong). Osteosarcoma xenograft sections from the PPTP were then stained with this method using rhabdomyosarcoma sections as an internal control in a blinded fashion.

RESULTS

In vivo testing

Glembatumumab vedotin was tested against the osteosarcoma xenograft panel as well as against two rhabdomyosarcoma xenografts. All of the osteosarcoma xenografts expressed GPNMB at the RNA level as evaluated using Agilent gene expression arrays (Figure 1). By contrast, none of the rhabdomyosarcoma xenografts expressed GPNMB at the RNA level. Immunohistochemistry (IHC) staining results for GPNMB for the osteosarcoma and rhabdomyosarcoma xenografts are shown in Table I. Six of 7 osteosarcoma xenografts showed GPNMB expression detectable by IHC (5-80 % positive cells, 1-3+ intensity), with only OS-31 being negative for GPNMB by IHC. None of the rhabdomyosarcoma xenografts showed tumor staining for GPNMB.

Glembatumumab vedotin was well tolerated with no deaths (0/79) in the treatment arms and minimal weight loss (~5%). As glembatumumab does not recognize murine GPNMB, this suggests that the toxicity may be a consequence of relatively low levels of free auristatin E being released from the antibody conjugate. Eight of 8 tested xenograft models were considered evaluable for efficacy. Complete details of testing are provided in Supplemental Table I including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

For the osteosarcoma xenografts, glembatumumab vedotin induced statistically significant differences in EFS distribution compared to control in each of the 6 osteosarcoma models studied and in the Rh36 rhabdomyosarcoma xenograft (Table II). For those xenografts with a significant difference in EFS distribution between treated and control groups, the EFS T/C activity measure additionally requires an EFS T/C value of > 2.0 for intermediate activity and indicates a substantial agent effect in slowing tumor growth. High activity further requires a reduction in final tumor volume compared to the starting tumor volume. Glembatumumab vedotin induced tumor growth inhibition meeting criteria for intermediate and high EFS T/C activity in 2 and 3 osteosarcoma xenografts, respectively, and intermediate activity in the Rh36 model.

Three of 6 osteosarcoma xenografts showed objective responses to glembatumumab vedotin, with each of the responding models achieving a maintained complete response (MCR). Two other xenografts showed progressive disease with growth delay (PD2), while the final xenograft showed progressive disease with no growth delay (PD1). The objective response results for solid tumor models are represented in Figure 2 using a 'heat map' and 'COMPARE' format, the latter is based on the objective response scoring criteria centered

around the midpoint score of 0 that represents stable disease. Representative responses for osteosarcoma models is presented in Figure 3. As expected, the two rhabdomyosarcoma xenografts showed limited responses to glembatumumab vedotin, although one did show significant tumor growth inhibition with an EFS T/C value of 2.6.

DISCUSSION

The drug administration schedule was chosen to provide good exposure to glembatumumab vedotin over several weeks, and while not exactly the same as used in previous studies, it is generally consistent with doses that were effective in melanoma xenograft studies. Relative to humans, the half-life is longer in mice (10.8 days at 1 mg/kg) because glembatumumab vedotin has lower affinity to the orthologous endogenous GPNMB. Interestingly, at the 1 mg/kg dose, the half-life in SCID mice bearing SK-MEL-2 tumor drops to 5.5 days, suggesting the importance of target-mediated clearance mechanisms. While overall exposure differs, the C_{max} observed in humans for the antibody- drug conjugate at 1.88 mg/kg (the MTD in melanoma, and breast clinical trials) is in the range of that observed in SCID mice in which efficacy was observed against SK-MEL-2 melanoma (T. Hawthorne, Celldex, *pers comm*).

Glembatumumab vedotin demonstrated intermediate to high activity in 5 of 6 osteosarcoma xenografts, with a maintained complete response in three of the lines. Both rhabdomyosarcoma models demonstrated progressive disease on treatment, although glembatumumab demonstrated intermediate activity against the Rh36 models (EFS T/C = 2.4). GPNMB expression, mined from an Agilent gene expression array, was high in each of the osteosarcoma lines but not the rhabdomyosarcoma lines (Figure 1). Immunohistochemical staining (Table I) showed a similar pattern as that observed for the gene expression dataset with GPNMB protein expression in the osteosarcoma lines but not in the rhabdomyosarcoma lines.

In each of the lines that demonstrated a maintained complete response to glembatumumab vedotin (OS2, OS17 and OS33) there is 2+ to 3+ staining for GPNMB by immunohistochemistry (Table 1). However, while mRNA expression for GPNMB in OS-33 xenografts was high and similar to OS-17 (Figure 1), the percentage of tumor cells positive was low at 5%. That OS-33 was sensitive to glembatumumab vedotin could suggest a robust bystander effect or an artificially low immunostaining in this tumor. Overexpression of GPNMB mRNA and GPNMB protein is also observed in the OS9 xenograft, despite low activity of the agent against this model. GPNMB protein is not detected in the OS31 xenograft, notwithstanding mRNA overexpression and tumor growth delay, but not tumor regression in response to glembatumumab vedotin. The two rhabdomyosarcoma xenografts do not express GPNMB at the protein level, and their best response to glembatumumab vedotin was progressive disease with significant growth delay for Rh36. This xenograft is highly sensitive to vincristine, showing complete response to treatment with this agent, and so the response to glembatumumab vedotin is much less than that achievable with vincristine. These observations support the position that while GPNMB expression is associated with high-level responses to glembatumumab vedotin, it is not sufficient for response to this agent, as OS-9 tumors that express GPNMB (30% tumor cells, 1+ staining

intensity), failed to respond. Of note, the PPTP has previously tested vincristine [18] and eribulin [19], and the 2 xenograft lines least responsive to glembatumumab vedotin, were also least responsive to vincristine and eribulin. The sensitivity of these tumor xenografts to vincristine, eribulin and glembatumumab vedotin are summarized in heat map format, Supplemental Figure 1. The PPTP results with vincristine, eribulin and glembatumumab vedotin suggest that microtubule inhibitors may have activity in osteosarcoma, but that at the same time a subset of osteosarcomas may demonstrate intrinsic resistance.

In general, microtubule inhibitors have not been incorporated into standard neoadjuvant therapy regimens or in common retrieval regimens for osteosarcoma. Limited single agent data on vincristine in osteosarcoma is available from the 1960s [20,21]. Several subsequent single arm and randomized trials combining vincristine with other conventional agents failed to clearly demonstrate a role for vincristine in neo-adjuvant chemotherapy [22-27]. There have been few recent clinical trials of microtubule targeted therapies in osteosarcoma. In an Italian pediatric solid tumor phase 2 study, a response to vinorelbine is observed in one of five patients with osteosarcomas [28]. Additional preclinical data for microtubule targeted drugs in osteosarcoma include ABT-751, an orally bioavailable sulfonamide that binds to the colchicine binding site on β -tubulin to inhibit microtubule polymerization. In contrast to glembatumumab vedotin, none of the four osteosarcoma xenograft lines demonstrated significant tumor growth delay in response to ABT-751 [29].

GPNMB expression levels can be modulated by a number of factors that suggest potential strategies for combination therapies involving glembatumumab vedotin. For example, MEK inhibitors increased GPNMB expression in selected melanoma cell lines, and the higher GPNMB expression levels were associated with greater sensitivity to glembatumumab vedotin [30]. Imatinib was also shown to increase GPNMB expression in melanoma cell lines [30]. Upregulation of GPNMB expression is not restricted to malignant cells, as the anti-inflammatory cytokine IL-10 and the BCR-ABL inhibitors imatinib, dasatinib, and nilotinib produced marked upregulation of GPNMB expression in monocyte-derived dendritic cells [6]. A potential area for future research is identifying strategies for increasing GPNMB expression in osteosarcoma cells to enhance the effectiveness of glembatumumab vedotin as an osteosarcoma therapeutic agent. Cell-surface localized GPNMB is expressed in both normal osteoclasts and osteoblasts, hence it is possible that glembatumumab vedotin could alter bone turnover, although of the more severe toxicities (epidermal necrolysis, hyperglycemia, acute renal failure and rash) using more frequent dose schedules only the skin toxicity may have been caused by the expression of GPNMB in healthy skin. However, monitoring of bone turnover, particularly in the osteosarcoma population may be warranted.

In conclusion, glembatumumab vedotin showed intermediate to high-level in vivo activity in 5 of 6 pediatric preclinical models of osteosarcoma. Further studies to define the expression of GPNMB in a large number of primary and metastatic osteosarcoma specimens will be required to determine the true incidence of positivity. In two previous reports, the PPTP has demonstrated that microtubule-targeted agents (vincristine and eribulin) have significant activity against osteosarcoma xenografts. These data suggest that this class of conventional chemotherapeutics is still of interest in osteosarcoma. Glembatumumab vedotin is of

particular interest owing to the ability of the antibody to target vedotin delivery to osteosarcoma cells likely to express high levels of transmembrane GPNMB.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 2.

Left: The colored heat map depicts group response scores. A high level of activity is indicated by a score of 6 or more, intermediate activity by a score of >2 but <6, and low activity by a score of <2. Right: representation of tumor sensitivity based on the difference of individual tumor lines from the midpoint response (stable disease). Bars to the right of the median represent lines that are more sensitive, and to the left are tumor models that are less sensitive. Red bars indicate lines with a significant difference in EFS distribution between treatment and control groups, while blue bars indicate lines for which the EFS distributions were not significantly different.

Figure 3. Glembatumumab vedotin *in vivo* objective response activity for osteosarcoma models. Kaplan-Meier curves for EFS (left), median relative tumor volume graphs (center), and individual tumor volume graphs (right) are shown for selected lines. Treated (black lines), statistical significance (p values) of the difference between treated and control groups are included.

Table I

GPNMB Expression Assessed by IHC Staining

Line	% Tumor cells expressing GPNMB	Tumor cell Intensity	Tumor component -
OS-1	5	2+	>99%
OS-2	40	2+	>99%
OS-9	30	1 +	90%
OS-17	80	2-3+	80%
OS-29	60	2+	90%
OS-31	0	0	95%
OS-33	5	2+	>99%
Rh10	0	0	>99%
Rh18	0	0	70%
Rh36	0	0	95%
Rh30R	0	0	95%
Rh41	0	0	>99%
Rh65	0	0	95%

Table IISummary of *in Vivo* Activity of Glembatumumab Vedotin

Line	Tumor Type	Median Time to Event	P-value	EFS T/C	Median RTV End of Study	Tumor Volume T/C	Response	EFS Activity
Rh10	Alveolar RMS	17.3	0.004	0.8	>4	1.17	PD1	Low
Rh36	Embryonal RMS	32.2	0.003	2.6	>4	1.00	PD2	Int
OS-1	Osteosarcoma	> EP	<0.001	> 2.1	1.4	0.44	PD2	Int
OS-2	Osteosarcoma	> EP	<0.001	> 2.3	0.0	0.20	MCR	High
OS-17	Osteosarcoma	> EP	<0.001	> 2.4	0.3	0.50	MCR	High
OS-9	Osteosarcoma	31.5	0.011	1.4	>4	0.57	PD1	Low
OS-33	Osteosarcoma	> EP	<0.001	> 2.5	0.1	0.30	MCR	High
OS-31	Osteosarcoma	38.9	<0.001	2.0	>4	0.44	PD2	Int

RMS, rhabdomyosarcoma; EFS T/C, ratio of time to event for treated versus control animals; PD1, progressive disease without growth delay; PD2, progressive disease with growth delay; MCR, maintained complete response; Int, Intermediate.