Supplementary Legends

Table S1: Specific primers for RT-PCR.

Table S2. Phosphorylation of downstream intracellular kinases. Cell lysates of three AML samples treated with or without PTK/ZK were subjected to a Human Phospho-Kinase Array Kit. The list displays the phosphorylation data of the downstream intracellular kinases.

Figure S1. Effect of PTK787/ZK 222584 and MK2206 on AML cell lines. (A) A time- and dose-dependent assay for PTK/ZK and control (DMSO) determined by Annexin/PI staining, measured by FACS analysis. Early apoptotic cells are Annexin-positive and PI negative, whereas cells that are in late apoptosis or already dead are both Annexin and PI positive. As shown for both cell lines, an induction of apoptosis was dose-dependent. (B) FACS analysis of the tyrosine kinase receptors VEGFR2, c-KIT, c-FMS and PDGFRß. Percentage cells positive for protein expression was measured after 24 hours of culture for both culture conditions. Expression of VEGFR3 was not detectable (not shown). No difference was seen on receptor expression when cultured with PTK/ZK compared with control cultures. (C) After 48 hours of incubation with PTK/ZK, western blot analysis showed a downregulation of pAKT in MOLM13 and THP-1 cells independent of total AKT levels. For MOLM13 a slight downregulation of pERK could be seen, independent of total ERK expression. pSRC and total SRC levels did not

significantly differ between cells treated with PTK/ZK compared with control. (D) HL-60, NB4 and THP-1 cells were treated with MK2206 (0.1-10 μM) for 48 hours, and cell survival percentages were measured by using a WST assay. MK2206 induced a dose-dependent decrease in cell survival in all three AML cell lines.

Figure S2. Effect of VEGFA-addition, Bevacizumab, specific VEGFR-inhibitor and MK2206 on CD34+ sorted pediatric AML cells (A) Relative mRNA expression of VEGFA for 11 AML samples is shown. (B) Relative mRNA expression of VEGFA165 in MS5-control compared with MS5-VEGFA. A 27-fold upregulation was found (C) ELISA confirmed secreted VEGFA protein levels in the supernatant of the MS5-VEGFA cells (751 pg/ml), whereas VEGFA was not detectable in supernatant of the MS5-control cells. ELISA of supernatant of AML samples cultured for 10 weeks showed that VEGFA protein production was sustained during culture. (D) Recombinant VEGFA induces expression of NUR77 in ECs in a dose dependent way. Supernatant of transduced MS5 cells contained functional VEGFA, indicated by the expression of NUR77. MS5-VEGFA showed a higher expression of NURR77 than MS5-control, demonstrating that VEGFA165 cells produce more functional VEGFA. Similar graphs were seen when investigating the expression of EGR3 and NOR1. (E) Median growth curve of sorted CD34+ cells of 6 pediatric patients cultured on MS5-control or MS5-VEGFA165. No significant difference in expansion was seen between both conditions (paired Wilcoxon signed rank test, week 2-10, $P>.05$). (F) Median growth curve of sorted CD34+ cells of 6 pediatric patients cultured with or without addition of Bevacizumab. No significant difference in expansion was seen between both conditions (paired Wilcoxon signed

rank test, week 2-10, P>.05). (G) CD34+ cells of AML6 were cultured on MS5 with a specific VEGFR2-inhibitor, specific VEGFR3-inhibitor or control up to 5 weeks. No decrease in cell expansion was seen after addition of the specific VEGFR-inhibitors. (H) CD34+ cells of AML14 were treated with MK2206 (0.1-10 μM) for 48 hours, and cell survival percentages were measured by using a WST assay. MK2206 induced a dose-dependent decrease in cell survival. (I) Annexin-V staining showed an increase of (early) apoptotic when CD34+ sorted AML cells of AML 6 and AML14 were incubated with 0.1 μM MS2206 compared with control (DMSO).