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Initial Testing (Stage 1) of the Antibody-Maytansinoid Conjugate, IMGN901 (Lorvotuzumab Mertansine), by the Pediatric Preclinical Testing Program

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Abstract

Background—IMGN901 (lorvotuzumab mertansine) is an antibody-drug conjugate composed of a humanized antibody that specifically binds to CD56 (NCAM, neural cell adhesion molecule) and that is conjugated to the maytansinoid, DM1 (a microtubule targeting agent).

Procedures—IMGN901 and DM1-SMe (unconjugated DM1 as a mixed disulfide with thiomethane to cap its sulfhydryl group) were tested *in vitro* at concentrations ranging from 0.01 nM to 0.1 μM and 0.3 pM to 3 nM, respectively. IMGN901 was tested against a subset of PPTP solid tumor xenografts focusing on those with high CD56 expression. The combination of IMGN901 with topotecan was also evaluated.

Results—Neuroblastoma models expressed CD56 at or above the median expression level for all PPTP xenografts and cell lines. Neuroblastoma cell lines demonstrated relatively low sensitivity to DM1-SMe compared to other cell lines, but the sensitivity of neuroblastoma cell lines to

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IMGN901 was comparable to that of non-neuroblastoma cell lines. *In vivo*, objective responses were observed in 9 of 24 (38%) models including, 3 of 7 neuroblastoma xenografts, and 2 of 7 rhabdomyosarcoma xenografts. All xenografts with objective responses showed homogeneous high-level staining by IHC for CD56, but not all xenografts with homogenous high-level staining had objective responses. Combined with topotecan, IMGN901 demonstrated therapeutic enhancement against 2 of 4 neuroblastoma models.

Conclusions—IMGN901 has anti-tumor activity against some CD56 expressing pediatric cancer models. High expression of CD56 is a biomarker for *in vivo* response, but resistance mechanisms to IMGN901 in some high CD56 expressing lines need to be defined.

Keywords

Preclinical Testing; Developmental Therapeutics; antibody-maytansinoid conjugate; microtubules

INTRODUCTION

IMGN901 (lorvotuzumab mertansine) is an antibody-drug conjugate (ADC) consisting of the antimetabolic maytansinoid, DM1, and a humanized monoclonal antibody (huN901) targeting CD56, joined through a disulfide linker [1-4]. ADCs exploit the binding specificity of monoclonal antibodies to target the antitumor activity of potent cytotoxics. On binding to the target tumor cell the ADC is internalized, the antibody-drug conjugate is then cleaved inside the cell, leading to localized and enhanced antitumor activity while reducing exposure of healthy tissues and limiting systemic toxicities [5].

DM1 is a synthetic derivative of maytansine, an *ansa* macrolide that binds to tubulin at the vinca alkaloid-binding site leading to inhibition of microtubule assembly and cell proliferation and to cell death[2,6,7]. Although early phase trials of maytansine showed broad anti-tumor activity, further development of the drug was halted after patients experienced severe gastrointestinal and neurological dose limiting toxicities [2,8-11]. The development of maytansinoids containing a free sulfhydryl group permitted conjugation to antibodies thus allowing targeted delivery of the cytotoxic to tumor and a reduction in systemic toxicities [6]. The feasibility of the ADC approach also improved with ongoing optimization of linkers that were sufficiently stable in blood to stay conjugated to a high maytansinoid payload, while allowing efficient internalization and intracellular release of the cytotoxic and its metabolites [12,13].

Several antibody-maytansinoid conjugates are now in clinical development. The lead compound is trastuzumab emtansine (T-DM1), an ADC designed for treatment of HER2-positive cancers[14]. A randomized phase 3 study comparing trastuzumab emtansine monotherapy to lapatinib and capecitabine for HER2-positive locally advanced or metastatic breast cancer (MBC) previously treated with trastuzumab and a taxane reported superior progression free survival and lower toxicity for the trastuzumab emtansine arm [15].

CD56 (NCAM, neural cell adhesion molecule) is a member of the immunoglobulin superfamily of cell surface adhesion glycoproteins and was originally identified due to its role in neural cell adhesion and migration [16,17]. It is also expressed in non-neural tissue

including natural killer cells [18] and cardiomyocytes [19] where it regulates homophilic and heterophilic cell-cell and cell-matrix interactions [20]. CD56 is highly expressed on a variety of adult human tumors including small-cell lung cancer (SCLC) [21], multiple myeloma (MM) [22], ovarian carcinoma [23], subtypes of lymphoma and leukemia, and neuroendocrine tumors such as Merkel cell carcinoma (MCC) [24]. Although CD56 is also expressed in some normal tissues, early phase clinical trials of IMGN901 have demonstrated safety, tolerability and clinical activity when used alone and in combination against CD56-positive disease [3,19].

CD56 is expressed by several cancers affecting pediatric populations including neuroblastoma, rhabdomyosarcoma, Wilms tumor, acute myeloid leukemia, glioma, and astrocytoma[25-29]. As the clinical activity of ADCs are dependent on expression of the target antigen, we investigated the *in vitro* preclinical activity of IMGN901 by comparing it against DM1-SMe, an unconjugated form of the maytansinoid in IMGN901, and by examining the *in vivo* anti-tumor activity of IMGN901 in xenograft models with high level expression of CD56. As antimicrotubule agents have demonstrated interesting synergy with camptothecin-based topoisomerase I poisons[30], we also evaluated the combination of IMGN901 and topotecan against several neuroblastoma models.

MATERIALS AND METHODS

In vitro testing: *In vitro* testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multi-well plates [31]. IMGN901, and its unconjugated cytotoxic moiety DM1-SMe[5], were both tested against the PPTP cell line panel at concentrations ranging from 0.01 nM to 0.1 μ M as previously described [32]. Absolute IC₅₀ values represent the concentration of IMGN901 that reduces cell survival to 50% of the control value, while relative IC₅₀ (rIC₅₀) values represent the concentration of test agent that reduces cell survival by 50% of the maximum effect [33]. Relative In/Out (I/O)% values represent the percentage difference between the Y_{min} value (the minimum T/C% value) and the estimated starting cell number and either the control cell number (for agents with Y_{min}> starting cell number) or 0 (for agents with Y_{min}< estimated starting cell number). Relative I/O% values range between 100% (no treatment effect) to -100% (complete cytotoxic effect), with a Relative I/O% value of 0 being observed for a completely effective cytostatic agent.

In vivo tumor growth inhibition studies

CB17SC *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), and neuroblastoma. 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. Ten mice were used in each control or treatment group. Tumor volumes (cm³) were determined as previously described [34] and responses were determined using three activity measures as

previously described [34]. 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 ProcStatXact 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. For the combination drug studies, therapeutic enhancement was determined as previously described [35].

Drugs and Formulation

IMGN901 was provided to the PPTP by ImmunoGen, Inc., through the Cancer Therapy Evaluation Program (NCI). IMGN901 was tested 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. For subsequent combination testing with topotecan, IMGN901 was tested using the same dose and schedule. Topotecan was administered at 0.6 mg/kg daily days 1-5 and 8-13, and IMGN901 was administered days 1, 8 and 15. Mice were observed for up to 12 weeks.

Immunohistochemical Studies

Formalin-fixed paraffin-embedded samples from 30 solid tumor xenograft models were sectioned and stained for CD56 expression at ImmunoGen, Inc. to determine appropriate xenograft models for *in vivo* testing with IMGN901. The staining protocol involved heat-induced antigen retrieval using Reveal buffer (Biocare Medical, Cat# RV1000G1), blocking with 2% horse serum, and staining with 0.75 µg/mL anti-human CD56 (Novocastra, Clone 1B6, Cat# NCL-CD56-1B6). Controls included staining of known CD56-positive and negative FFPE cell pellets.

RESULTS

IMGN901 *in vitro* testing

IMGN901 and DM1-SMe were tested against the PPTP's *in vitro* cell line panel at concentrations ranging from 0.01 nM to 0.1 µM and 0.3 pM to 3 nM, respectively, using the PPTP's standard 96 hour exposure period as previously described [32]. The median rIC₅₀ for IMGN901 and DM1-SMe were 35 nM and 0.06 nM, respectively (**Table I**). The two agents had distinctive activity patterns, with the neuroblastoma panel showing significantly higher rIC₅₀ values compared to non-neuroblastoma cell lines for DM1-SMe (2.5 nM and 0.039 nM, respectively), but comparable rIC₅₀ values to other cell lines for IMGN901 (36 nM and 35 nM, respectively).

A metric used to compare the relative responsiveness of the PPTP cell lines to test agents is the ratio of the median rIC₅₀ of the entire panel to that of each cell line (**Table I**). Higher ratios are indicative of greater sensitivity to IMGN901. In **Figure 1A** bars to the right represent cell lines with greater sensitivity, while bars to the left indicate cell lines with lesser sensitivity. Both the rhabdomyosarcoma and neuroblastoma panels have cell lines with greater sensitivity than the panel median, while the ALL cell lines are generally less

sensitive than the panel median. **Figure IB** shows the rIC_{50} values for DM1-SMe for the cell lines of the PPTP panel. In contrast to IMG901, the cell lines of the ALL panel show the greatest sensitivity to DM1-SMe (bars to the right for NALM-6 through CCRF-CEM), while the neuroblastoma cell lines are the least sensitive to this agent (bars to the left for NB-1643 through CHLA-136). The relationship between rIC_{50} and CD56 expression as assessed by Affymetrix U133 Plus 2.0 array data was also examined (**Table I**). The cell lines with the highest CD56 RNA expression (Rh41, NB-1643, and CHLA-136) showed rIC_{50} values well below the median rIC_{50} for the panel. However, rIC_{50} values were similar for the cell lines with high CD56 RNA expression (expression values at least 3-fold higher than the minimum expression value) compared to the remaining cell lines with low expression (33 nM and 40 nM, respectively).

Another component of the *in vitro* response to test agents is the maximal effect at high concentrations, as assessed by Relative In/Out%. For DM1-SMe, there was a marked difference in Relative In/Out% values for the ALL cell lines and for some solid tumor cell lines (**Table I**). The ALL cell lines showed values approaching –100%, which indicate a profound cytotoxic effect. By contrast, some solid tumor cell lines (RD, Rh30, CHLA-9, and CHLA-10) showed plateau Relative In/Out% values at higher DM1-SMe concentrations greater than 0%, which is consistent with a cytostatic response during the 96-hour test period. The concentration range studied for IMG901 did not allow us to assess for a plateau effect.

Immunohistochemical studies

Sections from 30 xenograft models were stained for expression of CD56, with all 30 of the tumors positive for CD56 (**Supplemental Table I**). In addition, 28 of the tumors were strong positive, with staining levels of 2-3, 3, or 3+ and with either a heterogeneous or homogeneous staining pattern. Homogeneous strong staining was common for neuroblastoma (6 of 7), rhabdomyosarcoma (6 of 7), and Wilms tumor (3 of 3) models.

IMG901 *in vivo* testing

IMG901 was tested against a subset of PPTP solid tumor xenografts based on their CD56 expression using a dose of 15 mg/kg by the intravenous route using a weekly x 3 schedule. IMG901 was well tolerated with 9 of 248 treated mice dying (3.6%) compared to 0 of 246 control mice (0%). All 24 xenograft models studied were considered evaluable for efficacy. Complete details of testing are provided in **Supplemental Table II**, 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. Tail vein necrosis occurred in one xenograft (Rh30) requiring retesting.

IMG901 induced significant differences in EFS distribution compared to control in 18 of 24 (75%) of the evaluable solid tumor xenografts, including all 7 neuroblastoma xenografts, **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. IMG901 induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity in 5 of 19 (26%) and high activity in 6 of 19 (32%) evaluable solid tumor xenografts. Intermediate or high activity for the EFS T/C metric was observed in 4 of 7 neuroblastoma xenografts and 4 of 7 rhabdomyosarcoma xenografts.

Objective responses were observed for 9 of 24 (38%) solid tumor xenografts, including 2 complete responses (CR) and 6 maintained CRs (MCR). CR/MCR responses were observed for 2 of 2 Wilms tumors, 1 of 1 medulloblastoma xenografts, 3 of 7 neuroblastoma xenografts and 2 of 7 rhabdomyosarcoma xenografts. The *in vivo* testing results for the objective response measure of activity are presented in **Figure 2** in a heat-map format as well as a COMPARE-like format, based on the scoring criteria described in the Supplemental Response Definitions section. The latter analysis demonstrates relative tumor sensitivities around the midpoint score of 0 that represents stable disease.

The relationship between CD56 expression (both by IHC and Affymetrix U133 Plus 2 profiling) and *in vivo* activity is shown in **Supplemental Table III**. Affymetrix U133 Plus 2.0 array data, was generally concordant with the protein expression level, and all of the responding neuroblastoma xenografts showed RNA expression levels that were at least 2-fold above the median expression level for the entire PPTP panel. Each of the 9 xenografts achieving an objective response showed homogeneous staining by IHC for CD56 with expression levels of 2-3, 3 or 3+. Other xenografts also showed 3 or 3+ homogeneous staining but did not show tumor regression in response to IMG901 treatment (e.g., Rh10, Rh28, Rh18, Rh36, NB-1771, and NB-1691). Representative responses for neuroblastoma xenografts and their respective CD56 expression by IHC are presented in **Figure 3**.

Vincristine is used in the treatment of the histotypes that showed responses to IMG901, including high-risk neuroblastoma induction in both the COG and SIOP cooperative groups and standard regimens for Wilms tumor and rhabdomyosarcoma. As IMG901 is also an antimitotic agent, we compared the objective response results for IMG901 to those previously obtained for vincristine in the “heat map”, **Supplemental Figure 1**. For the entire neuroblastoma panel, the objective response activity of IMG901 was greater than that of vincristine for xenografts with data available for both agents. The neuroblastoma xenograft NB-1643 showed a CR to IMG901 but only a PD1 response to vincristine. The Wilms tumor xenograft KT-10 also responded better to IMG901 than vincristine (MCR versus PD2, respectively), while the rhabdomyosarcoma xenograft Rh28 showed the opposite result, with an MCR response to vincristine and a PD1 response to IMG901.

Testing of IMG901 combined with topotecan

Previously we noted synergistic activity in models of sarcoma when the tubulin-binding agent vincristine was administered in combination with topotecan or irinotecan, both topoisomerase I poisons derived from camptothecin[30,36]. To test whether IMG901 showed therapeutic enhancement when administered with topotecan, we selected four neuroblastoma models that are refractory to treatment with vincristine [37], but showed sensitivity to two cycles of topotecan [38]. In the current study, topotecan was given for a single cycle (days 1-5 and 8-13) and IMG901 was administered days 1, 8 and 15. Mice were observed for up to 12 weeks. Tumor bearing mice received IMG901, topotecan as

single agents or in combination. Complete details are given in **Supplemental Table IV**. The combination treatment was significantly better than IMGN901 against all four neuroblastoma models and superior to topotecan against three models. The combination demonstrated ‘Therapeutic Enhancement’ (i.e., significant differences in EFS distribution for the combination compared to both agents used alone) against NB-1691 and NB-EBc1 models but not against NB-SD and NB-1771 neuroblastoma xenografts (**Figure 4**, and **Supplemental Table V**).

DISCUSSION

IMGN901 is an ADC that combines the pharmacological potency of the maytansinoid DM1 with the specificity of an anti-CD56 monoclonal antibody. IMGN901 has demonstrated activity in early phase adult clinical trials against CD56 positive malignancies such as Merkel cell carcinoma and SCLC[39,40]. Although CD56 is expressed in some normal tissues, localization of the highly potent cytotoxic payload to the CD56 antigen has been sufficient to reduce the systemic toxicities expected with a potent inhibitor of tubulin polymerization including nausea, diarrhea, and neuropathy [3,19]. As CD56 is expressed in several pediatric malignancies [25-29], we sought to provide preclinical evidence for IMGN901 activity against CD56-expressing PPTP cell lines and xenografts.

The PPTP *in vitro* results are reasonably comparable to those previously reported for DM1-SMe, for which adult cancer cell lines commonly have IC₅₀ values in the 10 to 40 pM range [5,6]. The PPTP cell lines appear somewhat less sensitive to IMGN901 than a CD56-expressing ovarian cancer cell line (OVCAR-3), which showed an IC₅₀ of approximately 4 nM [41]. The PPTP rIC₅₀ values for IMGN901 are also greater than those reported for other DM1--based antibody-drug conjugates such as trastuzumab-DM1 and huC242-SPPDM1 that show IC₅₀ values in the 0.1 to 3 nM range against cell lines over-expressing their target surface antigen [42,43].

Inherent in ADC design is a responder hypothesis that the target of the monoclonal antibody can serve as a biomarker to predict treatment response to the ADC. Consistent with this hypothesis, our *in vitro* data show that neuroblastoma and rhabdomyosarcoma cell lines that expressed high levels of CD56 showed the greatest evidence for sensitization by antibody targeting, as shown by lower ratios of IMGN901 rIC₅₀ to DM1-SMe rIC₅₀ (Table I, Column 6). The neuroblastoma cell lines all had low values for this ratio, consistent with *in vitro* cytotoxicity being significantly enhanced when DM1 is concentrated within an antigen expressing tumor cell. Conversely, ALL cell lines displayed approximately 1000-fold greater sensitivity to the cytotoxic moiety alone than to IMGN901, and were generally less sensitive to IMGN901 than other cell lines. It should be noted, however that overall the rIC₅₀ values for IMGN901 were similar for cell lines with high and low CD56 expression. A potential explanation is that CD56 expression is necessary, but not sufficient, for preferential sensitivity to IMGN901 as the benefit of targeting CD56 may be counterbalanced by the low intrinsic sensitivity to DM1-SMe of some CD56-expressing cell lines.

The *in vivo* studies were enriched for xenografts with high-level CD56 expression. IMGN901 produced a significant increase in EFS versus untreated controls in 18 of 24

xenograft models, with complete xenograft regression seen in 8 of 24 models (including neuroblastoma, 3 of 7; rhabdomyosarcoma, 2 of 7; and Wilms tumor, 2 of 2). Within this preselected group of xenografts with high-level CD56 expression, there was an inconsistent relationship of CD56 expression to treatment response. Of the 16 xenograft models with high-level homogenous CD56 expression (2-3, 3, or 3+), IMG901 achieved complete or partial responses in 9, stable disease in 2 models, PD2 responses in 3 models, and no response (PD1) in 2 models (Rh18, Rh28). We hypothesize that over a certain threshold, CD56 expression may become a weaker predictor of treatment response as other modifiers of treatment response become more prominent such as mitotic rate, chemoresistance to tubulin binding agents, and/or intracellular processing of IMG901. Alternatively, CD56/NCAM is variably expressed as different isoforms and with extensive post-translational modifications, and these factors may influence ADC binding and/or internalization. Support for further evaluation of IMG901 for Wilms tumor comes from a report describing Wilms tumor cancer stem cells (CSCs) that express CD56 and have a primitive undifferentiated-“blastema” phenotype [44]. Like the data presented herein, the report described Wilms tumor xenografts that showed complete responses to IMG901.

The combination of IMG901 with topotecan demonstrated little toxicity and therapeutic enhancement in 2 of 4 neuroblastoma models. The combination was significantly better than IMG901 alone against all the models and superior to topotecan in three neuroblastoma models, consistent with previous preclinical studies in which vincristine was combined with topotecan [30].

Development of IMG901 for pediatric malignancies will require ongoing positive results from adult clinical trials. However, the encouraging *in vivo* results presented here should prompt future work to refine a responder hypothesis and to develop rational combinatorial approaches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

In vitro activity of IMGN901 and DM1-SMe. *Left panel:* The median rIC_{50} ratio graph for IMGN901 shows the relative IC_{50} values for the cell lines of the PPTP panel. *Right panel:* The median rIC_{50} ratio graph for DM1-SMe shows the rIC_{50} values for the cell lines of the PPTP panel. For both graphs each bar represents the ratio of the panel IC_{50} to the IC_{50} value of the indicated cell line. Bars to the right represent cell lines with higher sensitivity, while bars to the left indicate cell lines with lesser sensitivity.

Figure 2.

IMGN901 *in vivo* objective response activity, 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.

A. IMGN901 activity against individual neuroblastoma xenografts. Kaplan-Meier curves for EFS, median relative tumor volume graphs, and individual tumor volume graphs are shown for selected lines, NB-1643, NB-1382, NB-1691, and NB-SD xenografts. Controls (gray lines); Treated (black lines). B. Immunohistochemical staining for CD56 in neuroblastoma xenografts. CD56 expression in NB-1643, NB-1382, NB-1691, and NB-SD xenografts, as determined by immunostaining as described in Materials and Methods.

Figure 4. Individual tumor growth curves for Control (untreated), topotecan (0.6 mg/kg days 1-5, 8-13), IMGN901 (15 mg/kg days 1,8,15) or the combination. The relative tumor volume plots (far right panels) show the median tumor volumes for each group relative to the start of treatment. Control (○), topotecan (▲), IMGN901 (◆), combination (□).

Table 1

In vitro activity of IMG901 and DM1-SMe against PPTP *in vitro* cell lines

Cell Line	Histotype	IMG901 MC50 (nM)	IMG901 Panel rIC ₅₀ /Line rIC ₅₀	DM1-SMe rIC50 (nM)	DM1-SMe Panel rIC ₅₀ /Line rIC ₅₀	IMG901 rIC ₅₀ /DM1-SMe rIC50	DM1-SMe Relative In/Out (Observed * Ymin)	Relative CD56 expression **
RD	Rhabdomyosarcoma	28	1.2	0.042	1.45	677	23%	5.8
Rh41	Rhabdomyosarcoma	7	4.8	0.380	0.16	19	-13%	13.8
Rh18	Rhabdomyosarcoma	110	0.3	>3.00	0.02	<35	51%	5.1
Rh30	Rhabdomyosarcoma	35	1.0	0.019	3.24	1865	4%	11.4
BT-12	Rhabdoid	59	0.6	1.473	0.04	40	14%	1.1
CHLA-266	Rhabdoid	45	0.8	1.903	0.03	24	23%	1.1
TC-71	Ewing sarcoma	22	1.6	0.026	2.38	874	-85%	1.3
CHLA-9	Ewing sarcoma	8	4.2	0.002	34.59	4693	42%	1.1
CHLA-10	Ewing sarcoma	34	1.0	0.036	1.70	955	9%	1.3
CHLA-258	Ewing sarcoma	NE	NE	>3.00	<0.02	NE	75%	1.1
SI-GBM2	Glioblastoma	33	1.1	1.200	0.05	27	23%	6.2
NB-1643	Neuroblastoma	20	1.7	0.341	0.18	59	-4%	13.8
NB-EBc1	Neuroblastoma	63	0.5	>3.00	<0.02	<20	57%	4.3
CHLA-90	Neuroblastoma	49	0.7	1.820	0.03	27	21%	3.1
CHLA-136	Neuroblastoma	23	1.5	>3.00	<0.02	<7	29%	13.9
NALM-6	ALL	56	0.6	0.034	1.80	1644	-93%	1.0
COG-LL-317	ALL	36	1.0	0.007	8.28	4860	-100%	1.0
RS4;11	ALL	30	1.1	0.058	1.05	527	-91%	1.1
MOLT-4	ALL	51	0.7	0.064	0.95	803	-98%	1.1
CCRF-CEM (1)	ALL	54	0.6	0.030	2.06	1840	-99%	1.1
CCRF-CEM (2)	ALL	58	0.6	0.031	1.94	1844	-99%	1.1
Kasumi-1	AML	45	0.8	0.313	0.19	145	-22%	1.2
Karpas-299	ALCL	22	1.6	0.024	2.59	917	-70%	1.3
Ramos-RA1	NHL	24	1.4	0.002	29.65	11839	-100%	1.1

Cell Line	Histotype	IMGN901 MC50 (nM)	IMGN901 Panel rIC ₅₀ /Line rIC ₅₀	DM1-SMe rIC50 (nM)	DM1-SMe Panel rIC ₅₀ /Line rIC ₅₀	IMGN901 rIC ₅₀ /DM1-SMe rIC50	DM1-SMe Relative In/Out (Observed Ymin) [*]	Relative CD56 expression ^{**}
Median		35	1.0	0.06	1.00	677	0%	1.2
Minimum		7	0.3	0.00	<0.02	7	-100%	1.0
Maximum		110	4.8	>3.00	34.59	11839	75%	13.9

^{*} Bolded cells indicate cell lines that show model-estimated Rel In/Out% > 0%.

^{**} Relative CD56/NCAM1 expression is based on Affymetrix U133 Plus 2.0 results using probeset“212843_at” and is the ratio of the expression value for the cell line to that of the cell line with the lowest expression value.

Table II

Activity of IMG901 against selected Solid Tumor Xenografts

Xenograft	Tumor Type	Median Time to Event	P-value	EFS T/C ³	Median RTV at End of Study	Tumor Volume T/C ¹	Median Group Response ²	T/C Volume Activity	EFS Activity ³
KT-12	Rhabdoid	11.0	0.855	1.1	>4	1.05	PD1	Low	Low
KT-10	Wilms	> EP	<0.001	>2.7	0.0	0.08	MCR	High	High
KT-11	Wilms	> EP	<0.001	>2.6	0.0	0.08	MCR	High	High
Rh10	ALV RMS	27.8	0.389	1.3	>4	0.72	PD1	Low	Low
Rh28	ALV RMS	34.7	0.783	1.1	>4	0.67	PD1	Low	Low
Rh30	ALV RMS	> EP	<0.001	>3.3	0.0	0.14	MCR	High	High
Rh41 ^a	ALV RMS	25.4	<0.001	2.5	>4	0.40	PD2	Int	Int
Rh65	ALV RMS	> EP	<0.001	>2.7	0.0	0.09	MCR	High	High
Rh18	EMB RMS	11.9	0.570	0.9	>4	1.45	PD1	Low	Low
Rh36	EMB RMS	> EP	<0.001	>4.8	2.3	0.55	SD	Low	Int
BT-50	Medulloblastoma	> EP	0.033	-	0.0	0.12	MCR	High	NE
BT-36	Ependymoma	> EP	0.474	-	0.5	0.47	SD	Low	NE
BT-44	Ependymoma	> EP	1.000	-	0.8	0.23	PR	Int	NE
NB-SD	Neuroblastoma	> EP	<0.001	>3.6	3.1	0.39	CR	Int	Int
NB-1771	Neuroblastoma	15.1	<0.001	1.6	>4	0.55	PD2	Low	Low
NB-1691	Neuroblastoma	12.6	0.002	2.3	>4	0.54	PD2	Low	Int
NB-EBc1	Neuroblastoma	7.6	<0.001	1.7	>4	0.32	PD2	Int	Low
CHLA-79	Neuroblastoma	11.8	<0.001	2.0	>4	0.39	PD2	Int	Low
NB-1643	Neuroblastoma	> EP	0.002	>4.5	0.5	0.57	CR	Low	High
NB-1382	Neuroblastoma	> EP	<0.001	>2.8	0.0	0.21	MCR	Int	High
OS-1	Osteosarcoma	> EP	<0.001	>1.7	0.6	0.28	SD	Int	NE
OS-9	Osteosarcoma	28.9	0.027	1.3	>4	0.83	PD1	Low	Low
OS-33	Osteosarcoma	> EP	<0.001	>1.7	0.8	0.29	SD	Int	NE
OS-31	Osteosarcoma	> EP	<0.001	>2.3	1.9	0.34	PD2	Int	Int

^aRepeat experiment.

¹ *Tumor Volume T/C value:* Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days). The T/C value is the mean RTV for the treatment group divided by the mean RTV for the control group. High activity = $T/C \geq 0.15$; Intermediate activity = $T/C \geq 0.45$ but < 0.15 ; and Low activity = $T/C < 0.45$.

² Objective response measures are described in detail in the Supplemental Response Definitions. PD1 = progressive disease with EFS $T/C \leq 1.5$, and PD2 = progressive disease with EFS $T/C > 1.5$.

³ EFS T/C values = the ratio of the median time to event of the treatment group and the median time to event of the respective control group. High activity requires: a) an EFS $T/C > 2$; b) a significant difference in EFS distributions, and c) a net reduction in median tumor volume for animals in the treated group at the end of treatment as compared to at treatment initiation. Intermediate activity = criteria a) and b) above, but not having a net reduction in median tumor volume for treated animals at the end of the study. Low activity = EFS $T/C < 2$.