

PDL192, a Novel, Humanized Antibody to TWEAK Receptor, Shows Potent Anti-Tumor Activity in Preclinical Models

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ABSTRACT

Background: TWEAK receptor (TweakR) is a member of the tumor necrosis factor receptor superfamily, a group of receptors that has garnered significant interest as therapeutic targets in cancer and autoimmune disease. TweakR expression has been reported in some solid tumors. In addition, signaling via TweakR can induce apoptosis in certain cancer cell lines.

Methods: Expression of TweakR in primary tumors was assessed by immunohistochemistry (IHC). PDL192, a novel, humanized, IgG1 monoclonal antibody to TweakR, was assessed for its ability to inhibit the growth of cancer cell lines both *in vitro* (soft agar and anchorage-dependent proliferation assays) and *in vivo* (xenograft models in SCID mice). In addition, antibody-dependent cellular cytotoxicity (ADCC) assays were performed with PDL192 on tumor cell lines using peripheral blood mononuclear cells from normal human donors in a ⁵¹Cr release assay.

Results: TweakR was found to be expressed by IHC in a variety of solid tumors, including pancreatic, breast, lung, and renal cancer. In *in vitro* assays, the anti-TweakR antibody, PDL192, inhibited the growth of approximately 30% of TweakR-expressing cancer cell lines tested. PDL192 also potently induced ADCC against TweakR-expressing cancer cell lines *in vitro*. PDL192 significantly inhibited tumor growth in several xenograft models representing a variety of tumor types. In an orthotopic model of breast cancer, PDL192 inhibited both primary tumor growth as well as the growth of lung metastases. In a pancreatic model, PDL192 or gemcitabine significantly slowed tumor growth; however, the two agents in combination caused complete tumor regression.

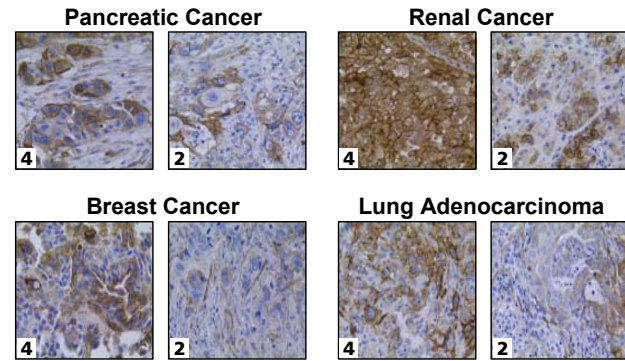
Conclusions: The humanized anti-TweakR antibody, PDL192, has been found to have anti-tumor effects against multiple TweakR-expressing tumor cell lines both *in vitro* and in xenograft models. In addition, PDL192 has been found to enhance the anti-tumor activity of some chemotherapeutic agents in xenograft models. These data, together with the histological data showing that TweakR is expressed on a variety of tumor types, suggest that PDL192 has the potential to be a therapy for patients with solid tumors. This data is the basis for an ongoing Phase I safety study of PDL192 in patients with solid tumors.

INTRODUCTION

- TWEAK receptor (aka TweakR, Fn14, CD266, TNFRSF12A), a cell surface protein, is a member of the TNF receptor superfamily.
- TweakR is overexpressed on a number of cancer cells as compared with normal tissues¹⁻³.
- Inhibition of TweakR expression by RNAi inhibited the growth of some tumor cells *in vitro*³.
- Binding of the natural ligand, TWEAK, to TweakR results in pleiotropic biological activities, including cancer cell killing⁴.
- Mouse anti-TweakR antibodies have been reported to have anti-tumor cell growth properties *in vitro*^{3,5}.
- PDL192 is a humanized IgG1 monoclonal antibody to TweakR, with a binding affinity of 5.5 nM.

RESULTS

TweakR Protein Is Expressed in a Range of Solid Tumor Types



Formalin-fixed paraffin embedded tumor samples were stained for TweakR protein using the 374.2 antibody and BORG antigen retrieval solution with MACH4 detection system (Biocare). Examples of two different tumor samples from four different cancer types are shown. The score each sample received is indicated (the scoring system is described below).

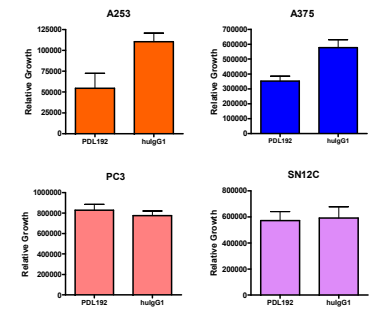
TweakR Immunohistochemistry Summary

Cancer	N	IHC Score					≥2	% pos.	
		0	1+	2+	3+	4+			
Pancreatic	87	17	18	30	17	5	52/87	60	
Colon	121	62	34	21	3	1	25/121	21	
Ovarian	142	103	17	16	6	1	23/142	16	
RCC	67	26	21	11	2	7	20/67	30	
Melanoma	40	21	11	3	3	2	8/40	20	
Gastric Adeno	68	38	17	9	4	0	13/68	19	
Breast:	Ductal	135	68	38	15	11	3	29/135	21
	Lobular	29	19	9	0	1	0	1/31	3
	LN mets	59	40	9	6	3	1	10/59	17
Lung:	Adeno	83	29	18	16	13	7	36/83	43
	Squamous Cell	16	1	5	10	0	0	10/16	63
Bladder	31	19	5	5	2	0	7/31	23	
Head & Neck	30	10	11	6	2	1	9/30	30	
Esophageal	49	22	12	5	3	5	13/49	27	
Sarcoma	36	24	5	2	1	4	7/36	19	
Hepatocellular	20	12	4	1	2	1	4/20	20	
Prostate	61	58	3	0	0	0	0/5	0	

TweakR staining was scored by board-certified pathologists using the following system: 4 = 76-100% of tumor cells stained positive; 3 = 51-75% of tumor cells stained positive; 2 = 26-50% of tumor cells stained positive; 1 = 11-25% of tumor cells stained positive; 0 = 0-10% of tumor cells stained positive

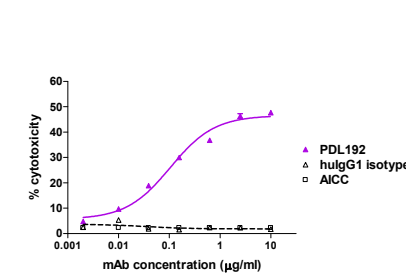
TweakR mRNA was previously shown to be overexpressed in a number of cancers compared to cognate normal tissue³. TweakR protein is also more widely expressed in cancers than in normal tissues (data not shown).

PDL192 Inhibits Tumor Cell Growth *in vitro*



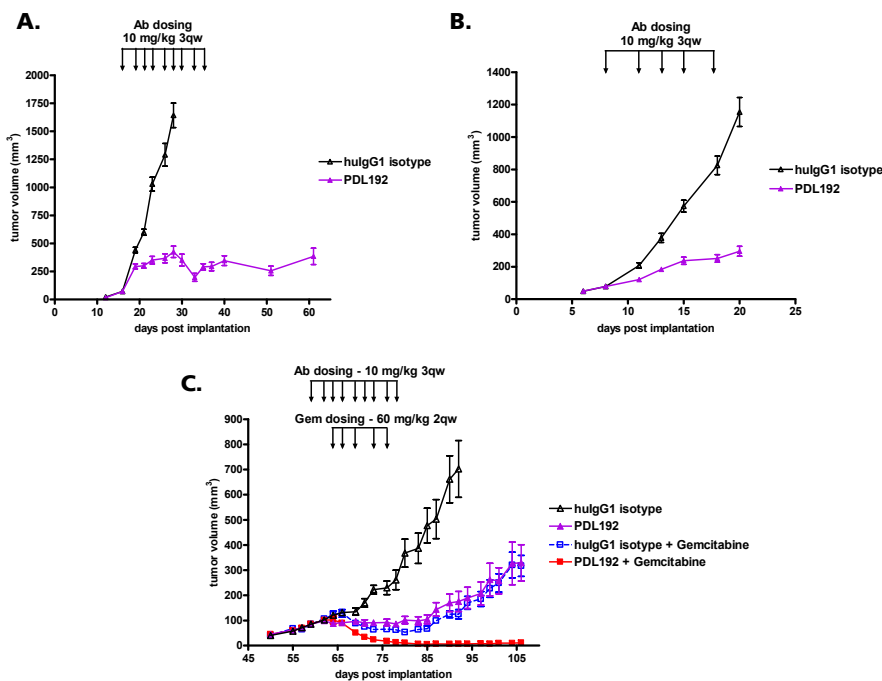
Tumor cell lines were grown in soft agar in the presence of PDL192 or an isotype control antibody. PDL192 inhibited the growth of A253 head and neck cancer cells and A375 melanoma cells, but not PC3 prostate cancer cells or SN12C renal cancer cells.

Induction of ADCC by PDL192



⁵¹Cr labeled SN12C renal cancer cells were incubated with human peripheral blood mononuclear cells and PDL192 or an isotype control antibody. Percent cytotoxicity indicates the extent to which PDL192 induces killing of the SN12C tumor cells.

PDL192 Inhibits Tumor Growth in SCID Mice



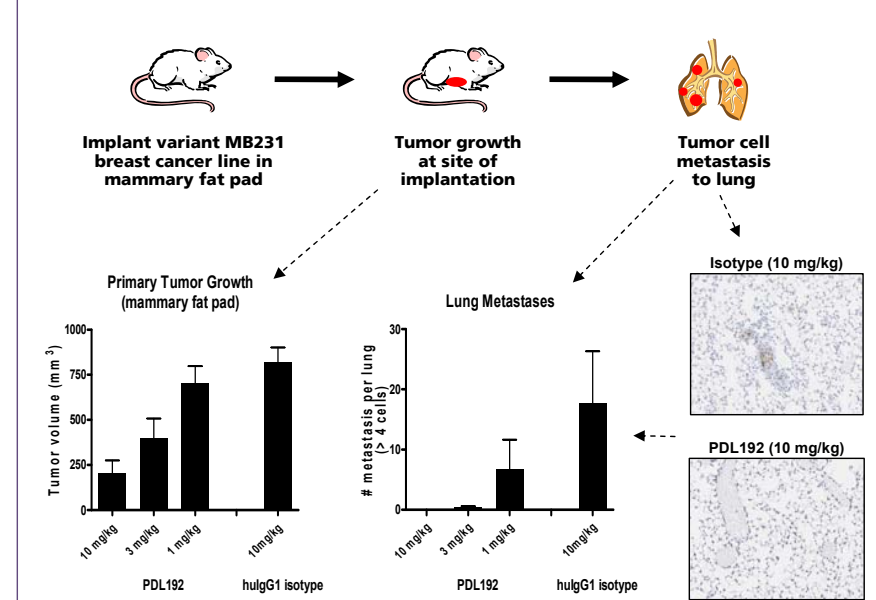
ICR SCID mice bearing established xenograft tumors were dosed with PDL192 or human IgG1 isotype control antibody.

A. HT-29 model of colorectal cancer. PDL192 treatment resulted in a prolonged inhibition of tumor growth.

B. ES-2 model of ovarian cancer. PDL192 treatment significantly inhibited tumor growth.

C. Panc-1 model of pancreatic cancer. Monotherapy with PDL192 or gemcitabine treatment inhibited tumor growth; the combination of the two agents resulted in complete tumor regression.

PDL192 Inhibits Both Primary Tumor Growth and the Formation and Growth of Metastases



Lung sections were stained with CAM 5.2 to identify cytokeratin-positive cells. Lung metastases were quantified by whole slide scanning using the Aperio ScanScope System, followed by manual enumeration of CAM 5.2-positive cell clusters. (Performed at Mosaic Laboratories, Lake Forest, CA)

CONCLUSIONS

- PDL192 is a novel, humanized IgG1 monoclonal antibody that targets TweakR, which is expressed on many solid tumors, including pancreatic, lung, renal, colorectal, and breast cancers.
- In *in vitro* assays, PDL192 directly inhibited the growth of some tumor cell lines and recruited NK cells to kill TweakR-expressing tumor cells via ADCC.
- PDL192 exhibited potent anti-tumor activity in animal models and also inhibited the formation and growth of tumors at metastatic sites.
- PDL192 enhanced the anti-tumor activity of gemcitabine in the Panc-1 xenograft model, providing a rationale for clinical investigation of this potential combination therapy.

References

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