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), 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 via subcutaneous injection 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 diameter. 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 \( \frac{4}{3} \pi r^3 \), where \( r \) represents the mean diameter.

The primary endpoint measures were the objective response rate (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.

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 anti-cancer drug conjugate 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 vivo 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, NCT00764158, NCT10156753) 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 expression experience suggests that GPNMB gene 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).
- Testing was supported by NCI NO1CM42216.

#### In vivo responses

<table>
<thead>
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<th>Line</th>
<th>Tumor Type</th>
<th>Median Time to Event (days)</th>
<th>P-value</th>
<th>EFS T/C</th>
<th>Median RTV at End of Study</th>
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</tr>
</tbody>
</table>

#### 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 etoposide, 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-targeting agent, etoposide, 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.