Correlation of TweakR Expression with Her2+ Ductal Breast Cancer and the Preclinical Therapeutic Effect of PDL192, a Humanized anti-TweakR antibody

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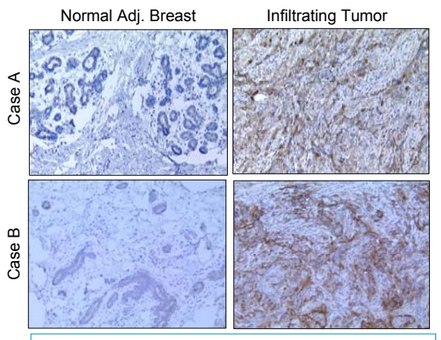
INTRODUCTION

- TWEAK receptor (TweakR), also known as Fn14, is a cell surface protein and member of the tumor necrosis factor receptor superfamily
- TweakR is a potential target for the treatment of solid tumors based on these data:
  1) Overexpression in many solid tumors
  2) RNAi knockdown of TweakR resulted in significant decrease in cancer cell proliferation
- PDL192 is a humanized IgG1 anti-TweakR antibody currently in a phase 1 clinical trial for solid tumors
- Here, we investigate the expression of TweakR and the therapeutic potential of PDL192 in various forms of breast cancer

TweakR Expression in Invasive Breast CA

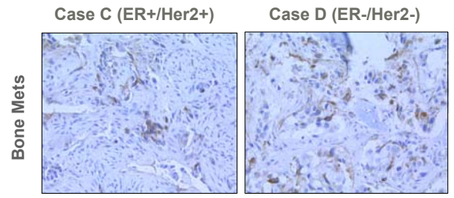


Minimal TweakR Expression in Normal Breast or DCIS
Elevated TweakR Expression in Invasive Tumors

Type	N	0	1	2	3	4	≥2	%
Normal	16	16	0	0	0	0	0/16	0
Hyperplasia	12	12	0	0	0	0	0/12	0
Benign	12	12	0	0	0	0	0/12	0
DCIS	18	16	1	1	0	0	1/18	6
Invasive Ductal	378	183	76	41	46	32	119/378	32
Invasive Lobular	46	45	0	0	1	0	1/45	2
LN Mets	30	22	5	2	1	0	3/30	10
Bone Mets	10	2	3	0	5	0	5/10	50

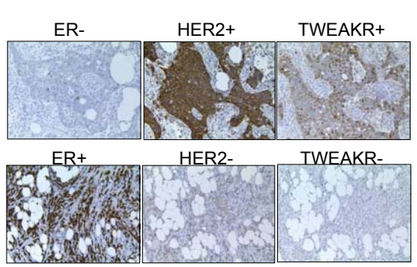
RESULTS

TweakR Expression in Bone Metastasis



Archived FFPE Bone metastatic tissues from primary Breast cancer patients were treated with BORG antigen retrieval buffer prior to staining with anti-TweakR mAb 374.2 and MACH4 system (BioCare) was used for detection.

Correlation of TweakR Staining by IHC with Her2 Expression

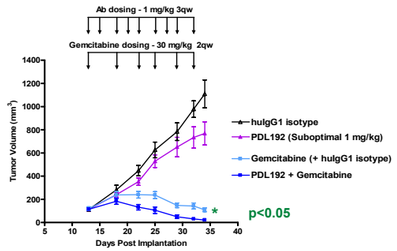


Serial sections of FFPE tissues were stained with ER, Her2 and TweakR mAbs

		IHC Score						
Type	N	0	1	2	3	4	≥2	%pos
ER+/Her2+	63	14	12	11	16	10	37/63	59
ER+/Her2-	51	34	5	5	4	3	12/51	24
ER-/Her2+	57	9	10	7	17	14	38/57	67
ER-/Her2-	66	37	16	6	3	4	13/66	20
Total	237	94	43	29	40	31	100/237	42

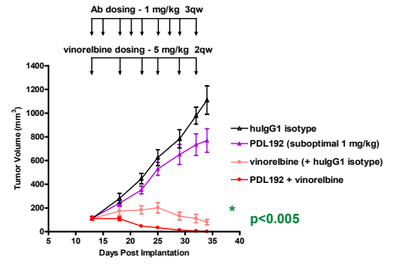
(Chi-square test for correlation p=1.129e-10)

PDL192 Enhances Chemo in vivo

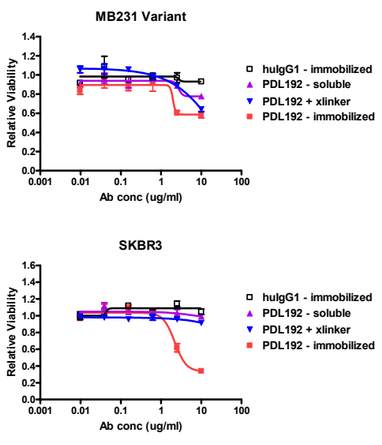


MB231 Variant– Breast Cancer Model

PDL192 dosed at 1 mg/kg (sub-efficacious monotherapy dose)



PDL192 Inhibits Breast CA Growth In Vitro

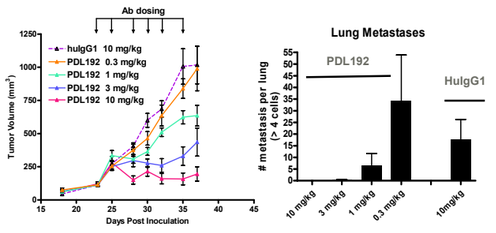


Cells were incubated for 5 days with soluble PDL192 ± cross-linker (+xlinker) or in plates pre-coated with PDL192 (Immobilized).
Cell viability was measured by Alamar Blue.

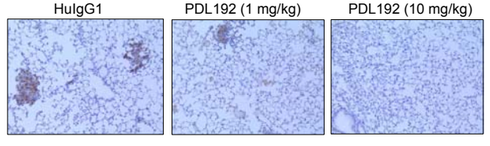
Cells	TweakR expression (fold shift)	ER	HER2	PDL192 treatment			TWEAK Tx
				soluble	+xlinker	Imm.	
MB231 Variant	7.1	-	-	+	+	+	+
BT549	2.7	-	-	+/-	+	+	+/-
SKBR3	2.7	-	+	-	-	+	+
MCF7-ATCC	6.2	+	-	-	-	+	+
MCF7-NCI	4.5	+	-	-	-	+	-
BT474	2.2	+	+	-	-	+	-
MDA-MB453	2.1	-	+	-	+/-	+	-
BT20	5.8	-	-	-	-	-	-
T47D	2.5	+	-	-	-	-	-
MDA-MB231	6.4	-	-	+/-	+	n.d	+
MDA-MB468	2.6	-	-	+	+	n.d	+
HCC38	13.4	-	-	+	+	n.d	+
HCC1143	21.3	-	-	-	+/-	n.d	+/-
HCC1937	5.7	-	-	-	+/-	n.d	+/-
ZR-75-30	4.2	+	+	-	-	n.d	-

+ >25% inhibitory activity; +/- 10-25% Inhibitory activity; - no effect;
n.d.: not determined; Imm: immobilized
TweakR Expression determined by flow cytometry (fold shift over isotype control)
ER &Her2 status (adapted from Neve et al., 2006)

PDL192 Inhibits Tumor Growth and Mets in vivo



NW231, a derivative of MDA231 breast cancer cells were inoculated into mammary pads of CB17.Scid mice and treatment started after the tumor reached 50 mm^3. Lungs were harvested and metastases were counted and stained for TweakR expression. Representative images are shown.



CONCLUSIONS

- **TweakR was over-expressed in 32% of invasive ductal breast cancer and 50% of bone metastases**
- **Strong positive correlation of TweakR with Her2 expression in breast cancer**
- **PDL192 inhibited cell growth of various types of breast cancer cell lines in vitro**
- **PDL192 inhibited tumor growth and enhances the activity of several chemotherapeutics in a xenograft model of breast cancer.**
- **PDL192 is a potential therapy for multiple subtypes of breast cancer**

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