Abstract C105

# Pediatric Preclinical Testing Program (PPTP) Stage 1 Evaluation of the CD56-Targeting Antibody-Drug Conjugate Lorvotuzumab Mertansine (IMGN901)

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## Lorvotuzumab Mertansine (IMGN901)

- Lorvotuzumab mertansine (LM, IMGN901) is an antibody-drug conjugate composed of the following:
  - The cytotoxic maytansinoid, DM1, a potent antimitotic agent that inhibits tubulin polymerization.
  - The humanized monoclonal antibody lorvotuzumab (huN901), which selectively binds to CD56 (NCAM1, neural cell adhesion molecule).
  - A disulfide linker covalently joining DM1 to lorvotuzumab (3-4 DM1 linked per antibody molecule).
- LM shows high level preclinical activity against CD56-expressing adult cancer xenografts.
- LM is currently in clinical trials for patients with CD56-positive cancers [e.g., small-cell lung cancer (SCLC), multiple myeloma, and Merkel cell carcinoma].
- The activity of LM was evaluated against the Pediatric Preclinical testing Program (PPTP) *in vitro* panel and against selected CD56-expressing xenografts from the PPTP *in vivo* panels.

## Methods

***In vitro***: *In vitro* testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system (Keshelava, et al. Methods Mol.Med., *110*: 139-153, 2005). LM and its cytotoxic moiety L-DM1-SMe were tested at concentrations ranging from 0.01 nM to 0.1 μM using the PPTP's standard 96 hour exposure period. Relative $IC_{50}$ ($rIC_{50}$) values were used as a measure of the potency of LM and L-DM1-SMe against the PPTP cell lines.

Immunohistochemistry for NCAM (CD56): Standard IHC methods were employed using anti-human CD56 (Novocastra, Clone 1B6, Cat# NCL-CD56-1B6). Staining intensity was scored (0=negative, 1=weak, 2=moderate, 3= strong) and uniformity of staining was scored (<25% of cells stained = focal, 25% to 75% of cells stained = heterogeneous, and >75% of cells stained = homogeneous).

***In vivo***: Standard PPTP methods for *in vivo* testing were employed (see http://pptp.nchresearch.org/documents.html). LM was tested against a subset of PPTP solid tumor xenografts at a dose of 15 mg/kg by the intravenous route using a weekly x 3 schedule with a total planned testing and observation period of 6 weeks. Models were selected for testing based on their CD56 expression levels.

Solid tumor testing: 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.

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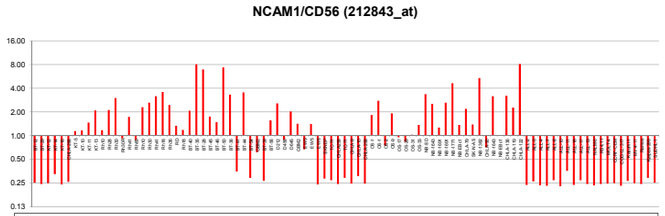
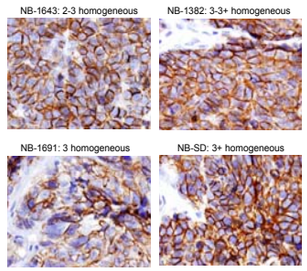
## NCAM1 (CD56) Expression for PPTP Cell Lines and Xenografts

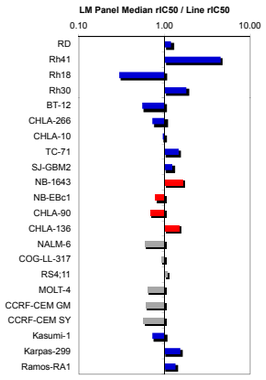


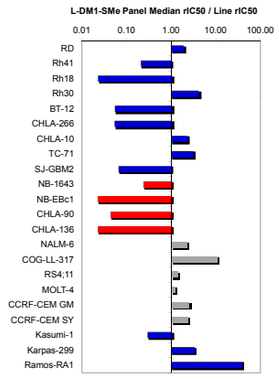




- CD56 RNA expression was highest for the neuroblastoma, rhabdomyosarcoma, and Wilms tumor panels.
- CD56 RNA expression was generally low for the rhabdoid tumor, Ewing sarcoma, and hematopoietic malignancy panels
- Neuroblastoma and rhabdomyosarcoma xenografts commonly showed 3-3+ homogeneous CD56 expression by IHC.

## Lorvotuzumab Mertansine *In Vitro* Activity

| Cell Line | LM rIC50 (nM) | L-DM1-SMe rIC50 (nM) | L-DM1-SMe rIC50/ LM rIC50 |
|---|---|---|---|
| RD | 27 | 1.3 | 0.05 |
| Rh41 | 7 | 12.0 | 1.67 |
| Rh18 | >100 | >100 | 1.00 |
| Rh30 | 18 | 0.6 | 0.03 |
| BT-12 | 59 | 46.1 | 0.78 |
| CHLA-266 | 45 | 47.0 | 1.04 |
| CHLA-9 | NE | NE | NE |
| CHLA-10 | 34 | 1.1 | 0.03 |
| CHLA-258 | NE | >100 | NE |
| TC-71 | 22 | 0.8 | 0.04 |
| SJ-GBM2 | 26 | 38.3 | 1.45 |
| NB-1643 | 20 | 10.7 | 0.53 |
| NB-EBc1 | 42 | >100 | 2.63 |
| CHLA-90 | 48 | 58.5 | 1.21 |
| CHLA-136 | 22 | >100 | 4.95 |
| NALM-6 | 56 | 1.1 | 0.02 |
| COG-LL-317 | 35 | 0.2 | 0.01 |
| RS4;11 | 30 | 1.8 | 0.06 |
| MOLT-4 | 51 | 2.0 | 0.04 |
| CCRF-CEM GM | 54 | 1.0 | 0.02 |
| CCRF-CEM SY | 58 | 1.0 | 0.02 |
| Kasumi-1 | 45 | 8.5 | 0.19 |
| Karpas-299 | 21 | 0.7 | 0.04 |
| Ramos-RA1 | 24 | 0.1 | 0.00 |
| **Median** | **35** | **2.0** | **0.05** |
| **Minimum** | **7** | **0.1** | **0.00** |
| **Maximum** | **>100** | **>100** | **4.95** |



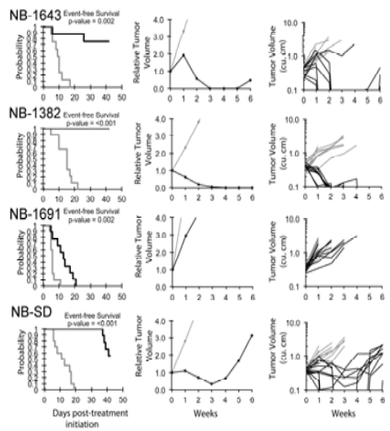




- The median $rIC_{50}$ for LM and L-DM1-SMe were 35 nm and 2 nM, respectively.
- LM showed higher activity against the neuroblastoma panel (red bars) compared to the ALL panel (gray bars), while L-DM1-SMe showed its highest activity against the ALL panel and low activity for the neuroblastoma panel. Bars to the right in the two COMPARE-like graphs indicate greater sensitivity to the test agent.
- For L-DM1-SMe, there was 79-fold greater potency against the ALL panel (median $rIC_{50}$ 1.1 nM) compared to the neuroblastoma panel (median $rIC_{50}$ 84 nM). By contrast, LM showed 1.7-fold greater potency against the neuroblastoma panel (median $rIC_{50}$ 32 nM) compared to the ALL panel (median $rIC_{50}$ 53 nM).

## Lorvotuzumab Mertansine *in Vivo* Activity

| Line | Histology | Median Time to Event | EFS T/C | Log-rank p-value | Tumor Volume T/C | Response | CD56 (NCAM) IHC | NCAM Expression Ratio[1] |
|---|---|---|---|---|---|---|---|---|
| KT-12 | Rhabdoid | 11 | 1.1 | 0.855 | 1.05 | PD1 | 2-3 HETERO | 0.25 |
| KT-10 | Wilms | > EP | > 2.7 | <0.001 | 0.08 | MCR | 3 HOMO | 1.17 |
| Rh28 | ALV RMS | 34.7 | 1.1 | 0.783 | 0.67 | PD1 | 3 HOMO | 2.12 |
| Rh30 | ALV RMS | > EP | > 3.3 | <0.001 | 0.14 | MCR | 3 HOMO | 3.01 |
| Rh41 | ALV RMS | > EP | > 1.9 | 0.008 | 0.59 | MCR | 3 HOMO | 1.73 |
| Rh41R | ALV RMS | 25.4 | 2.5 | <0.001 | 0.4 | PD2 | 3 HOMO | 1.73 |
| Rh18 | EMB RMS | 11.9 | 0.9 | 0.570 | 1.45 | PD1 | 3 HOMO | 3.58 |
| Rh36 | EMB RMS | > EP | > 4.8 | <0.001 | 0.55 | SD | 3 HOMO | 2.45 |
| NB-SD | Neuroblastoma | > EP | > 3.6 | <0.001 | 0.39 | CR | 3+ HOMO | 3.37 |
| NB-1771 | Neuroblastoma | 15.1 | 1.6 | <0.001 | 0.55 | PD2 | 3+ HOMO | 4.63 |
| NB-1691 | Neuroblastoma | 12.6 | 2.3 | 0.002 | 0.54 | PD2 | 3 HOMO | 1.27 |
| NB-EBc1 | Neuroblastoma | 7.6 | 1.7 | <0.001 | 0.32 | PD2 | 3 HETERO | 1.37 |
| CHLA-79 | Neuroblastoma | 11.8 | 2 | <0.001 | 0.39 | PD2 | 3-3+ HOMO | 2.19 |
| NB-1643 | Neuroblastoma | > EP | > 4.5 | 0.002 | 0.57 | CR | 2-3 HOMO | 2.53 |
| NB-1382 | Neuroblastoma | > EP | > 2.8 | <0.001 | 0.21 | MCR | 3-3+ HOMO | 5.39 |
| OS-1 | Osteosarcoma | > EP | > 1.7 | <0.001 | 0.28 | SD | 3 HETERO | 1.83 |
| OS-9 | Osteosarcoma | 28.9 | 1.3 | 0.027 | 0.83 | PD1 | 1 FOCAL | 1.92 |
| OS-33 | Osteosarcoma | > EP | > 1.7 | <0.001 | 0.29 | SD | 2 HETERO | 1.37 |
| OS-31 | Osteosarcoma | > EP | > 2.3 | <0.001 | 0.34 | PD2 | 3 HETERO | 1.03 |

[1] NCAM1 expression data are from Affymetrix U133 Plus 2.0 arrays (probe set 212843_at). The NCAM1 expression ratio is the expression value for the xenograft to the median expression value for all PPTP xenografts and cell lines. Molecular characterization of the PPTP models is in Neale, et al. (Clin Cancer Res 2008;14:4572-83).




## *In Vivo* Results Summary &Conclusions

- Lorvotuzumab mertansine (LM) induced significant differences in EFS distribution compared to control in 15 of 18 (83%) of the evaluable solid tumor xenografts, including all 7 neuroblastoma xenografts.
- Objective responses were observed in 6 of 18 (33%) solid tumor xenografts [2 complete responses (CR) and 4 maintained CR (MCR)]:
  - 3 of 7 neuroblastoma xenografts
  - 2 of 5 rhabdomyosarcoma xenografts
  - One Wilms tumor xenograft
- Each of the 6 xenografts achieving CR or MCR had homogeneous staining by IHC for NCAM (CD56) with expression levels of 3 or 3+ (excepting NB-1643 which had 2-3 level homogeneous staining).
- High NCAM (CD56) expression was not sufficient for response to LM treatment, as some xenografts with 3 or 3+ homogenous staining did not show tumor regression.
- Comparison of LM *in vivo* activity to that previously described for vincristine showed that neuroblastoma xenografts with data for both agents were more responsive to LM than to vincristine.
- LM demonstrated target-directed activity *in vitro* and promising activity *in vivo* against CD56-expressing childhood cancers.

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