

Anti-TweakR Antibodies Inhibit Tumor Growth *In Vivo* Through Dual Mechanisms

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ABSTRACT

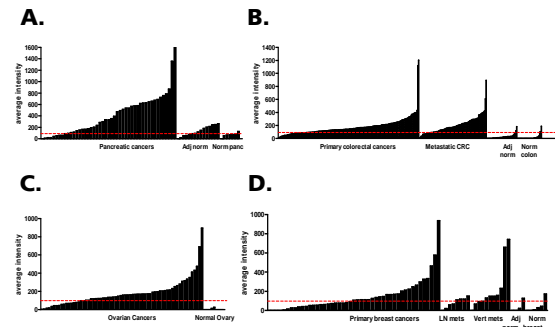
TWEAK receptor (TweakR, Fn14, TNFRSF12A) was identified in a genome-wide screen as over-expressed in a number of solid tumors, including pancreatic, lung, renal, breast, and head & neck cancers. In addition to its mRNA being up-regulated in multiple cancer types, TweakR also scored in an RNAi screen designed to identify those over-expressed genes that might play a functional role in carcinogenesis. Based on these two lines of data, and supported by published literature demonstrating that TWEAK, the natural ligand for TweakR, and antibodies to TweakR can induce apoptosis of the HT29 colorectal carcinoma cell line, TweakR was selected as a target for the development of therapeutic monoclonal antibodies.

We generated a mouse monoclonal antibody to TweakR, 19.2.1, that induced apoptosis of HT29 cells and moderately stimulated other TWEAK-mediated functions. 19.2.1 does not interfere with normal TWEAK signaling but similar to TWEAK, this antibody inhibited cancer cell proliferation and slowed the growth of approximately one-third of TweakR-expressing cancer cell lines tested in both anchorage-dependent and anchorage-independent assays. In mouse xenograft models, 19.2.1 showed significant anti-tumor activity in a range of tumor models, including models of colorectal, breast, renal, and head & neck cancers. 19.2.1 is a murine IgG2a antibody, an isotype which is predicted to have strong antibody-dependent cellular cytotoxicity (ADCC) activity. 19.2.1 was found to have potent ADCC activity *in vitro* using either human peripheral blood mononuclear cells or mouse splenocytes as effector cells. Thus, it is likely that both ADCC and the direct anti-proliferative activity of 19.2.1 contribute to its *in vivo* anti-tumor activity and that the combination of these two mechanisms results in greater tumor growth inhibition *in vivo*. Based on these findings, PDL192, a humanized IgG1 antibody, was derived from 19.2.1. PDL192 and 19.2.1 were found to have comparable binding affinities for TweakR and displayed similar *in vitro* and *in vivo* anti-tumor activities. Both ADCC and direct anti-proliferative activity contribute to the anti-tumor activity of PDL192 and are likely to enhance its potential as a treatment for solid tumors.

INTRODUCTION

- TWEAK receptor (aka TweakR, Fn14, TNFRSF12A), a cell surface protein, is a member of the TNF receptor superfamily, a family of receptors which has garnered significant interest as potential targets for the treatment of oncology and autoimmune diseases.
- TweakR is expressed on some cancer cells^{1, 2}
- Binding of the natural ligand, TWEAK, to TweakR results in pleiotropic biological activities, including cancer cell killing³.
- Anti-TweakR antibodies have been reported to have *in vitro* anti-tumor cell growth properties⁴.
- Murine monoclonal antibodies to TweakR were generated
- 19.2.1 was selected based on its ability to inhibit the proliferation of tumor cell lines *in vitro*
- 19.2.1 is a murine IgG2a, an isotype predicted to exert potent antibody-dependent cellular cytotoxicity (ADCC)
- 19.2.1 showed potent anti-tumor activity in multiple xenograft models
- 19.2.1 was humanized to PDL192
- The affinity of PDL192 to TweakR (5.5 nM) is not significantly different from that of 19.2.1 (7.1 nM); thus the humanization process did not significantly impact the binding affinity of the antibody to its target

Figure 1



TweakR mRNA is Overexpressed in Multiple Solid Tumors
mRNA isolated from primary tumors, metastases, cognate adjacent normal tissue, and tissues from normal donors was converted to cRNA using standard protocols and hybridized to Eos Hu03, a customized Affymetrix gene chip containing approximately 59,000 probesets representing 46,000 genes, EST clusters, and predicted exons. The Y axis on each graph represents the intensity of mRNA hybridization to the TweakR probeset, the X axis indicates the sample identity. The red dashed line indicates the estimated level of background (100 intensity units).

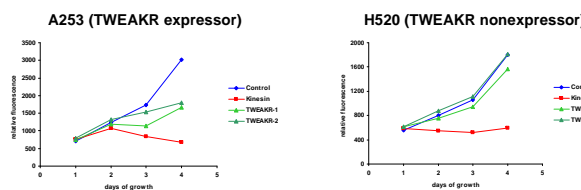
A. 79% of pancreatic cancers (37/47) expressed TweakR. In contrast, 57% of adjacent normal pancreatic tissues (8/14), and 17% of normal pancreatic tissue samples (1/6) were positive.

B. 75% of primary colorectal cancers (128/171) and 76% of metastatic colorectal cancer samples (62/82) expressed TweakR. In contrast, 5% of adjacent normal tissues (2/36) and 7% of normal colorectal tissue samples (2/29) were positive.

C. 73% of ovarian cancers (48/66) expressed TweakR. No adjacent normal tissue or normal ovarian tissues expressed TweakR (0/4 and 0/2, respectively).

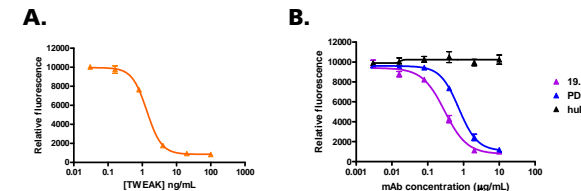
D. 51% of primary breast cancers (24/47), 57% of lymph node metastases (4/7), and 70% of metastases to the spinal column (7/10) expressed TweakR. In normal adjacent and normal breast tissue samples, just 1/4 and 1/5 samples were positive for TweakR, respectively.

Figure 2



TweakR Plays a Critical Role in Cancer Cell Growth
siRNAs were transfected into tumor cell lines, after which cell viability was monitored daily. Two different TweakR siRNAs inhibited the proliferation of A253 head and neck cancer cell line, which expresses TweakR. Knockdown of cell surface TweakR protein was confirmed by flow cytometry (data not shown). The TweakR siRNAs had no effect on the growth of H520, a lung cancer cell line, which does not express TweakR. A negative control siRNA, which does not recognize any known cellular mRNA, had no effect on cell viability, while a positive control siRNA to kinesin-1 prevented growth of both cell lines.

Figure 3

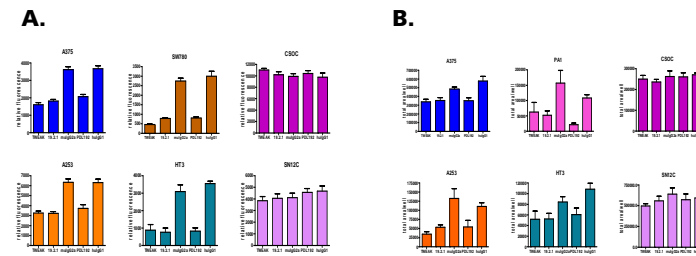


Anti-TweakR Antibodies Kill HT-29 Colon Cancer Cells
The murine monoclonal anti-TweakR antibody, 19.2.1, and its humanized counterpart, PDL192, were assessed for their ability to kill HT29 colorectal cancer cells. HT29 cells were incubated in the presence of 20 ng/mL IFN γ for 24 hours, after which TWEAK or TweakR antibodies were added. Cell viability was assessed four days later.

A. TWEAK kills HT29 colon cancer cells in a dose-dependent manner, as previously reported³.

B. Both 19.2.1 and PDL192 displayed the ability to kill HT29 cells. A human IgG1 isotype control antibody had no effect on HT29 viability.

Figure 4

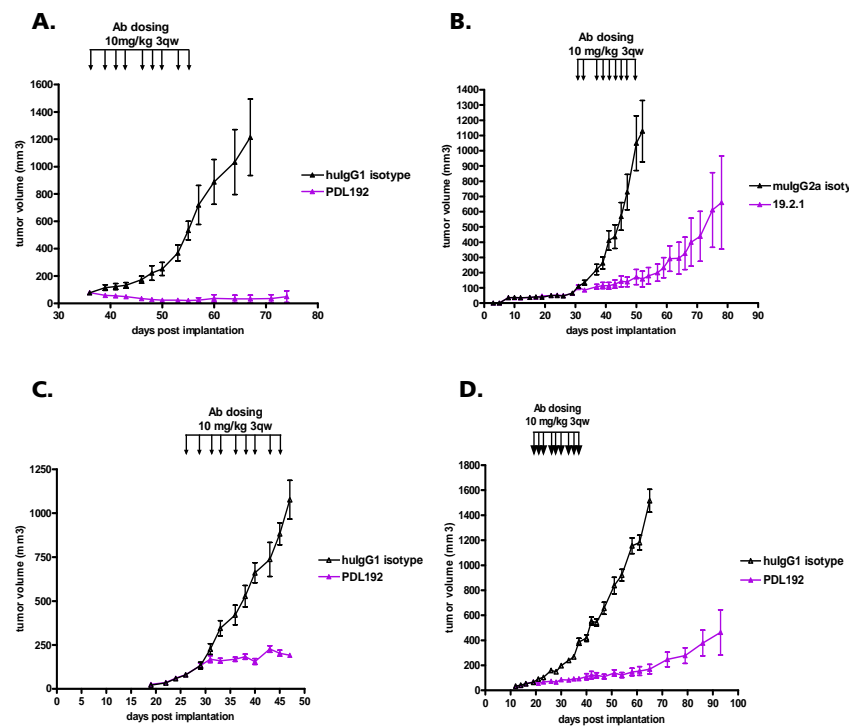


TweakR Antibodies Inhibit the Growth of Multiple Cancer Cell Lines in *in vitro* Assays

A. In a cell proliferation assay, TWEAK, 19.2.1, and PDL192 significantly inhibited the growth of A375 melanoma cells, A253 head and neck cancer cells, SW780 bladder cancer cells, and HT3 cervical cancer cells ($p \leq .05$), but had no effect on the growth of CSOC ovarian cancer cells or SN12C renal cancer cells.

B. In a soft agar assay, TWEAK, 19.2.1, and PDL192 significantly inhibited the growth of A375 melanoma cells, A253 head and neck cancer cells, HT3 cervical cancer cells, and PA-1 ovarian cancer cells ($p \leq .05$), but not CSOC ovarian cancer cells or SN12C renal cancer cells.

Figure 5



ICR SCID mice bearing established xenograft tumors were dosed with PDL192, 19.2.1, or isotype control antibody at 10 mg/kg, three times a week for a total of nine doses.

A. In the SN12C model of renal cancer, PDL192 treatment resulted in tumor regression in all animals. PDL192 and 19.2.1 also displayed potent anti-tumor activity in additional xenograft models, including:

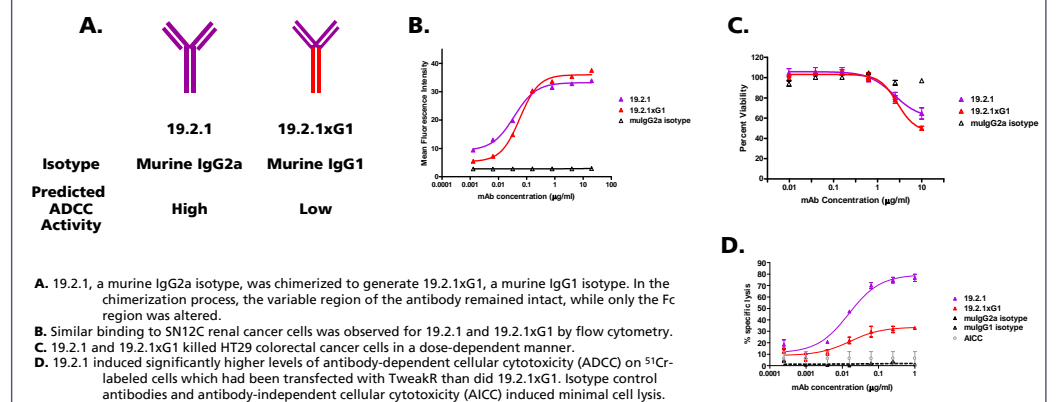
B. The A253 model of head and neck cancer

C. The A375 model of melanoma

D. The CSOC model of ovarian cancer

RESULTS

Figure 6



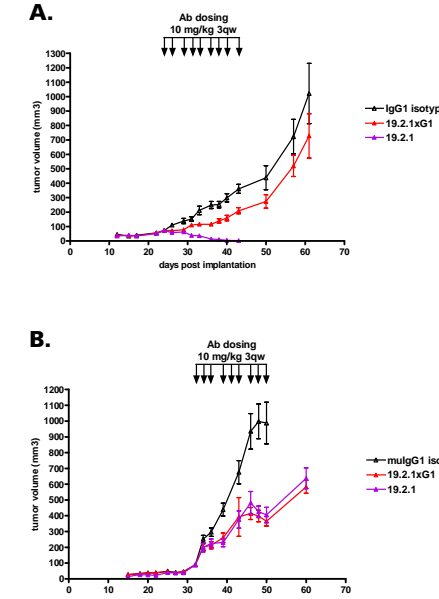
A. 19.2.1, a murine IgG2a isotype, was chimerized to generate 19.2.1xG1, a murine IgG1 isotype. In the chimerization process, the variable region of the antibody remained intact, while only the Fc region was altered.

B. Similar binding to SN12C renal cancer cells was observed for 19.2.1 and 19.2.1xG1 by flow cytometry.

C. 19.2.1 and 19.2.1xG1 killed HT29 colorectal cancer cells in a dose-dependent manner.

D. 19.2.1 induced significantly higher levels of antibody-dependent cellular cytotoxicity (ADCC) on ⁵¹Cr-labeled cells which had been transfected with TweakR than did 19.2.1xG1. Isotype control antibodies and antibody-independent cellular cytotoxicity (AICC) induced minimal cell lysis.

Figure 7



19.2.1 Exerts its Anti-Tumor Activity *in vivo* Through Multiple Mechanisms of Action

ICR SCID mice bearing established xenograft tumors were dosed with 19.2.1, 19.2.1xG1, or a murine IgG1 isotype control antibody at 10 mg/kg, three times a week for a total of nine doses.

A. In the SN12C model of renal cancer, 19.2.1 treatment resulted in complete tumor eradication in all mice. In contrast, although 19.2.1xG1 inhibited tumor growth on days 26, 29, 33-50 ($p \leq .05$), this isotype exhibited significantly less anti-tumor activity than 19.2.1 ($p < .001$).

B. In the A375 model of melanoma, both 19.2.1 and 19.2.1xG1 exerted similar levels of anti-tumor activity, with significant tumor growth inhibition, compared to an isotype control antibody, on days 39-50 ($p \leq .05$).

CONCLUSIONS

- TweakR is overexpressed in many solid tumors, including pancreatic and ovarian cancers, and primary and metastatic colorectal and breast cancers
- TweakR plays a critical role in the growth of a number of tumor cell lines *in vitro*
- PDL192, a novel, humanized, IgG1 mAb to TweakR, exhibits potent anti-tumor activity against multiple TweakR-expressing solid tumor cell lines, *in vitro* and *in vivo*
- PDL192 exerts its potent anti-tumor cell activity through multiple mechanisms of action, including direct inhibition of proliferation and recruitment of immune effector cells to induce ADCC
- The dual mechanism of action of PDL192 may make it effective against tumor cells which are resistant to either mechanism

References

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