Supplemental Figures

Figure S1 Mean vessel densities of tumours at study endpoint. There is no significance difference in mean vessel densities between tumours in any of the cohorts. The vehicle and gemcitabine treated cohorts are presented together (V/G), as are the PEGPH20 and gemcitabine/PEGPH20 treated cohorts (P/P+G).

Figure S2 Plasma gemcitabine pharmacokinetics from control mice treated with either vehicle (black) or PEGPH20 (green) 1 hour prior to gemcitabine. There is no significance difference in plasma dFdC or dFdU either thirty or sixty minutes after dosing with gemcitabine.

Figure S3 Immunofluorescence micrographs from vehicle or PEGPH20 treated genetic control (PC) mice that were injected with biotinylated lectin (green) and two different molecular weight dextrans (magenta = 40kDa, yellow = 2MDa, n=4 mice per cohort). Normal tissues were not different in interstitial dextran distribution between treatment groups. (Scale bar = 100mm).

Figure S4 Tumour growth curves based on volume for each KPC mouse on each arm of the survival trial. Some mice progressed and were removed from study prior to obtaining an accurate volumetric measurement (e.g. MH812). From the top, vehicle, gemcitabine, PEGPH20, and gemcitabine/PEGPH20 treated cohorts. Y axis = Percent Tumour Volume (0-600%), X axis = Time (-20- to +40 days).

Figure S5 Absence of HA expression at endpoint in PEGPH20 and gemcitabine/PEGPH20 treated cohorts. HA is detectable in vehicle and gemcitabine treated cohorts (top panel, left three images). (Scale bars 4X = 250 μm, 40X = 100 μm). Quantification indicates an enrichment of low-HA expressing tumours in the PEGPH20 and gemcitabine/PEGPH20 treated cohorts at endpoint.

Supplemental Methods

Agent Formulation and Dosing

PEGPH20 (3.5mg/mL solution, Halozyme) was administered intravenously, one dose of 4.5mg/kg for the gemcitabine delivery assay, two doses of 4.5mg/kg 72 hours apart for the vascular function and doxorubicin delivery assays, and two doses of 5mg/kg per week on days 1 and 4 for the survival study.

Lyophilised streptavidin – AlexaFluor633 (Cat.no. S21375, Invitrogen) and lyophilised biotinylated *Lycopersicon esculentum* lectin (Cat.no. B1175, Vector Labs) were reconstituted with sterile 0.85% saline and mixed to give a working labelling agent (7), administered by slow intravenous injection at 20mg/kg lectin. FITC-conjugated dextran of molecular weight 40kDa and TRITC-conjugated dextran of 2MDa (Cat.no. D1845 and D7139, Invitrogen) were reconstituted with sterile 0.85% saline to 10 mg/ml and a single dose was administered by slow intravenous injection at 40mg/kg.

Doxorubicin hydrochloride powder (Cat.no. D1515, Sigma) was reconstituted with sterile 0.85% saline to 4mg/mL and a single dose was administered by slow intravenous injection at 20mg/kg.

Gemcitabine hydrochloride powder (~48% dFdC, Eli Lilly) was reconstituted with sterile 0.85% saline to 5mg dFdC/mL and administered intraperitoneally, one dose of 100mg dFdC/kg for the aflibercept pharmacodelivery study, and two doses of 100mg dFdC/kg per week on days 1 and 4 for survival studies. For the $VEGF^{164}$ and PEGPH20 pharmacodelivery studies, gemcitabine hydrochloride was reconstituted to 40mg dFdC/mL and a single dose was administered intravenously at 100mg dFdC/kg.

Electron Microscopy

Mice were terminally perfused one hour after the final dose of PEGPH20. Terminal perfusion consisted of 0.9% saline containing heparin (1U/ml) followed by 150mL of 3% glutaraldehyde and 1% formaldehyde in 0.1 M HEPES buffer. The tumours were excised and fixed by immersion in the 3% glutaraldehyde/1% formaldehyde solution for a further 24 hours. The tumours were cut into slices and processed for either scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

Tissue used for SEM was dehydrated in 100% ethanol and frozen in liquid nitrogen, followed by fracturing with razor blades cooled in liquid nitrogen. The fractured tissue fragments were rehydrated in deionized water and incubated with 1% osmium ferricyanide at 4°C for 18 hours. The tissue was then treated with 2% uranyl acetate in 0.05M maleate buffer at pH 5.5 for 18 hours at 4°C, before being dehydrated in an ascending series of ethanol solutions. Coverslips were placed in a Polaron critical point dryer (Quorum/Emitech) and glued to SEM stubs with colloidal silver. The stubs were coated with 10nm of gold in a Quorum/Emitech K575X sputter coater and viewed in an FEI-Philips XL30 FEGSEM at 5kv. At least seven tumour core vessels were imaged per mouse, and at least four mice were evaluated for each experiment. Images were recorded at 20,000X magnification and a quadratic lattice was overlain on each image. Point counting was used to determine the percentage of endothelial surface occupied by fenestrae.

Slices used for TEM were dehydrated in 100% ethanol, rinsed twice in acetonitrile, and embedded in Quetol epoxy resin. They were sectioned at 50-70nm using a Leica Ultracut UCT, mounted on 300 mesh grids, stained with uranyl acetate and lead citrate and viewed in a FEI Tecnai G2, TEM operated at 120kv. Images were captured with an AMT XR80B digital camera running Deben software.

HA Tissue Microarray

A blinded pathologist confirmed tissue quality and tissue cores in the arrays with less than 10% of tumour cells or more than 50% of necrotic tissue were excluded for the evaluation. PC3 xenograft tumour tissues served as positive control tissue to determine intensity threshold for positive pixel quantity. HA staining specificity was confirmed by digesting a subset of sections with recombinant human hyaluronidase PH20 (1000 U/mL in PIPES buffer) before addition of bHABP.