SUPPLEMENTARY INFORMATION

Therapeutic paradigm of dual targeting VEGF and PDGF for effectively treating FGF-2 off-target tumors

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Supplementary Figure 1. Cell proliferation and growth factor production in vitro. a. In vitro proliferation of E0771-vector and E0771-FGF-2 cancer cells (n = 6). b. In vitro proliferation of T241-vector and T241-FGF-2 cells (n = 6). c. ELISA measurement of VEGF levels in E0771-vector and E0771-FGF-2 cancer cell lysates (n = 3 individual samples) and their corresponding conditioned media (n = 4 individual samples), and in T241-vector and T241-FGF-2 cell lysates (n = 3 individual samples) and conditioned media (n = 4 individual samples). d. ELISA measurement of PDGF-B levels in E0771-vector and E0771-FGF-2 cell lysates (n = 3).
and conditioned media (n = 4), and in T241-vector and T241-FGF-2 cell lysates (n = 3) and conditioned media (n = 4). e. In vitro proliferation of E0771-vector and E0771-FGF-2 cancer cells treated with VEGF blockade (1 and 2 µg mL\(^{-1}\); n(Vector, at Day6) = 6; 6; n(FGF-2, , at Day6) = 5; 6). f. In vitro proliferation of T241-vector and T241-FGF-2 cells treated with VEGF blockade (1 and 2 µg mL\(^{-1}\); n = 6). g. In vitro proliferation of E0771-vector and E0771-FGF-2 cancer cells treated with PDGFRβ blockade (0.1, 0.5, and 1 µg mL\(^{-1}\); n = 6; P = 0.0019, 0.0036, 0.0004) h. In vitro proliferation of T241-vector and T241-FGF-2 treated with PDGFRβ blockade (0.1, 0.5, and 1 µg mL\(^{-1}\); n = 6; P = 0.0014, 0.0016, 0.0003). i. In vitro proliferation of mouse endothelial cells treated with conditioned media from E0771, T241, and recombinant FGF-2 protein (5 ng mL\(^{-1}\)) (n = 5; P < 0.0001, P = 0.046, P = 0.0085). j. In vitro proliferation of mouse pericytes treated with conditioned media from E0771, T241 (n = 5), and recombinant FGF-2 protein (50 ng mL\(^{-1}\)) (n = 6; P = 0.0075, P = 0.0003, P = 0.0095). FGF-2 = vector tumor cells; FGF-2\(^+\) = FGF-2 tumor cells; CM = conditioned media; Rec FGF-2 = recombinant FGF-2; n.s. = Not significant; *P < 0.05; ** P < 0.01; *** P < 0.001; two-tailed t-test. Data presented as mean ± s.e.m. Experiments were repeated two-three times. Source data are provided as a Source Data file.
Supplementary Figure 2. Necrosis and cancer-associated fibroblasts in E0771 tumors. a. Quantification of the total necrotic areas in vehicle- and anti-VEGF + imatinib dual-treated E0771 breast cancers (n = 4 samples per group; \( P(\text{Vehicle-treated vector vs imatinib plus anti-VEGF-treated vector}) = 0.0290 \)). b. Quantification of the total necrotic areas in vehicle- and anti-VEGF + imatinib dual-treated E0771-FGF-2 breast cancers (n = 4 samples per group). c. CD31\(^{+}\) microvessels (red) and fibroblast specific protein-1 (FSP-1\(^{+}\)) stromal fibroblasts (green) of anti-VEGF-, imatinib-, and anti-VEGF plus imatinib-treated E0771-vector and E0771-FGF-2 breast cancers. Bar = 50 \( \mu \text{m} \). Arrowheads indicate fibroblasts. d. Quantification of FSP-1\(^{+}\) fibrotic signals of anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated E0771-vector and E0771-FGF-2 breast cancers (n(Vector) = 8/11/8/11; n(FGF-2) = 11/12/12/14). e. CD31\(^{+}\) microvessels (red) and \( \alpha \)-smooth muscle actin (\( \alpha \)-SMA\(^{+}\)) smooth muscle cells and myofibroblasts (green) of anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated E0771-vector and E0771-FGF-2 breast cancers. Bar = 50 \( \mu \text{m} \). Arrowheads point fibroblasts. f. Quantification of \( \alpha \)-SMA\(^{+}\) myofibrotic signals of anti-VEGF-, imatinib-, and anti-VEGF plus imatinib-treated E0771-vector and E0771-FGF-2 breast cancers (n(Vector) = 9/9/10/13; n(FGF-2) = 12/12/12/13). FGF-2\(^{-}\) = vector cancers; FGF-2\(^{+}\) = FGF-2 cancers; n.s. = Not significant; \(* P < 0.05\); two-tailed t-test. Data presented as mean ± s.e.m. Experiments were repeated two times. Source data are provided as a Source Data file.
Supplementary Figure 3. Tumor inflammatory cells and immune cells in E0771 breast cancers. a, b. Immunohistochemical analysis of Iba 1\(^+\) inflammatory macrophages (green), CD3\(^+\) total T cell population (red), CD4\(^+\) subpopulation of T cells (red), and CD8\(^+\) subpopulation of T cells (red) in anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated E0771-vector and E0771-FGF-2 breast cancers. Bar = 50 \(\mu\)m. c. Quantification of Iba 1\(^+\) inflammatory macrophages of anti-VEGF-,
imatinib- and anti-VEGF plus imatinib-treated E0771-vector and E0771-FGF-2 breast cancers (n(Vector) = 12/15/15/16; n(FGF-2) = 16 each; P(Vector vs FGF-2) < 0.0001).  

**d** Quantification of CD3^+ T cells of anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated E0771-vector and E0771-FGF-2 breast cancers (n(Vector) = 10 each; n(FGF-2) = 8/9/8/9; P(Vector vs FGF-2) = 0.0052).  

**e** Quantification of CD4^+ T cells of anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated E0771-vector and E0771-FGF-2 breast cancers (n(Vector) = 10 each; n(FGF-2) = 8/9/8/9; P(Vector vs FGF-2) = 0.0060).  

**f** Quantification of CD8^+ T cells of anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated E0771-vector and E0771-FGF-2 breast cancers (n(Vector) = 10/9/9/10; n(FGF-2) = 9/9/8/9 each).  

**g-j.** FACS measurement of F4/80^+ inflammatory macrophages (n = 4 each; P(Vector vs FGF-2) = 0.0007) (g), CD3^+ T cells (n(Vector) = 4/4/3/3; n(FGF-2) = 3/4/3/4; P(Vector vs FGF-2) = 0.0211) (h), CD4^+ T cells (n = 4 each; P(Vector vs FGF-2) = 0.002) (i), and CD8^+ T cells (n(Vector) = 4 each; n(FGF-2) = 4/3/3/4). (j) of anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated E0771-vector and E0771-FGF-2 breast cancers. The values are presented as the percentage of positive signals versus the total gated events. FGF-2^= vector cancers; FGF-2^+ = FGF-2 cancers; n.s. = Not significant; *P < 0.05, **P < 0.01, ***P < 0.001; two-tailed t-test. Data presented as mean ± s.e.m. Experiments were repeated two times. Source data are provided as a Source Data file.
Supplementary Figure 4. Tumor angiogenesis and perivascular coverage of anti-VEGF-, imatinib-, and anti-VEGF plus imatinib-treated fibrosarcomas.

a. CD31+ (red) and NG2+ (blue) microvessels in anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated fibrosarcomas. Bar = 100 μm.

b. Quantification of microvessels (n = 7 each; P(Vector vs FGF-2) = 0.0275; P(Vehicle-treated vector vs anti-VEGF-treated vector) < 0.001), pericyte coverages (n = 7/7/8/8; P(Vector vs anti-FGF-2) = 0.0016) and pericyte area (n = 7/7/8/8; P(Vector vs anti-FGF-2) = 0.0009; P(Vehicle-treated vector vs anti-VEGF-treated vector) = 0.0313) of vehicle-anti-VEGF-treated fibrosarcomas.

c. Quantification of microvessels (n = 7 each; P(Vehicle-treated vector vs imatinib-treated vector) = 0.0004), pericyte coverages (n = 7/7/8/8; P(Vehicle-treated vector vs imatinib-treated vector) = 0.0084; P(Vehicle-treated FGF-2 vs imatinib-treated FGF-2) = 0.0001) and pericyte area (n = 7/7/8/8; P(Vehicle-treated vector vs imatinib-treated vector) < 0.0001; P(Vehicle-treated FGF-2 vs imatinib-treated FGF-2) = 0.0008) of vehicle- and imatinib-treated fibrosarcomas.

d. Quantification of microvessels (n = 7 each; P(Vehicle-treated vector vs combination-treated vector) = 0.0004; P(Vehicle-treated FGF-2 vs combination-treated FGF-2) = 0.00096), pericyte coverages (n = 7/7/8/8; P(Vehicle-treated FGF-2 vs combination-treated FGF-2) = 0.0031) and pericyte area (n = 7/8/8/7; P(Vehicle-treated vector vs combination-treated vector) = 0.0059; P(Vehicle-treated FGF-2 vs combination-treated FGF-2) < 0.0001) of vehicle- and anti-VEGF plus imatinib-treated fibrosarcomas. FGF-2– vector cancers; FGF-2+ = FGF-2 cancers; n.s. = Not significant; *P < 0.05; **P < 0.01; ***P < 0.001; two-tailed t-test. Data presented as mean ± s.e.m. Experiments were repeated two times. Source data are provided as a Source Data file.
Supplementary Figure 5. Necrosis and cancer-associated fibroblasts in fibrosarcomas. **a.** Quantification of the total necrotic areas in vehicle- and anti-VEGF + imatinib dual-treated T241-fibrosarcomas (n = 4 samples per group; P(Vehicle-treated vector vs imatinib plus anti-VEGF-treated vector) = 0.0398). **b.** Quantification of the total necrotic areas in vehicle- and anti-VEGF + imatinib dual-treated T241-FGF-2 fibrosarcomas (n = 5 samples per group). **c.** CD31+ microvessels (red) and fibroblast specific protein-1 (FSP-1)+ stromal fibroblasts (green). Arrowheads indicate fibroblasts. Bar = 50 μm. **d.** Quantification of FSP-1+ fibrotic signals of anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated T241-vector and T241-FGF-2 fibrosarcomas (n = 14 samples per group). **e.** CD31+ microvessels (red) and α-smooth muscle actin (α-SMA)+ smooth muscle cells and myofibroblasts (green) in anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated T241-vector and T241-FGF-2 fibrosarcomas. Arrowheads point fibroblasts. Bar = 50 μm. **f.** Quantification of α-SMA+ myofibrotic signals of anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated T241-vector and T241-FGF-2 fibrosarcomas (n(Vector) = 11/10/9/10; n(FGF-2) = 10/10/11/11). FGF-2* = vector cancers; FGF-2* = FGF-2 cancers; n.s. = Not significant; *P < 0.05; two-tailed t-test. Data presented as mean ± s.e.m. Experiments were repeated two times. Source data are provided as a Source Data file.
Supplementary Figure 6. Tumor inflammatory cells and immune cells in T241 fibrosarcomas. a, b. Immunohistochemical analysis of Iba 1+ inflammatory macrophages (green), CD3+ total T cell population (red), CD4+ subpopulation of T cells (red), and CD8+ subpopulation of T cells (red) in anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated T241-vector and T241-FGF-2 fibrosarcomas. Bar = 50 µm. c. Quantification of Iba 1+ inflammatory macrophages of anti-VEGF-,
imatinib- and anti-VEGF plus imatinib-treated T241-vector and T241-FGF-2 fibrosarcomas (n(Vector) = 16/14/16/16; n(FGF-2) = 16/15/16/15; P(Vector vs FGF-2) < 0.0001). d Quantification of CD3+ T cells of anti-VEGF-, imatinib-, and anti-VEGF plus imatinib-treated T241-vector and T241-FGF-2 fibrosarcomas (n(Vector) = 10 each; n(FGF-2) = 10/10/10/9). e Quantification of CD4+ T cells of anti-VEGF-, imatinib-, and anti-VEGF plus imatinib-treated T241-vector and T241-FGF-2 fibrosarcomas (n(Vector) = 10 each; n(FGF-2) = 10/10/10/9). f Quantification of CD8+ T cells of anti-VEGF-, imatinib-, and anti-VEGF plus imatinib-treated T241-vector and T241-FGF-2 fibrosarcomas (n = 8 samples per group). g-j. FACS measurement of F4/80+ inflammatory macrophages (n(Vector) = 4/4/3/4; n(FGF-2) = 4 each; P(Vector vs FGF-2) = 0.0176) (g), CD3+ T cells (n(Vector) = 4/4/3/3; n(FGF-2) = 4 each) (h), CD4+ T cells (n(Vector) = 3/4/3/3; n(FGF-2) = 3 each) (i), and CD8+ T cells (n(Vector) = 4/4/3/3; n(FGF-2) = 4 each) (j) of anti-VEGF-, imatinib- and anti-VEGF plus imatinib-treated T241-vector and T241-FGF-2 fibrosarcomas. The values are presented as the percentage of positive signals versus the total gated events. FGF-2- = vector cancers; FGF-2+ = FGF-2 cancers; n.s. = Not significant; *P < 0.05, ***P < 0.001; two-tailed t-test. Data presented as mean ± s.e.m. Experiments were repeated two times. Source data are provided as a Source Data file.
Supplementary Figure 7. Tumor growth, vascular function, and hypoxia in various drug-treated established FGF-2\(^+\) fibrosarcomas. a. Drug treatment was initiated at day 9 when tumors took off. Tumor growth of vehicle-, anti-VEGF-, imatinib- and anti-VEGF plus imatinib- treated T241-FGF-2 fibrosarcomas (n = 7/5/6/5; P(Vehicle vs imatinib plus anti-VEGF) = 0.0245). b. CD31\(^+\) microvessels (red) and NG2\(^+\) pericytes (blue) in various drug-treated T241-FGF-2 fibrosarcomas. Bar = 100 \(\mu m\). c. Quantification of microvessels (n = 10/10/9/10; P(Vehicle vs imatinib plus anti-VEGF) < 0.0001), pericycle coverages (n = 8/8/9/10; Vehicle vs imatinib) = 0.0290; Vehicle vs imatinib plus anti-VEGF) = 0.0336) and pericycle area
(n = 9/10/9/10; Vehicle vs imatinib) = 0.0052; P(Vehicle vs imatinib plus anti-VEGF) = 0.0001) of vehicle-, anti-VEGF-, imatinib- and anti-VEGF plus imatinib- treated T241-FGF-2. d. Vascular perfusion of 2000 kDa dextran (red) and vascular permeability of 70 kDa dextran (red) of various therapy-treated T241-FGF-2. Bar = 50 μm. e. Quantification of vascular perfusion and permeability of vehicle-, anti-VEGF-, imatinib- and anti-VEGF plus imatinib- treated T241-FGF-2 (Perfusion: n = 13/12/12/13; P(Vehicle vs imatinib plus anti-VEGF) < 0.0001; permeability: n = 11/9/9/11; P(Vehicle vs anti-VEGF) = 0.0342 ; P(Vehicle vs imatinib) = 0.0373 ; P(Vehicle vs imatinib plus anti-VEGF) = 0.0067). f. CAXI+ signals (green) of tumor hypoxia. Bar = 100 μm. g. Quantification of CAXI+ hypoxic signals of various therapy-treated T241-FGF-2 (n = 13 each; P(Vehicle vs imatinib plus anti-VEGF) = 0.0036). h. Ki67+ proliferative cell signals (green) stained with CD31+ microvessels (red) and DAPI (blue) of various therapy-treated T241-FGF-2. i. Quantification of Ki67+ signals in various therapy-treated T241-FGF-2 (n = 11 each; P(Vehicle vs imatinib plus anti-VEGF) = 0.0042). j. Quantification of Ki67+ and CD31+ double positive signals in various therapy-treated T241-FGF-2 (n = 9/10/11/11; P(Vehicle vs imatinib plus anti-VEGF) < 0.0001). k. Micrographs of caspase-3+ apoptotic cells (green) in various therapy-treated T241-FGF-2. Bar = 50 μm. l. Quantification of caspase-3 signals in various therapy-treated T241-FGF-2 fibrosarcomas (n = 15 each; P(Vehicle vs imatinib plus anti-VEGF) = 0.0005). n.s. = Not significant; *P < 0.05; **P < 0.01; ***P < 0.001; two-tailed t-test. Data presented as mean ± s.e.m.
Supplementary Figure 8. Triple Combination therapy with chemotherapeutics.

**a.** Tumor growth of vehicle-, anti-VEGF plus imatinib-, 5-FU (10 mg kg⁻¹)-, and the triple combination of 5-FU and anti-VEGF plus imatinib-treated E0771-FGF-2 breast cancers (n = 4 animals per group; \( P(\text{Vehicle vs imatinib plus anti-VEGF}) = 0.0005; \)
\( P(\text{Vehicle vs the triple combination of 5-FU and anti-VEGF plus imatinib}) = 0.0002; \)
\( P(\text{5FU vs imatinib plus anti-VEGF}) = 0.0054; \)
\( P(\text{5-FU vs the triple combination of 5-FU and anti-VEGF plus imatinib}) = 0.0056). \)

**b.** Tumor growth of vehicle-, anti-VEGF plus imatinib-, 5-FU (60 mg kg⁻¹)-, and the combination of 5-FU and anti-VEGF plus imatinib-treated E0771-FGF-2 breast cancers (n = 4 animals per group; \( P(\text{Vehicle vs imatinib plus anti-VEGF}) = 0.0005; \)
\( P(\text{Vehicle vs the triple combination of 5-FU and anti-VEGF plus imatinib}) < 0.0001; \)
\( P(\text{Vehicle vs 5-FU}) = 0.0003; \)
\( P(\text{5-FU vs the triple combination of 5-FU and anti-VEGF plus imatinib}) = 0.0024). \)

**c.** Tumor growth of vehicle-, anti-VEGF plus imatinib-, 5-FU (10 mg kg⁻¹)-, and the combination of 5-FU and anti-VEGF plus imatinib-treated T241-FGF-2 fibrosarcomas (n = 4 animals in vehicle- and anti-VEGF plus imatinib- treated groups. n = 5 animals in 5-FU and the combination of 5-FU and anti-VEGF plus imatinib-treated groups. two-site injections per animal; \( P(\text{Vehicle vs imatinib plus anti-VEGF}) < 0.0001; \)
\( P(\text{Vehicle vs 5-FU}) < 0.0001; \)
\( P(\text{Vehicle vs the triple combination of 5-FU and anti-VEGF plus imatinib}) < 0.0001; \)
\( P(\text{5-FU vs anti-VEGF plus imatinib}) = 0.0211; \)
\( P(\text{5-FU vs the triple combination of 5-FU and anti-VEGF plus imatinib}) = 0.0195). \)

**d.** Tumor growth of vehicle-, anti-VEGF plus imatinib-, 5-FU (60 mg kg⁻¹)-, and the combination of 5-FU and anti-VEGF plus imatinib-treated T241-FGF-2 fibrosarcomas. (n = 4 animals in vehicle- and anti-VEGF plus imatinib-treated groups. (n = 5 animals in 5-FU-, and the
combination of 5-FU and anti-VEGF plus imatinib-treated, two-site injections per animal; $P$(Vehicle vs imatinib plus anti-VEGF) < 0.0001; $P$(Vehicle vs 5-FU) < 0.0001; $P$(Vehicle vs the triple combination of 5-FU and anti-VEGF plus imatinib) < 0.0001; $P$(Imatinib plus anti-VEGF vs the triple combination of 5-FU and anti-VEGF plus imatinib) = 0.0403); two-tailed $t$-test. Data presented as mean ± s.e.m.
Supplementary Figure 9. Systemic effects of anti-VEGF plus imatinib therapy on healthy vasculatures. (a-d) Immunohistochemical images of CD31<sup>+</sup> microvessels (red) and NG2<sup>+</sup> pericytes (green) and quantification of microvessel density and pericyte coverage of vehicle- and anti-VEGF plus imatinib-treated healthy mice. a. Treatment impact on thyroid tissues (n = 7 samples per groups; P(CD31<sup>+</sup> microvessels: Vehicle-treated vs imatinib plus anti-VEGF-treated) = 0.0153). b. Treatment impact on skeletal muscle tissues (n = 6 samples per groups). c. Treatment
impact on renal cortex (n = 6 samples per groups). d. Treatment impact on glomeruli (n = 4 in vehicle, n = 5 in combination-treatment group). (e-h) Vascular permeability of fluorescein-labeled lysinated 70 kDa dextran (green) and vascular perfusion of fluorescein-labeled lysinated 2000 kDa dextran (green) stained with CD31+ microvessels (red). Quantification of vascular permeability and perfusion of vehicle- and anti-VEGF plus imatinib-treated groups in various organs. e. Treatment impact on thyroid tissues (n = 8 samples per groups; P(Vascular permeability: Vehicle-treated vs combination) = 0.0102). f. Treatment impact on skeletal muscle tissues (n = 8 in permeability, n = 7 in perfusion). g. Treatment impact on renal cortex (n = 8 in vehicle, n = 7 in combination-treatment). h. Treatment impact on glomeruli (n = 3 samples each). i. Total body weight of C57BL/6 of vehicle- and anti-VEGF plus imatinib-treated groups (n = 5 animals in vehicle, n = 4 animals in combination-treatment group). j. Systolic and diastolic blood pressure changes after 2-week treatment with vehicle, and anti-VEGF plus imatinib (n = 4 animals per group; P(Systolic: Vehicle-treated vs imatinib plus anti-VEGF-treated) = 0.0212; P(Diastolic: Vehicle-treated vs imatinib plus anti-VEGF-treated) = 0.02150). k. SDS-PAGE gel electrophoresis of urine samples. BSA was used as a marker for albumin. Quantification of the urine proteins (n = 5 animals in vehicle, n = 4 animals in combination-treatment group). Urine from non-treated healthy mice (n = 4 animals) was used as a control. All scale bar = 25 μm. n.s. = Not significant; *P < 0.05; two-tailed t-test. Data presented as mean ± s.e.m. Experiments were repeated two times. Source data are provided as a Source Data file.
Supplementary Figure 10. Tumor growth of anti-FGFR-treated FGF-2+ fibrosarcomas. Tumor growth of vehicle- (n = 4 animals per group; \( P = 0.0223 \)) and BGJ398-treated (n = 6 animals per group) T241-FGF-2 fibrosarcomas. *\( P < 0.05 \); two-tailed \( t \)-test. Data presented as mean ± s.e.m. Experiments were repeated two times. Source data are provided as a Source Data file.
Supplementary Figure 11. Gating strategies for flow cytometry and a full scan image. Gating strategy used for flow cytometry analysis. a. FSC-H/SSC-H gate for exclusion of debris. Gates for F4/80+ inflammatory macrophages (b), CD3+ T cells (c), CD4+ T cells (d), and CD8+ T cells (e). NC= negative control. f. Full scan data of immunoblot used for supplementary figure 9k.