C207 Pediatric Preclinical Testing Program (PPTP) Stage 1 Evaluation of Glembatumumab Vedotin (CDX-011, CR011-vcMMAE)

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GPNMB AND GLEMBATUMUMAB VEDOTIN

- Glycoprotein NMB (GPNMB), or osteoactivin, is a transmembrane glycoprotein primarily expressed in intracellular compartments (e.g., lysosomes and melanosomes) in non-malignant cells such as melanocytes, osteoclasts, and osteoblasts.
- Membrane GPNMB is overexpressed in some adult cancers, including hepatocellular carcinoma, breast cancer, and melanoma.
- Glembatumumab vedotin is an antibody-drug conjugate (ADC) that combines an anti-GPNMB antibody with the anti-mitotic agent monomethyl auristatin E (MMAE, vedotin).
- When glembatumumab vedotin is internalized, MMAE is released and results in cell cycle arrest and cell death.
- 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.
- Three clinical trials of glembatumumab vedotin (NCT00412828, NCT00704158, NCT01156753) have completed enrollment, and the agent has shown activity against breast cancer and melanoma.
- Considering the results from all three clinical trials, the initial glembatumumab vedotin experience suggests that GPNMB expression may correlate with response.

PPTP IN VITRO & IN VIVO TESTING METHODS

In vivo: Standard PPTP methods for in vivo testing were employed (http://pptp.nchresearch.org/documents/detailedAnalysisMethods.pdf).

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 x 3 schedule with an additional 3 weeks of observation.

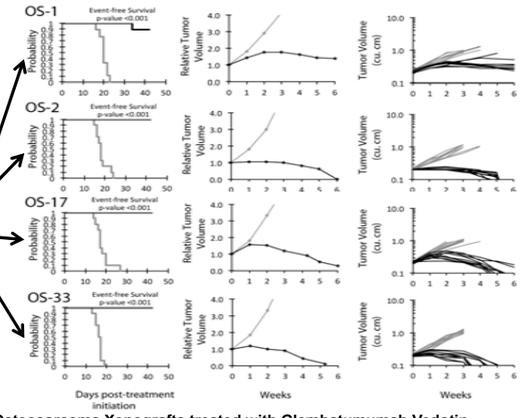
For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm^3. Two perpendicular tumor diameters were measured at either once or twice weekly intervals with digital vernier calipers. Assuming tumors to be spherical, volumes were calculated from the formula $(\pi/6) \times d3$, where d represents the mean diameter.

The primary activity measures were the objective response measure (see legend to figure at right) and the EFS T/C measure. The EFS T/C value is defined by the ratio of the median time to event of the treatment group and the median time to event of the respective control group.

GLEMBATUMUMAB VEDOTIN IN VIVO ACTIVITY

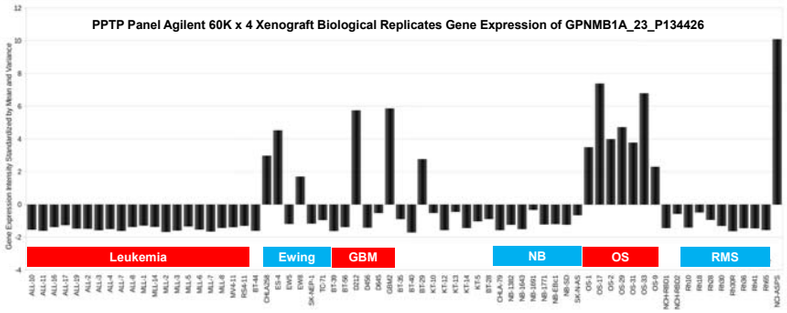
Line	Tumor Type	Median Time to Event (days)	P-value	EFS T/C	Median RTV at End of Study	Median Group Response
Rh10	Rhabdomyosarcoma	17.3	**0.004**	0.8	>4	PD1
Rh36	Rhabdomyosarcoma	32.2	**0.003**	**2.6**	>4	PD2
OS-1	Osteosarcoma	> EP	**<0.001**	**> 2.1**	1.4	PD2
OS-2	Osteosarcoma	> EP	**<0.001**	**> 2.3**	0.0	MCR
OS-9	Osteosarcoma	31.5	**0.011**	1.4	>4	PD1
OS-17	Osteosarcoma	> EP	**<0.001**	**> 2.4**	0.3	MCR
OS-31	Osteosarcoma	38.9	**<0.001**	**2.0**	>4	PD2
OS-33	Osteosarcoma	> EP	**<0.001**	**> 2.5**	0.1	MCR

- **Event:** 4-fold increase in tumor volume
- **> EP**: Time to event longer than the evaluation period (42 days)
- **EFS T/C**: Ratio of median time to event for treated and control animals.
- **RTV**: Relative tumor volume (ratio to day 1 tumor volume)
- **PD1 (Progressive Disease 1):** >25% ↑ in tumor volume, EFS T/C value ≤1.5;
- **PD2 (Progressive Disease 2):** >25% ↑ in tumor volume, EFS T/C value >1.5;
- **CR (Complete response):** disappearance of measurable tumor mass (< 0.10 cm^3)
- **MCR (Maintained CR):** tumor volume <0.10 cm^3 at the end of the study period



Osteosarcoma Xenografts treated with Glembatumumab Vedotin
- Controls (gray lines), Treated (black lines)
- Glembatumumab vedotin was administered at 2.5 mg/kg weekly x 3. For comparison, the clinical dose/schedule is 1.88 mg/kg IV q 3 weeks.
- OS-2, OS-17, and OS-33 eventually reached CR status, while OS-1 showed marked slowing of tumor growth during and after treatment.
- OS-9 and OS-31 each reached 4X relative tumor volume (RTV) status during the treatment and observation period (not shown).

GPNMB (OSTEOACTIVIN) GENE EXPRESSION



- GPNMB expression was consistently observed for the PPTP osteosarcoma (OS) xenografts. Most other xenografts showed low expression (NB = neuroblastoma, RMS = rhabdomyosarcoma, GBM = glioblastoma)

GPNMB PROTEIN EXPRESSION

Line	% Tumor cells expressing GPNMB	Tumor cell Intensity	% Stroma Cells expressing GPNMB	Stroma Intensity	Tumor component -
OS-1	5	2+	N/A	N/A	>99%
OS-2	40	2+	N/A	N/A	>99%
OS-9	30	1+	1%	3+	90%
OS-17	80	2-3+	0%	0	80%
OS-29	60	2+	5%	1+	90%
OS-31	0	0	1%	3+	95%
OS-33	5	2+	N/A	N/A	>99%
Rh10	0	0	N/A	N/A	>99%
Rh18	0	0	30%	2-3+	70%
Rh36	0	0	0%	0	95%
Rh30R	0	0	0%	0	95%
Rh41	0	0	N/A	N/A	>99%
Rh65	0	0	20%	2-3+	95%

- GPNMB expression was observed for the PPTP osteosarcoma xenografts, with the exception of OS-31. OS-9, which showed the lowest GPNMB gene expression, showed only 1+ staining by IHC.
- Rhabdomyosarcoma xenografts did not show GPNMB protein expression by IHC.

IN VIVO RESULTS AND CONCLUSIONS

- Three of six osteosarcoma (OS) xenografts demonstrated a maintained complete response (MCR) to glembatumumab vedotin. Two other OS xenografts showed progressive disease with growth delay, while the final xenograft showed progressive disease with no growth delay.
- Two of the OS xenografts with MCRs showed the highest GPNMB gene expression. Conversely, the xenograft with the lowest GPNMB gene expression had the poorest response to glembatumumab vedotin.
- The two OS xenografts with the least favorable responses to glembatumumab vedotin also showed the least favorable responses to vincristine and eribulin, suggesting that intrinsic resistance to tubulin-binding agents may affect response to glembatumumab vedotin.
- Two rhabdomyosarcoma xenografts (Rh36 and Rh10) that did not express GPNMB showed limited responses to glembatumumab vedotin. Both xenografts showed MCRs to the tubulin-targeted agent, eribulin, documenting sensitivity of both lines to tubulin-targeted agents.
- The glembatumumab vedotin-induced objective responses against 3 of 6 OS xenografts, support further evaluation of the agent for OS. The observation that GPNMB expression levels may be related to response should be considered in designing potential clinical trials of glembatumumab vedotin for OS.

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This poster will be available at: http://pptp.nchresearch.org/presentations.html