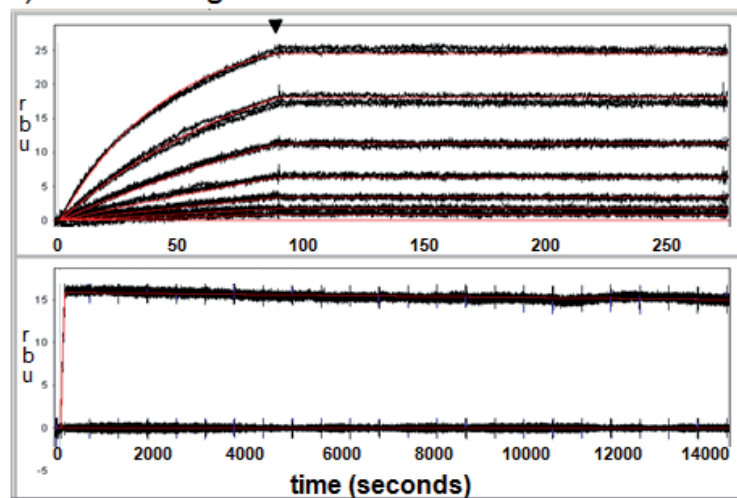


Molecular Pharmacology – Supplemental Methods and Figures

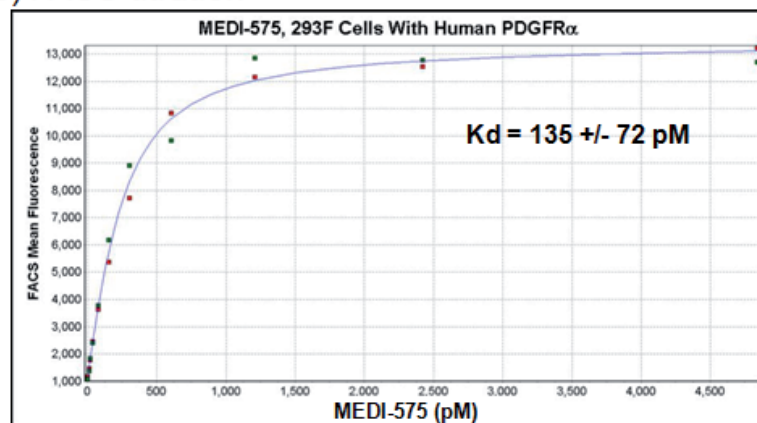
Inhibition of PDGFR α by MEDI-575 reduces tumor growth and stromal fibroblast content in a model of non-small cell lung cancer

Naomi Laing, Brenda McDermott, Shenghua Wen, David Yang, Deborah Lawson, Mike Collins, Corinne Reimer, Peter A Hall, Harriet Andersén, Michael Snaith, Xin Wang, Vahe Bedian, Zhu A. Cao, David Blakey

A) The binding of sPDGFR α to immobilized MEDI-575



B) FACS titration



Supplemental Figure 1. Affinity determinations for MEDI-575 using Biacore and FACS analysis. (a) Biacore binding curves. MEDI-575 was immobilized on Biacore chips and solutions of various fixed concentrations of sPDGFR α (0.8-52 nM) were flowed over the chip for the 90-second association phase, followed by a 3-minute dissociation phase (top panel). The bottom panel shows the dissociation that occurred over a 4-hour period after a chip was preloaded with MEDI-575 (90-second exposure to 25.8 nM of the mAb). (b) FACS titration of 293F cells expressing human PDGFR α with MEDI-575.

Supplemental Methods for Figure 1.

Biacore analysis

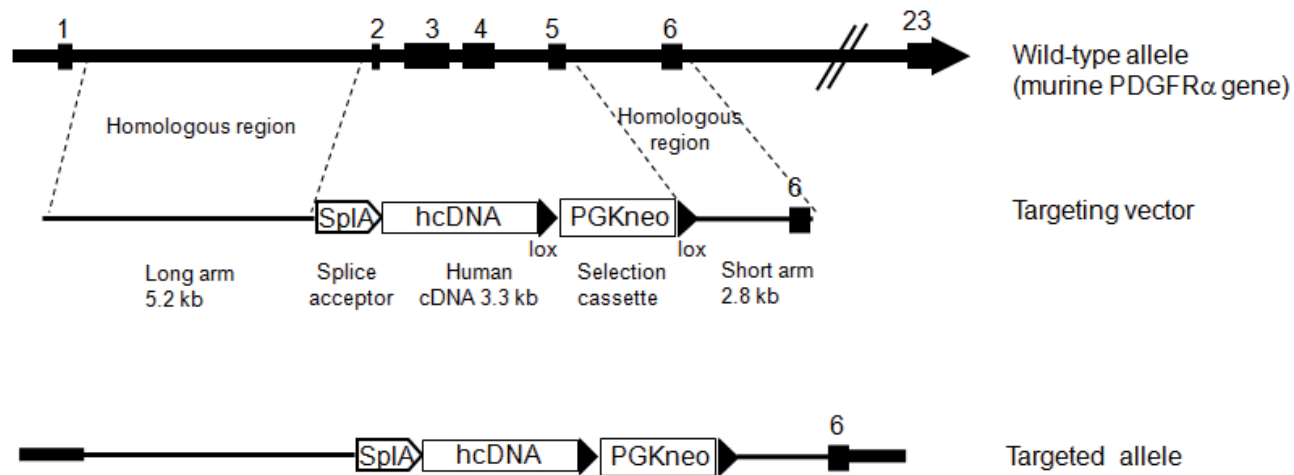
Standard aldehyde coupling was used to bind MEDI-575 to the surface of a CM5 biosensor chip (Biacore). A Biacore T100 instrument was injected with soluble recombinant human PDGFR α extracellular domain to bind to MEDI-575-coated CM5 biosensor chips for 90 seconds at a concentration range of 0.806 nM to 51.6 nM. This was followed by three minutes of buffer injection to monitor dissociation. The apparent slow dissociation of sPDGFR α from immobilized MEDI-575 was further characterized with additional injections using 25.8 nM sPDGFR α , followed by dissociation phase of 14,400 seconds (4 hours). The sample was randomly injected in triplicate, with over ten buffer injections interspersed for double referencing. All sensorgram data were processed with the Scrubber 2.0 program (Biologic Software) and globally fit to a 1:1 interaction model including a term for mass transport, using the CLAMP program.

Fluorescence-activated cell sorting affinity determination

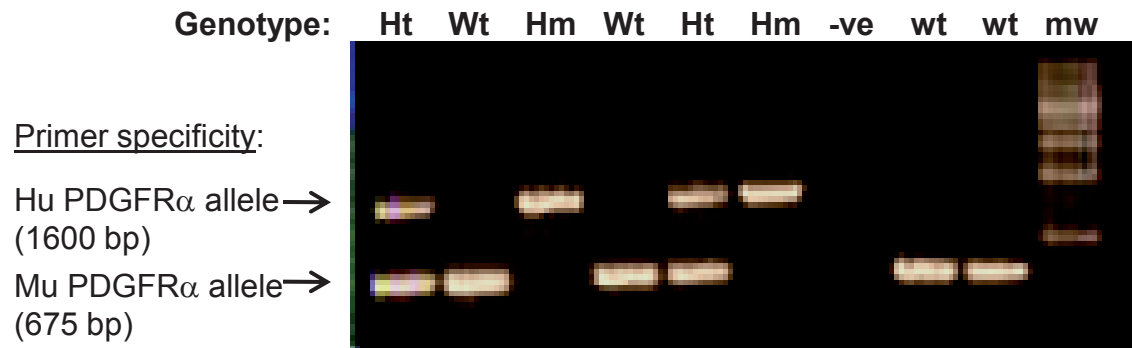
Human embryonic kidney 293F cells (Invitrogen) ectopically expressing the human PDGFR α gene were incubated with a range of MEDI-575 concentrations or with buffer alone. Mock transfected 293F cells served as a negative control and any binding observed to these cells was subtracted from that observed with the transfected cells. Cells (2 million cells per ml) were incubated with a range of MEDI-575 concentrations (10 pM to 5 nM) for 4h at 4C and then rinsed multiple times. Binding of MEDI-575 was detected by resuspending the cell pellet (2 million cells per ml) in a solution containing 100 nM Cyanine-5-labeled goat anti-human polyclonal antibody (Caltag) followed by rinsing and fluorescence-activated cell sorting (FACS) using a Canto II HTS flow cytometry instrument. F was graphed as a function of the concentration of MEDI-575 and fitted with Scientist 3.0 software (Micomath). All data were fit to a nonlinear fitting equation to yield an equilibrium dissociation constant (K_d):

$$F=P' \frac{(K_D + L_T + n \cdot M) - \sqrt{(K_D + L_T + n \cdot M)^2 - 4n \cdot M \cdot L_T}}{2} + B$$

where F = mean fluorescence, L_T = total molecular mAb concentration, P' = proportionality constant that relates arbitrary fluorescence units to bound antibody, M = cellular concentration in molarity, n = number of receptors per cell, B = background signal, and K_d = equilibrium dissociation constant. For each titration curve with MEDI-575, an estimate for K_d was obtained as P' ; n , B , and K_d were allowed to float freely in the nonlinear analysis (Drake AW, Klakamp SL. A rigorous multiple independent binding site model for determining cell-based equilibrium dissociation constants. J Immunol Methods 2007;318:147-52).

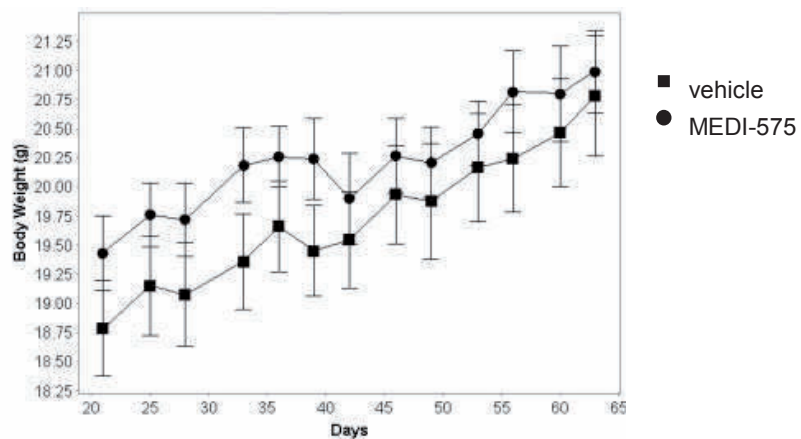


Supplemental Figure 2. The targeting vector used to generate transgenic mice expressing human PDGFR α in place of murine PDGFR α . The numbers represent the exon numbers within the murine PDGFR α gene. Abbreviations: SpA, splice acceptor; hcDNA = the human PDGFR α cDNA, PGKneo= neomycin-resistance gene.

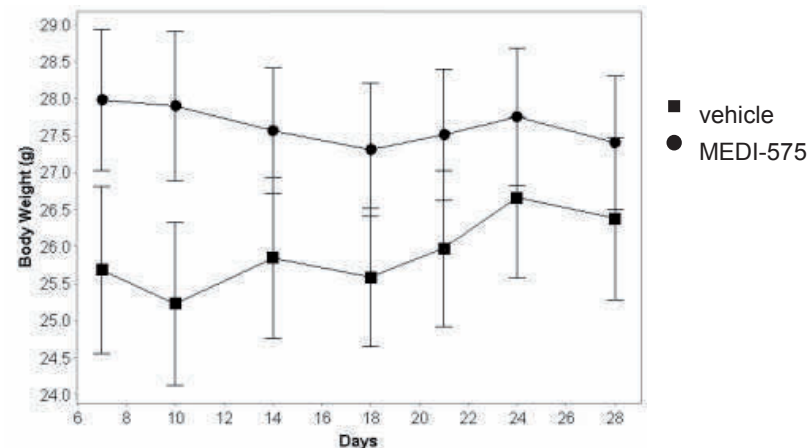


Supplemental Figure 3: Genotyping of C57BL/6 transgenic littermates that were homozygous (Hm), heterozygous (Ht), or wild-type (WT) at the PDGFR α locus by PCR. The primer pairs were designed to detect the murine (mu) or human (hu) PDGFR α alleles. mw= molecular weight markers.

U118



Calu-6/ huPDGFR α /SCID



Supplemental Figure 4: Body weight graphs from two efficacy studies. The graphs start on the first day of dosing, and initial body weights were determined before dosing on that day.

Supplemental Table 1. The Sequences and Specificity of Primers used for PCR Genotyping and Gene Expression Analysis

Purpose	Forward primer sequence	specificity	Reverse primer sequence	specificity	Predicted size (bp)
Genotyping	5'-GACGCACGCCAGACTGTGTATAA-3'	Mouse PDGFR α gene, exon 5	5'-TGAGGCCCCCATGCACAGTATTA-3'	Undeleted region in murine gene	675
	5'-ACACAGCTCGCAGACCTCT-3'	Human PDGFR α gene, cDNA	5'-TGAGGCCCCCATGCACAGTATTA-3'	Undeleted region in murine gene	1600
RNA Expression	5'-CCTCCCACCAGGTCTTTCTGGTCCTCA-3'	Murine PDGFR α gene, exon 2	5'-GGAGGCGTTAACCACTTCCAGCACT-3'	Murine exon 3	266
	5'-GAGGACGTTCAAGACCAGCGAG-3'	Murine PDGFR α gene, exon 4	5'-CAAAGGTGGGCTCAATCTCGAC-3'	Murine exon 7	380
	5'-TCCGGCGTTCCTGGTCTTAG-3'	Human PDGFR α gene, exon 2	5'-TGTGTGGGCCCGCCGAGGCACTGCT-3'	Human exon 3	271
	5'-TCCGGCGTTCCTGGTCTTAG-3'	Human PDGFR α gene, exon 2	5'-CAAAGGTGGGCTCAATCTCGAC-3'	Human exon 7	960