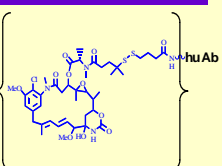


## ABSTRACT

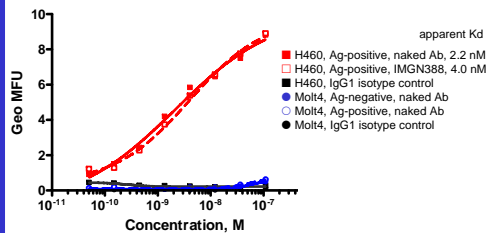
IMGN388 is an immunoconjugate composed of an integrin-targeting monoclonal antibody with the maytansinoid DM4, a potent cytotoxic agent, covalently attached. IMGN388 is being developed for the treatment of solid tumors. Its integrin target has been found by immunohistochemical staining to be present on a wide range of human solid tumors, with high expression observed in lung carcinomas, renal cell carcinomas, thyroid carcinomas, bladder carcinomas, melanomas, and sarcomas. The binding affinity of IMGN388 was in the range of 1 to 8 nM (EC50 values) for several human tumor cell lines, as determined by flow cytometry. The activity of IMGN388 has been evaluated in xenograft models in nude rats using a variety of human tumor cell lines. The antibody portion of IMGN388 does not cross-react with the murine integrin ortholog, but does bind to the rat ortholog, albeit at an affinity approximately 40-fold less than to the human integrin molecule. In one study, rats bearing established A549 human non-small cell lung tumors were treated with IMGN388 at 0.5, 1, 3, or 10 mg/kg, given weekly for six weeks. The response to IMGN388 was dose-dependent, with the minimum efficacious dose found to be 1 mg/kg. Tumor regressions were observed in animals treated at 1, 3 or 10 mg/kg with 5 complete responses and one partial response in the 7 animals treated at 10 mg/kg. IMGN388 also demonstrated efficacy against established human tumors of colon (HT-29), large cell lung (A460), pancreatic (AsPC-1), ovarian (A2780, SKOV-3), and breast (MDA-MB-231.OT.F2) carcinomas in nude rat models. Additionally, IMGN388 has been found to inhibit angiogenesis using an *in vivo* model of basic fibroblast growth factor-induced angiogenesis in nude rats. Thus, the anti-tumor effects of IMGN388 can be attributed to two distinct mechanisms of action: direct tumor-cell killing and anti-angiogenic activity. In conclusion, the broad expression of the target integrin among solid tumors and the observed anti-tumor efficacy of IMGN388 in xenograft models of pancreatic, colon, lung, breast, and ovarian carcinomas in rats support the clinical evaluation of IMGN388 for the treatment of solid tumors.

### Structure of IMGN388

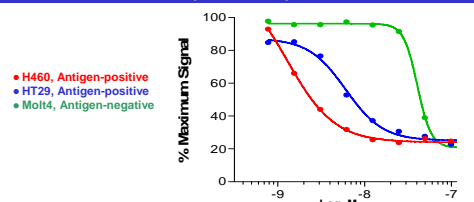


## 1) IMGN388 Retains Binding Affinity of Unconjugated Antibody

FACS binding comparison of unconjugated antibody vs IMGN388 on antigen-positive vs antigen-negative cells.



## 2) IMGN388 Demonstrates Antigen-Specific Cytotoxicity



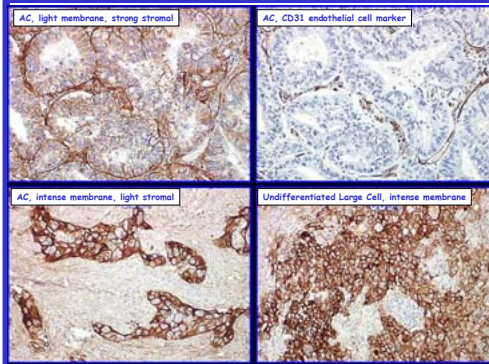
Cell Line	A460	HT29	Molt4
IMGN388 EC50	6.20 E-09	8.08 E-09	3.82 E-08

## 3) Antigen Target is Expressed in Many Tumor Types

Tumor type	# samples tested	# scored 3 or 4 (mottling)	% scored as 3 or 4
Breast carcinomas	21	8	38%
Ovarian carcinomas	22	6	27%
Prostate carcinomas	21	8	38%
Lung carcinomas	15	13	87%
Pancreatic carcinomas	10	4	40%
Renal Cell carcinomas	10	7	70%
Thyroid carcinomas	11	10	91%
Gastric carcinomas	10	3	30%
Esophageal carcinomas	10	2	20%
Head & Neck Tumors	12	7	58%
Bladder carcinomas	10	9	90%
Testicular tumors	10	3	30%
Basal Cell carcinomas	10	1	10%
Melanomas	11	7	64%
Sarcomas	10	8	80%
All Tumors	193	96	50%

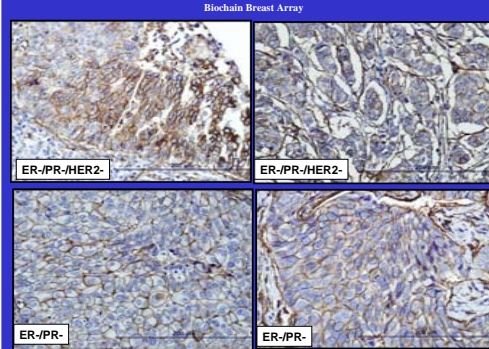
## 4) Lung Tumors Strongly Express Target

4/4 ACs, 7/9 SCCs had moderate or intense antigen staining



## 5) Breast Tumors Strongly Express Target

Staining of tumor cell membranes, stroma, & neovasculature.



## 6) ER-PR-/HER2- Breast Cancers Express Target

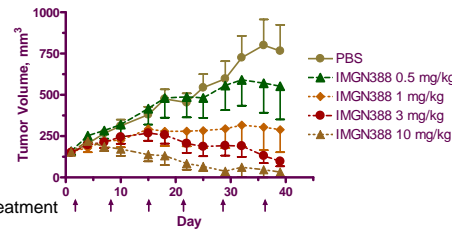
Biochain Breast Tissue Array - 67% Triple Negative Cancers have Intense/Moderate Staining

	Triple Negative	ER/PR Negative	HER2 Negative	PR/HER2 Negative	PR Negative	ER/HER2 Negative	Triple Positive
Total #	15	22	10	5	5	3	15
3 Homogeneous	4	7	1	0	1	0	4
3 Heterogeneous	0	2	0	1	2	0	2
2 Homogeneous	6	7	3	1	1	1	5
2 Heterogeneous	1	0	0	2	0	0	1
1 Homogeneous	1	3	1	1	1	0	2
Negative	2	2	3	0	0	1	1
Poor cores	1	1	2	0	0	1	0

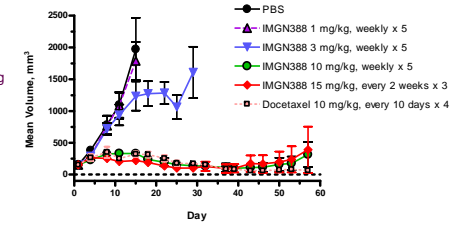
## 7) Anti-Tumor Activity of IMGN388 *in Vivo*

Nude rats bearing established subcutaneous human tumor xenografts were treated with IMGN388 as repeated IV bolus injections of 0.5 to 15 mg/kg (1 mg conjugate protein/kg, ~5.2 mg/m<sup>2</sup> in rat, equivalent to 15 µg linked DM4/kg).

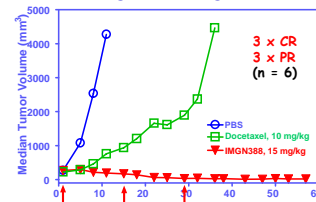
### A549, Human Lung Carcinoma



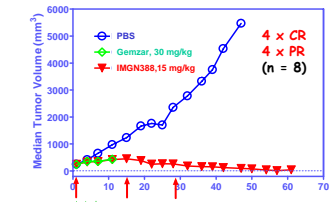
### MDA-MB-231.OT.F2, Human Breast Carcinoma



### A460, Large Cell Lung Carcinoma



### AsPC-1, Human Pancreatic Carcinoma



## 8) Estimated IMGN388 Pharmacokinetic Parameters

Estimates were made using a conjugate assay on samples taken after the first iv dose in Cynomolgus monkeys.

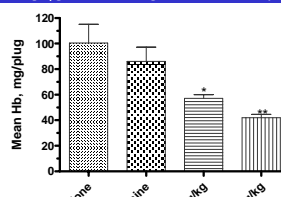
Dose	Gender	Parameter	C <sub>max</sub>		AUC		CL	V <sub>d</sub>	t <sub>1/2</sub>
			ng/ml	ng·h/ml	(h·h)	ml/kg			
1 mg/kg (weekly)	Male	N	4	4	4	4	4	4	4
	Mean	33.76	33.96	29.81	23.27	0.64			
2.5 mg/kg (weekly)	Male	N	5	5	5	5	5	5	5
	Mean	25.48	26.34	39.29	49.2	0.83			
5 mg/kg (every 2 weeks)	Male	N	5	5	5	5	5	5	5
	Mean	74.24	90.99	28.27	49.54	1.07			
10 mg/kg (every 2 weeks)	Male	N	5	5	5	5	5	5	5
	Mean	77.58	106.03	22.52	36.9	1.17			
2.5 mg/kg (every 3 weeks)	Male	N	5	5	5	5	5	5	5
	Mean	75.96	98.89	25.29	38.72	1.09			
5 mg/kg (every 3 weeks)	Male	N	5	5	5	5	5	5	5
	Mean	55.31	74.66	32.31	46.08	1.00			
10 mg/kg (every 3 weeks)	Male	N	5	5	5	5	5	5	5
	Mean	54	116.09	21.05	35.7	1.15			
2.5 mg/kg (every 3 weeks)	Female	N	5	5	5	5	5	5	5
	Mean	72.66	95.67	26.68	40.89	1.08			
5 mg/kg (every 3 weeks)	Female	N	5	5	5	5	5	5	5
	Mean	142.93	179.52	22.45	45.1	1.4			
10 mg/kg (every 3 weeks)	Female	N	5	5	5	5	5	5	5
	Mean	182.81	243.88	39.68	67.6	1.64			
2.5 mg/kg (every 3 weeks)	Female	N	5	5	5	5	5	5	5
	Mean	153.37	202.43	30.89	41.10	1.37			

Similar PK data were obtained following the final dose.

- Serum concentrations were similar, whether determined by IgG backbone or conjugate assays, indicating IMGN388 was not substantially cleaved.
- Unconjugated [DM4] at mean peak was minimal: 2.3 ng/ml after 2.5 mg/kg dose, 4.0 ng/ml after 5.0 mg/kg dose
- C<sub>max</sub> and AUC increased with dose in the 1 to 5 mg/kg range.
- Accumulation of IMGN388 in the systemic circulation was not observed.
- The data suggest that antigen-dependent clearance influences IMGN388 PK at low doses.

## 9) IMGN388 is Anti-Angiogenic

Tumor-free, growth factor-stimulated *in vivo* model, in which nude rats were injected with IMGN388 prior to implantation with Matrigel plugs containing 5 µg/ml bFGF. Hemoglobin content was analyzed after 1 wk.



\* significantly different from bFGF alone, p<0.05; \*\* significantly different from bFGF alone, p<0.01.

## Conclusions

1. IMGN388 binds to an integrin target present on a wide variety of solid tumors, as well as on some tumor neovascular and stromal tissues.
2. ~67% of "triple negative" invasive ductal breast carcinomas stained moderately or strongly positive for the IMGN388 target integrin.
3. Complete tumor regressions were achieved with IV administration of IMGN388 in several antigen-positive tumor xenograft models.
4. IMGN388 showed anti-angiogenesis activity as well as tumor cell cytotoxicity.
5. The findings support advancement of IMGN388 into clinical testing, and this occurred in mid-2008.