

# Abstract 558 Pediatric Preclinical Testing Program (PPTP) evaluation of the fully human anti-IGF-1R antibody IMC-A12



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## Abstract

**Background:** IMC-A12 is a fully human IgG1 monoclonal antibody that specifically blocks the insulin-like growth factor 1 receptor (IGF-1R). IGF-1R signaling may be especially important in the childhood cancer setting, with preclinical data supporting its role in the growth and survival of multiple pediatric cancers. The activity of IMC-A12 was evaluated against the *in vitro* and *in vivo* panels of the PPTP.

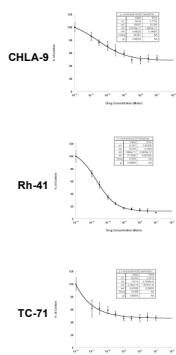
**Methods:** The PPTP includes a molecularly characterized *in vitro* panel of cell lines (n=27) and *in vivo* panel of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood ALL. IMC-A12 was tested against the PPTP *in vitro* panel at concentrations from 0.01 nM to 100 nM using culture medium supplemented with 20% FBS. It was tested against the PPTP *in vivo* panels at a dose of 1 mg per mouse administered twice weekly for six weeks via I.P. injection. IMC-A12 was not evaluated against the ALL *in vivo* panel. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event (4-fold increase in tumor volume) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).

**Results:** IMC-A12 induced 50% or greater *in vitro* growth inhibition in 3 of 23 cell lines (1 rhabdomyosarcoma and 2 Ewing sarcoma cell lines). IMC-A12 significantly increased event-free survival in 24 of 34 (71%) solid tumor xenograft models with tumor regressions in one rhabdomyosarcoma (RMS) model (maintained complete response). Although objective responses were not noted in the remaining RMS or osteosarcoma panels, tumor progression was significantly delayed with EFS T/C values > 2 for 9 out of 11 (82%) models. Using the time to event activity measure, IMC-A12 had intermediate (n=13) or high (n=1) activity against 33 evaluable xenografts, including xenografts from the rhabdoid tumor (1 of 3), Ewing (1 of 5), rhabdomyosarcoma (6 of 6), glioblastoma (1 of 4), neuroblastoma (2 of 5), and osteosarcoma panels (3 of 5).

**Conclusions:** IMC-A12 demonstrated broad antitumor activity against the PPTP's *in vivo* solid tumor panels. Further studies characterizing molecular predictors of response, as well as the activity of combinations of IMC-A12 with other agents are anticipated. (Supported by NCI N01CM42216)

## IMC-A12 In Vitro Activity

- 3 PPTP cell lines (2 Ewing sarcoma and 1 rhabdomyosarcoma) showed T/C values < 50% at the highest tested IMC-A12 concentration.
- The concentration-response curves for these 3 responsive cell lines are consistent with an anti-proliferative effect, similar to the *in vivo* effect reported for other anti-IGF-1R MABs.



## Methods for PPTP In Vivo Testing

Stage 1 testing involves testing an agent across the entire PPTP panel of childhood cancer xenograft lines at its MTD or at a dose selected based on PK/PD studies using adult preclinical models.

**Solid tumor testing:** For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm<sup>3</sup>. Two perpendicular tumor diameters were measured at once weekly intervals with digital vernier calipers. Assuming tumors to be spherical, volumes were calculated from the formula (π/6)xd<sup>3</sup>, where d represents the mean diameter.

**Drug:** IMC-A12 was provided by ImClone Systems (New York City, NY). IMC-A12 was administered intraperitoneally twice weekly for 6 consecutive weeks at a dose of 1mg per animal. IMC-A12 was provided to each testing site in coded vials for blinded testing according to the PPTP's standard operating procedures.

### Solid Tumor Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	>25% ↑ in tumor volume, TGD value ≤1.5	0
PD2 (Progressive Disease 2)	>25% ↑ in tumor volume, TGD value >1.5	2
SD (Stable Disease)	<25% ↑ in tumor volume, <50% regression	4
PR (Partial Response)	≥50% regression, but no CR	6
CR (Complete Response)	<0.1 cm <sup>3</sup> tumor volume	8
MCR (Maintained CR)	<0.1 cm <sup>3</sup> tumor volume at the end of study 10	10

**Median Group Response:** Each individual mouse in the treatment group was assigned a response score (see Tables above) and a median score for the treatment group was calculated and then each treatment group was assigned an overall response according to the table below.

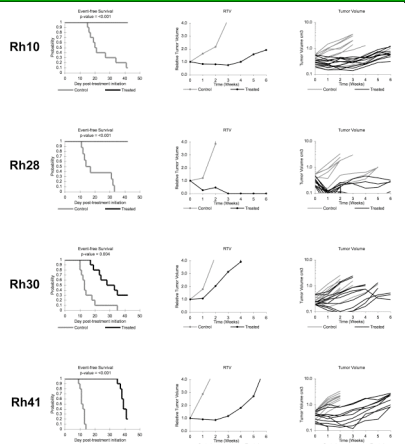
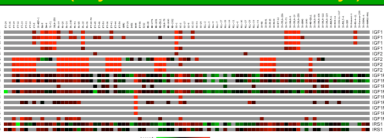
If Median Score (MS) from (1):	Overall Group Response
0 = MS 01	PD1
1 = MS 03	PD2
3 = MS 05	SD
5 = MS 07	PR
7 = MS 09	CR
9 = MS	MCR

**Statistical Methods:** Event-free survival (EFS) distributions of each treatment group were compared to the EFS distribution of the respective control group using the exact log rank test. P-values were 2-sided & were not adjusted for multiple comparisons given the exploratory nature of this study. P-values < 0.05 were considered to be significant.

## IMC-A12 In Vivo Activity

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	Overall Group Response
BT-29	Rhabdoid	<0.001	1.4	>4	0.66	0.023	PD1
KT-14	Rhabdoid	<0.001	>2.3	1.4	0.34	<0.001	PD2
KT-12	Rhabdoid	0.034	1.2	>4	0.81	0.165	PD1
KT-10	Wilms	0.006	1.6	>4	0.63	<0.001	PD2
KT-11	Wilms	0.056	1.4	>4	0.66	0.359	PD1
KT-13	Wilms	0.282	1.3	>4	0.74	0.436	PD1
SK-NEP-1	Ewing	0.043	1.2	>4	0.69	0.083	PD1
EW5	Ewing	<0.001	2.6	>4	0.39	<0.001	PD2
EW8	Ewing	0.032	1.2	>4	0.62	<0.004	PD1
TC-71	Ewing	0.698	1	>4	0.88	0.393	PD1
CHLA258	Ewing	0.03	1.3	>4	0.67	0.052	PD1
Rh10	ALV RMS	<0.001	>2.1	1.9	0.24	<0.001	PD2
Rh28	ALV RMS	<0.001	>2.8	0	0.15	<0.001	MCR
Rh30	ALV RMS	0.004	2.3	>4	0.41	0.007	PD2
Rh36R	ALV RMS	<0.001	2.4	>4	0.44	<0.001	PD2
Rh41	ALV RMS	<0.001	3.3	>4	0.17	<0.001	PD2
Rh18	EMB RMS	<0.001	3	>4	0.33	<0.001	PD2
BT-45	Medulloblastoma	0.612	1.1	>4	0.81	0.739	PD1
BT-50	Medulloblastoma	0.643	1	>4	1.04	0.684	PD1
BT-36	Ependymoma	1	1.8	0.89	0.853	0.02	PD2
GBM2	Glioblastoma	<0.001	1.4	>4	0.33	<0.001	PD1
BT-39	Glioblastoma	0.355	1.2	>4	0.84	0.529	PD1
D45	Glioblastoma	<0.001	2.3	>4	0.44	<0.001	PD2
D65	Glioblastoma	0.304	1.1	>4	0.67	0.28	PD1
NB-SD	Neuroblastoma	0.013	1.6	>4	0.58	<0.001	PD2
NB-1771	Neuroblastoma	<0.001	3.6	>4	0.12	<0.001	PD2
NB-1691	Neuroblastoma	0.268	1.4	>4	0.79	0.387	PD1
NB-EB1	Neuroblastoma	0.041	2	>4	0.42	0.01	PD2
NB-1643	Neuroblastoma	<0.001	4.2	>4	0.35	<0.001	PD2
OS-1	Osteosarcoma	<0.001	>2.3	3.4	0.47	<0.001	PD2
OS-2	Osteosarcoma	<0.001	2.1	>4	0.38	<0.001	PD2
OS-9	Osteosarcoma	<0.001	>2.3	3.1	0.41	<0.001	PD2
OS-33	Osteosarcoma	0.323	0.9	>4	1.02	0.481	PD1
OS-31	Osteosarcoma	0.367	1	>4	0.92	0.393	PD1

## Gene Expression for IGF-1, IGF-2, IGF-1R, and IRS-1 (Affymetrix HG-U133 Plus 2.0 Arrays)



## CONCLUSIONS

- IMC-A12 demonstrated limited activity against the PPTP *in vitro* panel, possibly due to the use of high serum concentrations.
- In vitro* activity was greatest for Ewing sarcoma and rhabdomyosarcoma cell lines.
- IMC-A12 demonstrated broad growth inhibitory activity against the PPTP *in vivo* solid tumor panels.
- The greatest activity observed was against rhabdomyosarcoma xenografts with complete remission observed for Rh28.
- Most PPTP solid tumor xenografts and cell lines express IGF-1R at some level, but differ in their ligand expression: the rhabdomyosarcoma, Wilms, ependymoma, and neuroblastoma xenografts primarily express IGF-2 while the Ewing xenografts express IGF-1.
- There is no clear correlation between expression of IGF-1R and its ligands and IMC-A12 *in vitro* and *in vivo* activity.

IMC-A12 was provided to the PPTP by ImClone Systems. Supported by NCI N01CM42216

## PPTP In Vitro Testing Methods

**Methods:** *In vitro* testing was performed using DIMSCAN, a semi-automated fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multiwell plates (Keshelava, et al. Methods Mol Med, 110: 139-153, 2005). Testing was for 96 hours at concentrations from 0.01 nM to 0.1 μM with replicates of 6 per data point. Data were analyzed using Kaleidagraph (Synergy), fitting a non-linear regression, sigmoidal dose-response model to the response, relative fluorescence values vs. the concentration. The PPTP *in vitro* panel contains cell lines for neuroblastoma (4), Ewing sarcoma (4), rhabdomyosarcoma (4), ALL (5), NHL (2), and others. Testing used culture medium supplemented with 20% FBS.

<sup>1</sup> EFS and T/C estimated only for cell lines with T/C > 50% at highest IMC-A12 concentration tested.