Nanoparticle-encapsulated siRNAs for gene silencing in the haematopoietic stem-cell niche

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Bone-marrow endothelial cells in the haematopoietic stem-cell niche form a network of blood vessels that regulates blood-cell traffic as well as the maintenance and function of haematopoietic stem and progenitor cells. Here, we report the design and in vivo performance of systemically injected lipid–polymer nanoparticles encapsulating small interfering RNA (siRNA), for the silencing of genes in bone-marrow endothelial cells. In mice, nanoparticles encapsulating siRNA sequences targeting the proteins stromal-derived factor 1 (Sdf1) or monocyte chemotactic protein 1 (Mcp1) enhanced (when silencing Sdf1) or inhibited (when silencing Mcp1) the release of stem and progenitor cells and of leukocytes from the bone marrow. In a mouse model of myocardial infarction, nanoparticle-mediated inhibition of cell release from the haematopoietic niche via Mcp1 silencing reduced leukocytes in the diseased heart, improved healing after infarction and attenuated heart failure. Nanoparticle-mediated RNA interference in the haematopoietic niche could be used to investigate haematopoietic processes for therapeutic applications in cancer, infection and cardiovascular disease.

Human bone marrow harbours about 10,000 bona fide haematopoietic stem cells as well as millions of downstream progenitors and releases billions of blood cells into the circulation every day\textsuperscript{1-3}. The organ produces a cellular ensemble that accomplishes vital tasks including oxygen transport, defence against pathogens and clotting\textsuperscript{4,5}. The activities of its inhabitants, such as cell quiescence, proliferation, differentiation and migration, are adjusted to current systemic needs and regulated by non-haematopoietic bone marrow niche cells\textsuperscript{3,4}. This cast of supporting cells includes endothelial cells, which instruct haematopoietic cell behaviour via a mix of soluble and cell-surface-bound signals\textsuperscript{6,7,8}. Niche cells receive circulating and neuronal signals from outside the marrow and relay them to haematopoietic stem and progenitor cells (HSPCs)\textsuperscript{8}.

Over the past decade, many niche cell steady-state functions have been discovered, leading to approved drugs for stem-cell mobilization before transplantation\textsuperscript{1}. Drugs such as filgrastim that disrupt the interactions between stromal-derived factor 1 (SDF1) and its receptor CXCR4 on leukocytes and HSPCs are now widely utilized as agents to mobilize stem cells into the bloodstream for bone marrow transplantation\textsuperscript{9}. Such agents have primarily been applied in the realm of haematology/oncology; however, recent evidence suggests that leukocyte and HSPC release from bone marrow plays an essential role in many other chronic inflammatory conditions, including cardiovascular disease\textsuperscript{10}. Broadly speaking, the number of circulating leukocytes and the production of blood components in the haematopoietic niche correlate closely with mortality\textsuperscript{11}, and if the bone marrow fails altogether, the organism succumbs within a week or two\textsuperscript{12,13}. Therefore, technologies that modulate cell behaviour within the haematopoietic niche could improve our fundamental understanding and treatment of a range of disease processes that are governed by bone-marrow-derived leukocytes.

RNA interference (RNAi) therapeutics are a potentially attractive means to influence protein expression within the haematopoietic niche, as they can be used to silence nearly any gene within the body to achieve therapeutic effects\textsuperscript{14}. Currently, the most advanced RNAi therapeutic is patisiran, a small interfering RNA (siRNA) lipid nanoparticle-based drug\textsuperscript{14}. Patisiran, recently approved by the FDA, inhibits hepatic transthyretin production as a form of transthyretin amyloidosis therapy\textsuperscript{14}. Because the gene sequences are known, siRNA drugs can be screened for in silico, produced and validated within very short time spans. However, although potent siRNAs can be rapidly identified, systemic delivery to the appropriate tissue can prove challenging. The use of RNAi to treat disease requires effective methods of targeted delivery, as ‘naked’ siRNAs are unstable in the bloodstream and do not readily traverse cell membranes\textsuperscript{15}.

With significant advantages over their non-formulated and free drug counterparts, nanoparticle delivery systems have been
**Fig. 1 | Composing a BMEC-targeting nanoparticle.**

**a.** Synthesis scheme of the epoxide-modified polymer–lipid hybrid material. **b.** Nanoparticles consisting of lipid hybrid, siRNA and a PEG–lipid conjugate were synthesized via microfluidic mixing. **c.** PEG–lipid conjugate parameters were varied to form a library of unique nanoparticles for siRNA delivery to BMECs. Values outlined by red dashed boxes indicate the best silencing efficiency. **d.** Tie2 silencing in femoral bone marrow of C57BL/6 mice 48 h after injection of each nanoparticle containing siRNA against Tie2 (dosage, 1.0 mg kg\(^{-1}\); \(n=4\) mice per group; data are normalized to mRNA expression in naive mice). The strongest silencing was seen for NicheEC-15 nanoparticles (highlighted in red). **e.** Cryo-transmission electron microscopy (TEM) of NicheEC-15 nanoparticles. **f.** Hydrodynamic diameter of NicheEC-15 nanoparticles. **g.** Tie2 silencing in bEnd.3 (**g**) and sorted BMECs (**h**) 24 h after addition of siLuc or siTie2 to the culture medium. Tie2 mRNA expression after exposure to siLuc was normalized to 100% (\(n=3\) independent experiments (g) or three mice (h) per group; two-sided t-test). **i.** Confocal microscopy of bEND.3 cells 2 h after adding NicheEC-15 to the culture medium. Nuclei were stained with SYTO 13 and cell membranes with wheat germ agglutinin (WGA). In the upper panels the nanoparticles were visualized by siRNA labelled with Alexa Fluor 647 (AF647). In the lower panels, an unlabelled control siRNA was used. This experiment was repeated twice with similar results. Data are shown as mean ± s.e.m.
used effectively as delivery vehicles in several medical settings\textsuperscript{18}. For siRNA delivery, nanoparticles’ key advantages are (1) preventing nucleic acid degradation by serum endonucleases in blood, (2) avoiding renal clearance from the bloodstream, (3) delivering cargo to specific cells by tailoring the nanoparticle surface chemistry and (4) mediating target cell entry and endosomal escape to enable nucleic acid release into the cytoplasm\textsuperscript{14,14}. Delivery materials differ in efficiency, toxicity and biodistribution, and certain nanoparticles have avidity to certain cell types, tissues and organs\textsuperscript{19}, particularly to hepatocytes, leukocytes and endothelial cells\textsuperscript{16-24}. Of note, our group previously reported a nanoparticulate formulation consisting of low-molecular-weight polyamines and lipids that mediated potent gene silencing in endothelial cells residing in the lung\textsuperscript{7}.

Here we describe the development of an siRNA formulation capable of delivering siRNA to endothelial cells in the haematopoietic niche. We first screened a library of nanoparticles based on a class of nanoparticle-forming materials that were generated by combinatorial chemical synthesis and deliver siRNA to lung endothelium in vivo\textsuperscript{17-24}. These materials were synthesized by reacting low-molecular-weight polyamines with epoxide-terminated lipids using an epoxide ring-opening reaction\textsuperscript{17}. By screening a library of these nanoparticles in vivo, we developed a polymer–lipid hybrid nanoparticle for delivery to bone marrow endothelial cells. In a series of proof-of-concept experiments, we silence endothelial cell expression of two quintessential haematopoietic niche factors, thereby altering HSPC behaviour and systemic leukocyte supply. Such an RNAi approach is of interest to modulating haematopoietic cell activity and abundance in fundamental science and to developing new therapeutics.

Results

Nanoparticle screen for in vivo siRNA delivery to the haematopoietic niche. Bone-marrow endothelial cells (BMECs) are an integral component of the haematopoietic niche and instruct stem cell and leukocyte behaviour. Cell-specific deletion experiments revealed that both progenitor proliferation and migration are governed by BMEC-derived signals\textsuperscript{17-21}. Based on previously reported endothelial-cell-avid delivery materials\textsuperscript{17}, we began developing suitable delivery materials for BMECs. We synthesized a polymer–lipid hybrid material by reacting C15 epoxide-terminated lipids with low-molecular-weight polyamines (PEI600) at a 14:1 molar ratio via Michael addition chemistry (Fig. 1a). The resulting material was combined with siRNA and a polyethylene glycol (PEG)–lipid conjugate in a high-throughput microfluidic mixing chamber\textsuperscript{22} to formulate nanoparticles via electrostatic interactions between the cationic polymeric material and the negatively charged nucleic acid (Fig. 1b). Using this nanomaterial as a starting point, we then tested different PEG surface coatings that alter the biodistribution and pharmacokinetics of various nanoparticle types\textsuperscript{19,23}. We hypothesized that modulating the nanoparticle PEG architecture enhances nanoparticle siRNA delivery to the bone marrow. By altering their PEG decoration, we created a library of 15 nanoparticle formulations, with three key parameters of the PEG–lipid conjugate modified in each candidate nanoparticle: (1) the molecular weight of the PEG surface coating (molecular weight range: 2,000–5,000), (2) PEG surface density, which was altered by varying the overall molar percentage of PEG within the formulation (molar percentage range, 2–20%) and (3) the length of the lipid chain that anchors PEG within the nanoparticle membrane (Fig. 1c). We injected this mini-library intravenously into mice (1.0 mg per kg body weight) and then assessed Tie2 silencing in the bone marrow. Tie2 was chosen as a silencing target purely for this delivery and silencing assay, and not for the gene’s function, which has roles beyond BMECs. Using a branched DNA assay, we identified the best nanoparticle formulation (termed NicheEC-15), which induced ~80% Tie2 in vivo gene silencing in bone marrow (Fig. 1d) without disrupting the vascular anatomy (Supplementary Fig. 1). Cryo-scanning electron microscopy and dynamic light scattering (DLS) showed that Niche-EC-15 formed multilamellar nanoparticles (Fig. 1e) with a 60–80-nm diameter (Fig. 1f). We next assessed Niche-EC-15 for Tie2 silencing in endothelial cells in vitro. Nanoparticles containing 60 nM Tie2 siRNA induced potent gene silencing in the mouse endothelial cell line bEnd.3 (Fig. 1g) and in primary murine BMECs (Fig. 1h). Confocal microscopic imaging confirmed efficient in vitro uptake of nanoparticles containing fluorescent siRNA (Fig. 1i).

In vivo behaviour of the lead particle NicheEC-15. The in vivo blood half-life of NicheEC-15 nanoparticles, containing a fluorescent siRNA, was measured to be 13.8 min by fitting the decrease in blood fluorescence intensity over time after a single intravenous (i.v.) injection (Fig. 2a). Using a range of 0.01–1.0 mg kg\textsuperscript{-1} siTie2, we observed dose-dependent knockdown of Tie2 messenger RNA (mRNA) expression by quantitative polymerase chain reaction (qPCR; Fig. 2b). At a dose of 1 mg kg\textsuperscript{-1} siTie2, the knockdown achieved with NicheEC-15 nanoparticles was ~50% stronger than 7C1 nanoparticles\textsuperscript{10}, a previously described nanoparticle formulation.

**Fig. 2** | In vivo uptake of NicheEC-15 nanoparticles in BMECs. a. The decline in blood fluorescence intensity was measured over time following a single injection of NicheEC-15 AF647-siRNA. The in vivo circulation half-life of NicheEC-15 nanoparticles was calculated to be 13.8 min (n=5 mice). b. Dose-response curve of Tie2 silencing in whole bone marrow after in vivo injection of NicheEC-15 or 7C1 nanoparticles encapsulating siTie2 by branched DNA (bDNA) assay (dose range 0.01–1.0 mg kg\textsuperscript{-1}; n=4 mice per group and time point). c. Time course of Tie2 expression in whole bone marrow following a single 1.0 mg kg\textsuperscript{-1} injection of NicheEC-15 or 7C1 encapsulating siTie2 by bDNA assay. Femurs were collected 1–18 days post-injection (n=4 mice per group and time point). The plots in b and c show fold changes in mRNA expression compared to naive mice. d. Intravital microscopy of the skull bone marrow 2 h after injection of NicheEC-15 with AF647-siRNA cargo. The vasculature was stained with PE-labelled CD31 and Sca-1 antibodies and OsteoSense was used to visualize bone. e, Mice were euthanized 2 h after i.v. injection of 2 mg kg\textsuperscript{-1} NicheEC-15 AF647-siRNA. BMECs were gated as shown in the FACS plots (left and middle). A histogram of endothelial cells (ECSs) is shown (right) with CD45\textsuperscript{+} leukocytes and endothelial cells of un.injected control mice. SCC indicates side scatter. f, ImageStream results of endothelial cells 2 h after injection of NicheEC-15 AF647-siRNA (top) compared to PBS-injected controls (bottom). g, h. Uptake of the nanoparticles in endothelial cells compared to control mice injected with PBS. Of 816 endothelial cells, 764 (93.4%) were positive for siAF647 based on mean AF647 positive pixel number (≈AF647+ pixel) (g) and 682 (83.6%) based on peak cellular signal intensity for AF647 (=AF647 SI) (h). i, ImageStream results of endothelial cells 2 h after injection of NicheEC-15 AF647-siRNA with additional staining for podoplanin, identifying sinusoidal endothelial cells. j, ImageStream results of leukocytes 2 h after injection of NicheEC-15 AF647-siRNA. k, l, Uptake of the nanoparticles in endothelial cells compared to leukocytes based on mean AF647 positive pixel number (k) and peak cellular signal intensity for AF647 (l). WBC indicates CD45\textsuperscript{+} leukocytes. Experiments d–l were repeated twice with similar results. m, Mean fluorescence intensity by flow cytometry of un.injected mice, CD45\textsuperscript{+} leukocytes, BMECs and lung endothelial cells 2 h after injection of 2 mg kg\textsuperscript{-1} AF647-siRNA encapsulated into the 7C1 or NicheEC-15 nanoparticles (n=4 mice each, except CD45\textsuperscript{+} n=8; one-way analysis of variance (ANOVA) with multiple comparisons). n, The mean fluorescence intensity (MFI) of the BMECs was divided by the MFI of endothelial cells for 7C1 and NicheEC-15, giving 1.39 for 7C1 and 3.17 for NicheEC-15. o, Comparison of Tie2 silencing in whole bone marrow and lung after in vivo injection of NicheEC-15 or 7C1 encapsulating siTie2 by bDNA assay (dose 1.0 mg kg\textsuperscript{-1}; n=4 mice each; two-sided t-test without multiple comparisons; fold changes in mRNA expression compared to naive mice). Data are shown as mean±s.e.m.
with excellent silencing in endothelial cells. A single injection of NicheEC-15 nanoparticles containing 1.0 mg kg\(^{-1}\) siTie2 induced long-lasting knockdown for more than two weeks (Fig. 2c). To better understand the uptake patterns of NicheEC-15 nanoparticles, we next directly visualized the haematopoietic niche using intravital microscopy of the skull bone marrow of mice. The vasculature was stained by intravenously injecting a cocktail of PE-labelled CD31 and Sca-1 antibodies, while OsteoSense, a molecular imaging probe that enriches in osteoblasts, outlined the bone surface surrounding haematopoietic niches\(^{29}\). Two hours after injecting NicheEC-15-containing fluorescently tagged siRNA, we primarily found the siRNA associated imaging signal in the bone marrow vasculature (Fig. 2d). The cellular distribution of NicheEC-15 nanoparticles was measured by flow cytometric analysis of the bone marrow. Although

**Figure 2:**

**a** Normalized fluorescence intensity over time after injection of NicheEC-15 nanoparticles. The half-life of Tie2 mRNA expression is 13.8 min.

**b** Tie2 mRNA expression over different doses of siRNA.

**c** Tie2 mRNA expression over days after injection.

**d** Intravital microscopy images showing CD31 + Sca1, siRNA, OsteoSense, and merged images.

**e** Flow cytometric analysis showing CD45, CD31, and Sca1 expression.

**f** Bright-field images of NicheEC-15 and PBS with siRNA and Merged.

**g** Flow cytometric analysis showing normalized frequency of CD1 and CD45* ECs.

**h** Flow cytometric analysis showing normalized frequency of AF647* pixel.

**i** Bright-field images of NicheEC-15 and PBS with siRNA and Podoplanin.

**j** Flow cytometric analysis showing normalized frequency of NicheEC-15 and PBS with siRNA and Podoplanin.

**k** Flow cytometric analysis showing normalized frequency of AF647* pixel.

**l** Flow cytometric analysis showing normalized frequency of AF647 Si.
we observed the highest fluorescence intensity in CD45−CD31+Sca-1+ endothelial cells, nanoparticle uptake into CD45+ leukocytes was minimal (Fig. 2e). Uptake into other bone marrow cells (Supplementary Fig. 2), and into leukocytes and endothelial cells in other organs (Supplementary Fig. 3a,b) was lower than for BMECs. Such uptake, especially in organs that eliminate drugs or nanoparticles from the bloodstream, is to be expected. Macroscopic fluorescence imaging of key organs 2 h after intravenous injection of NicheEC-15 encapsulating fluorescently labelled siRNA revealed strong fluorescence in the femur marrow (Supplementary Fig. 3c–f). Compared to a previously described lipidoid nanoparticle with dominant uptake in the liver and immune cells, NicheEC-15 bone marrow uptake was higher (Supplementary Fig. 3g). The avidity of NicheEC-15 nanoparticles for podoplanin+ arteriolar and podoplanin+ sinusoidal BMECs was confirmed by ImageStream (Fig. 2f–i), which indicates uptake into major bone marrow endothelial cell subsets. Cell imaging detected no NicheEC-15-associated fluorescence in bone marrow leukocytes (Fig. 2j–l).

We next benchmarked NicheEC-15 to 7C1 in vivo. Compared to 7C1, NicheEC-15 uptake into BMECs increased significantly, while uptake into lung endothelial cells decreased (Fig. 2m). Although 7C1 uptake was similar in both organs, NicheEC-15 exhibited a threefold higher uptake into bone marrow endothelial cells (Fig. 2n), a result that supports NicheEC-15's higher avidity for the marrow over the lung. Furthermore, directly comparing Tie2 in lung and bone marrow showed that NicheEC-15 enhances gene silencing in bone marrow while reducing such effects in lung, compared to 7C1 (Fig. 2o). Such a predilection may allow for high silencing efficiency in the marrow at lower doses.

**Sdf1/Mcp1 target screening.** Having identified NicheEC-15 as a delivery material, we proceeded to implement RNAi for modulating the haematopoietic bone marrow niche. We began by selecting two target proteins that influence haematopoietic stem cell and leukocyte behaviour: stromal-derived factor 1 (Sdf1, also known as Cxcl12) and monocyte chemotactic protein 1 (Mcp1, also known as Ccl2). These
Fig. 4 | Release of bone marrow monocytes and neutrophils after siSdf1 treatment. 

a, Representative dot plots 2 h after injection. The numbers shown indicate the percentage of cells of the parent gate. The experiment was repeated twice with similar results. 
b, Percentage of neutrophils (top) and monocytes (bottom) in the blood, assessed by flow cytometry (n = 10 mice for naive and 2 h, n = 5 mice for 6-h neutrophils and n = 7 mice for 6-h monocytes; two-way ANOVA with multiple comparisons). 
c, Intravital microscopy imaging of Cx3cr1+ cells in the skull bone marrow. Baseline images were taken before injection and cells were tracked by imaging every 10–30 min for a total of 2.5 h. The locations of cells compared to baseline and at the last imaging after 2.5 h are indicated with white circles. Red circles mark the areas of cells that have left the niche. 
d, Percentage of Cx3cr1+ cells in the same area after 2.5 h compared to baseline, which was adjusted to 100% (n = 4 mice each, four fields of view per mouse; two-sided t-test). 
e, The same set-up as in c, but neutrophils are labelled with Ly6g antibody. 
f, As in d, the percentage of neutrophils after 2.5 h compared to baseline (n = 4 mice each, four fields of view per mouse; two-sided t-test). Data are shown as mean ± s.e.m.
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Fig. 5 Effects of siMcp1 treatment during LPS-induced inflammation. a, Experimental set-up. b, Mcp1 expression in whole bone marrow by qPCR normalized to GAPDH expression (n = 3 mice per group, except ‘No LPS’ n = 5; two-way ANOVA with multiple comparisons). c, Mcp1 levels by enzyme-linked immunosorbent assay (ELISA) in bone marrow plasma (n = 4, 7 and 5 mice per group, respectively; two-way ANOVA with multiple comparisons). d, FACS plots of bone marrow neutrophils and monocytes. Numbers shown indicate the percentage of cells of the parent gate. Gating was performed from top to bottom as indicated by the arrows. The experiment was repeated twice with similar results. e, Number of monocytes per femur. f, Number of Ly6c\textsuperscript{high} monocytes per femur. g, FACS plots of blood neutrophils and monocytes. Numbers indicate the percentage of cells of the parent gate. Hasing was performed from top to bottom as indicated by the arrows. The experiment was repeated twice with similar results. h, Number of monocytes per ml of blood. i, Number of Ly6c\textsuperscript{high} monocytes per ml of blood. In e, f, h and i, n = 9 mice per group each; two-sided t-test. Data are shown as mean ± s.e.m.

Well-studied proteins\cite{1,2,3} were chosen because their known functional properties allowed us to use specific functional in vivo assays; additionally, because these proteins control cellular quiescence and migration, they are interesting drug targets. Viable siRNA sequences targeting Sdf1 (Supplementary Fig. 4) and Mcp1 (Supplementary Fig. 5) were identified by in vitro screening of in silico-predicted candidates. The top four siRNA sequences silencing Sdf1 (Supplementary Fig. 4a,b) and Mcp1 (Supplementary Fig. 5a,b) were then screened in vitro with a dose–response assay. The siRNAs with the lowest half maximal inhibitor concentration (IC\textsubscript{50}) and 80% of maximal inhibitor concentration (IC\textsubscript{80}) were selected for scale-up, nanoparticle formulation and modification to minimize immunostimulation and off-target gene silencing\cite{4}. The lead siRNA candidates encapsulated in NicheEC-15 nanoparticles induced poten, dose-dependent gene silencing of Sdf1 (Supplementary Fig. 4c) and Mcp1 (Supplementary Fig. 5c) at low dosages in endothelial cells in vitro.

SiSdf1 triggers bone marrow cell release. After tail-vein injection of NicheEC-15 nanoparticles loaded with siRNA cargo targeting Sdf1 (siSdf1), we found a greater than twofold decrease in Sdf1 expression (Fig. 3a) and significantly decreased Sdf1 protein in the femur (Supplementary Fig. 6a). To explore the cellular target responsible for this effect, using fluorescence-activated cell sorting (FACS), we isolated bone marrow endothelial cells from mice after treatment with NicheEC-15 nanoparticles containing siSdf1. Indeed, compared to controls, Sdf1 expression was strongly reduced in these cells (Fig. 3b) but not in other niche cells (Supplementary Fig. 6b–d). To evaluate the specificity of RNAi effects, we also examined the expression of two other genes that influence haematopoietic stem cell behaviour in the haematopoietic niche, Vcam1 and Sdf. As expected, no change in gene expression was observed in BMECs, osteoblasts or mesenchymal stromal cells after treatment with siSdf1 (Supplementary Fig. 6e,f).

Because Sdf1 retains HSPCs\cite{5} and leukocytes\cite{6} in the bone marrow, we proceeded to investigate HSPC migration to monitor the functional consequences of RNAi. Indeed, we observed higher lineage– sca1\textsuperscript{+} c-kit\textsuperscript{+} (LSK) cell numbers in blood of mice treated with siSdf1 (Fig. 3c), indicating release of these progenitor cells from...
the marrow. The increase in circulating HSPCs was confirmed by colony-forming unit (c.f.u.) assays in blood (Fig. 3d). Accordingly, siSdf1 injection decreased bone marrow LSK cells (Supplementary Fig. 6g,h). Similarly, downstream myeloid progenitors, including common myeloid progenitors (CMPs) and granulocyte macrophage progenitors (GMPs), declined in numbers in the femur after siSdf1 treatment (Supplementary Fig. 6i–k).

To investigate if RNAi leads to liberation of functional haematopoietic stem cells into the blood, we co-transplanted blood from siSdf1 and PBS-treated control mice into lethally irradiated recipients (Supplementary Fig. 7a). Eight weeks later, almost all leukocytes in the blood transplant recipients originated from siSdf1-treated mice (Supplementary Fig. 7b,c). The marrow of primary recipients was then used for a secondary transplantation into lethally irradiated recipient mice. Nine of the ten recipient mice survived for six months, after which they exhibited high blood chimerism for major leukocyte subsets (Supplementary Fig. 7d,e). In a head-to-head comparison of RNAi-triggered bone marrow stem cell release versus results with the clinical drug AMD3100, which inhibits CXCR4 (the cognate receptor of Sdf1), and granulocyte colony-stimulating factor (G-CSF), we found that the siSdf1 treatment effects were positioned between those of AMD3100 and G-CSF (Supplementary Fig. 7f).

In addition to HSPC release, we also found that siSdf1 treatment leads to monocyte and neutrophil departure from the bone marrow (Fig. 3k–m). As a consequence, neutrophils and monocytes may become more numerous in the circulation. Because the level of circulating myeloid cells regulates their presence at sites of inflammation

Fig. 6 | Effects of siMcp1 treatment on inflammatory cells 24 h after MI. a, Mcp1 expression in whole bone marrow by qPCR normalized to GAPDH expression. b, Mcp1 levels, by ELISA, in bone marrow plasma. c, FACS plots of bone marrow monocytes. Numbers shown indicate the percentage of cells of the parent gate. Gating was performed from top to bottom as indicated by the arrows. In a and b, n = 5 mice per group each; two-sided t-test. d, Number of monocytes per femur. e, Number of Ly6c<sup>high</sup> monocytes per femur. In d and e, n = 10 mice per group each; two-sided t-test. f, FACS plots of blood neutrophils and monocytes. Numbers shown indicate the percentage of cells of the parent gate. Gating was performed from top to bottom as indicated by the arrows. g, Number of monocytes per ml of blood. h, Number of Ly6c<sup>high</sup> monocytes per ml of blood. i, FACS plots of the infarct. Numbers shown indicate the percentage of cells of the parent gate. Gating was performed from top to bottom as indicated by the arrows. j, Number of monocytes per mg infarct tissue. k, Number of Ly6c<sup>high</sup> monocytes per mg infarct tissue. In g, h, j and k, n = 9 mice per group each; two-sided t-test. Data are shown as mean ± s.e.m.
and is associated with patient mortality, we studied the early dynamics of this leukocyte release. Two hours after siSdf1 injection, myeloid cells became significantly more abundant compared to baseline, and increased even further at 6 h (Fig. 4a,b). Such a boost in circulating leukocytes may also be caused by redistribution from other cell pools, such as in the lung or spleen. We thus used serial pre- and post-RNAi treatment intravital microscopy to survey the skull bone marrow. We visualized bone marrow macrophages and monocytes in Cx3cr1GFP/+ mice before and up to 2.5 h after siSdf1 injection. By monitoring specific bone marrow niches, we were able to track individual cell behaviour over time (Fig. 4c). Treatment with siSdf1 triggered departure of Cx3cr1+ cells, although this was not observed after treatment with control siRNA (Fig. 4d). A similar dynamic was observed for neutrophils that we labelled in vivo with Ly6g-APC antibody (Fig. 4e,f).

siMcp1 inhibits bone marrow cell release. Although Sdf1 silencing increased cell migration from the marrow, we next chose a gene target to explore the opposite functionality, that is, using RNAi to dampen leukocyte traffic. Such an intervention could be a therapeutic avenue to reduce systemic leukocyte supply in the setting of inflammation induced via the injection of LPS (Fig. 5a). As expected, LPS injection increased Mcp1 expression in the marrow, and such expression was significantly reduced by siMcp1 treatment (Fig. 5b). Mcp1 protein levels were similarly elevated by LPS, which was abrogated by siMcp1 treatment (Fig. 5c). As a consequence, monocyte numbers in the bone marrow were higher in siMcp1-treated mice (Fig. 5d–f), whereas blood monocyte numbers decreased (Fig. 5g–i).

Mcp1/Ccr2 signalling also regulates monocyte migration in sterile inflammation, including during cardiovascular disease. In particular, acute MI triggers blood monocytosis, followed by cell recruitment into the ischaemic myocardium. After MI, systemic oversupply of inflammatory monocytes may lead to heart failure and subsequent ischaemic events. We therefore tested if silencing Mcp1 in mice with acute MI may reduce inflammatory Ly6Chigh monocyte numbers in circulation and the heart. Treating mice with siMcp1 led to a lower bone marrow Mcp1 expression (Fig. 6a) and protein level (Fig. 6b). Consequently, monocyte bone marrow numbers rose (Fig. 6c,d), while circulating monocyte numbers remained lower (Fig. 6f–h), results that indicate attenuated cell release from the marrow. Ultimately, fewer monocytes, including the inflammatory Ly6Chigh subset, were recruited to the infarcted heart in mice treated with siMcp1 (Fig. 6i–k).

Fig. 7 | Cardiac effects of siMcp1 treatment seven days after MI. a, Immunohistochemical staining for myeloid cells (CD11b), anti-smooth muscle actin+ fibroblasts (anti-SMA) and collagen deposition (collagen I) in the border zone of the infarct. After 10 weeks of HFD starting at eight weeks of age, MI was induced by permanent ligation of the left anterior descending artery in ApoE−/− mice. Mice were either treated with siMcp1 or siLuc. b, Bar graphs showing the percentage of positive staining per region of interest (ROI) in the border zone of the infarct. c, Same analyses as in b but in the central infarct core. In a–c, n = 4 mice per group each and three fields of view per mouse; two-sided t-test. Data are shown as mean ± s.e.m.
We evaluated the therapeutic consequences of siMcp1 on cardiovascular outcomes by studying the recovery from MI in ApoE−/− mice with hypercholesterolaemia and atherosclerosis. In these mice, gene deletion and high-fat diet (HFD) lead to blood monocytosis, which arises from increased haematopoiesis36–38. We previously described that the oversupply of monocytes in the post-MI phase disrupts the resolution of inflammation after cardiac ischaemia, leading to poor infarct healing and increased severity of heart failure 39,40. Thus, these mice, like patients with atherosclerosis, hyperlipidaemia and monocytosis, hypothetically benefit from a dampened leukocyte supply to the infarct. ApoE−/− mice were given an atherogenic diet for 10 weeks before permanent coronary ligation. Mice were treated with three doses of control siRNA or siMcp1. We examined infarct healing histologically seven days after coronary ligation. In line with the flow cytometry data described above, RNAi treatment reduced staining for CD11b+ myeloid cells in the border zone and the infarct core (Fig. 7a–d). We also observed a reduction of fibroblast numbers and collagen content in the border zone, indicating that the treatment reduced inflammation and fibrosis.

Additional cohorts of ApoE−/− mice were examined three weeks after MI, when left ventricular remodelling gives rise to heart failure. Cardiac magnetic resonance imaging (MRI) reported that siMcp1 treatment preserved left ventricular anatomy and function when compared to siLuc-treated control ApoE−/− mice (Fig. 8a–e). Masson’s trichrome staining revealed that the percentage of the left ventricle occupied by scar was lower in siMcp1-treated mice (Fig. 8f,g). Taken together, these data indicate that reduction of systemic monocyte supply by RNAi augmented recovery from MI.

**Discussion**

Bone marrow niche cells regulate haematopoietic and leukocyte activity in numerous ways, including cell transit into the circulation12,14. We have developed a nanoformulation capable of delivering siRNA to bone marrow niche cells. These nanoparticles are derivatives of prior materials with avidity for lung endothelial cells13.
We enhanced nanoparticle siRNA delivery to the bone marrow by modulating nanoparticle PEI architecture, including (1) the molecular weight of the PEI surface coating, (2) the length of the lipid chain that anchors PEI within the nanoparticle membrane and (3) the PEG surface density. By screening polymer–lipid hybrid materials, combined with modulating PEG nanoparticle surface coatings, we obtained a nanoparticle, termed NicheEC-15, with superior avidity for and efficient silencing in bone marrow endothelial cells. We speculate that NicheEC-15, perhaps due to its denser PEG surface coat, escapes first-pass entrapment in the lung, which increases arterial circulation and uptake into bone marrow endothelial cells. The mechanism of uptake into bone marrow endothelial cells remains to be determined and may be subject to changes induced by pathologies or treatments such as irradiation or chemotherapy.

To test NicheEC-15, we silenced two prototypical haematopoietic niche factors. One of them, Sdf1, promotes stem cell quiescence and bone marrow retention of leukocytes via interaction with its cognate receptor Cxcr4. This pathway is currently targeted clinically using G-CSF and AMD3100, which trigger stem cell liberation into the blood.2 Silencing HSPC retention factors using nanoparticle-enabled RNAi may improve the cell yield in stem cell transplantation, especially in patients that are poor stem cell mobilizers due to comorbidities such as diabetes.2,3 Our second target, Mcp1, has the opposite effect, as this chemokine triggers bone marrow monocyte release. We found that silencing those proteins, using NicheEC-15 enabled RNAi, altered migration of HSPCs and leukocytes from bone marrow niches into the systemic blood pool. Our data provide a proof of principle that bone marrow HSPC and leukocyte behaviour may be addressed using RNAi therapeutics with avidity for the haematopoietic niche.

Owing to the modular character of RNAi, this approach can be adapted for silencing any other endothelial-cell-derived bone marrow niche factor, including growth hormones, cytokines and adhesion molecules that influence HSPC or leukocyte biology. Furthermore, silencing receptors may modulate the ability of endothelial cells to sense and relay information they receive from the circulation. The function of bone marrow niche cells in the steady state and disease is the subject of vibrant research, and large unbiased datasets (for example, obtained by RNA sequencing of bone marrow endothelial cells) are increasingly becoming available.4 The technology we describe here is suitable for rapidly testing the function of highly expressed genes and newly arising drug candidates in vivo.

Endothelial cells separate bone marrow niches from the blood pool. Their barrier function, which ultimately regulates cell release, makes them a particularly attractive target for modulating cell migration. However, there are other niche cells that regulate haematopoiesis. Future materials may deliver siRNA to bone marrow macrophages, mesenchymal stromal cells or osteoblasts, thereby targeting other niche components. Such additions to the target cell portfolio may also facilitate RNAi for niche-driven malignancies.

Although we have focused on bone marrow cell release, which is of clinical interest in collecting stem cells before autologous haematopoietic bone marrow transplantation for leukaemia treatment, other haematopoietic cell functions could also be influenced. Stem cell quiescence and lineage bias are of particular interest as they decisively influence the size and function of any systemic blood cell pool. As demonstrated by silencing Mcp1, reducing the release of short-lived leukocytes from their site of production may dampen inflammation at any inflammatory site, including the ischemic heart. Thus, reducing the oversupply of innate immune cells may curtail exuberant inflammatory activity in cardiovascular disease,5 the leading cause of death worldwide.

Methods

Synthesis of the polymer–lipid hybrid. Polymer–lipid hybrids were synthesized by reacting C15 alkyl epoxides (Tokyo Chemical Industry and Sigma) with PEI600 at 90 °C in 100% ethanol for 48–72 h at a 1:41 molar ratio. Because of the polydispersity index (PDI) of PEI600 (Mw = 600, PDI = 0.33), polymer–lipids were purified via high-performance liquid chromatography (HPLC) on a silica column with dichloromethane, MeOH and NH4OH washes after coupling PEI containing siRNA targeting one over a 45-min period to decrease polarity, which enabled HPLC separation of the polymer–lipid into fractions related to the hydrophobic C15:hydrophilic PEI ratio. Mixtures were split into five fractions and individually assessed for their ability to induce gene silencing in mice.

siRNA nanoparticle synthesis. To formulate nanoparticles, polymer–lipid materials were combined with polyethylene (PEG)–lipid conjugates (Avanti Polar Lipids) and mixed with siRNA in a microfluidic device. Polymer–lipids and PEG–lipid conjugates were dissolved in 200-proof ethanol and loaded into a gas-tight Hamilton glass syringe, with siRNA held in 10 mM citrate buffer (pH 3.4). Solutions were connected to the inlet of a microfluidic device containing static mixers, and the contents were perfused through the device to form nanoparticles. The nanoparticle formulations were sterile-filtered against PBS using a 20,000-molecular-weight-cutoff dialysis cassette (Thermo Scientific) to remove citrate and ethanol. Nanoparticle size and structure were determined by both DLS and the ZetaPALS (Nano Instruments) and cryo-TEM using a Tecnai G2 transmission electron microscope (FEI). Nanoparticle samples were prepared in a vitrification system (25 °C, ~100% humidity), and images of samples were recorded on an UltraScan 1000 charge-coupled device camera (Gatan) in low-dose conditions. For zeta potential measurements, nanoparticles (NicheEC-15) at a polydispersity index of 0.15 ± 0.03 were added to PBS (1 ml) and measured using a Malvern Zetasizer Nano ZS machine (mean, −0.15 mV; s.d., 9.28). The concentration of siRNA used for in vitro and in vivo treatment was quantified using both a Quant-iTTM Ribogreen assay (Invitrogen) and NanoDrop measurements (Thermo Scientific).

Cell culture. The endothelial cell line bEnd.3 (CRL-2299, ATCC) was cultured in Dulbecco’s modified Eagle’s medium, supplemented with 10% (vol/vol) fetal bovine serum and 1% (vol/vol) penicillin/streptomycin under humidified conditions at 37 °C and 5% CO2 until cell confluence. To investigate knockdown efficiency, bEnd.3 cells were plated in 24-well plates (150,000 cells per well) and incubated for 24 h before treatment with nanoparticles formulated with siRNA targeting one of the following genes: luciferase (luc), monocyte chemoattractant protein 1 (Mcp1) or stromal-derived factor-1 (Sdf1). A serial dilution of each siRNA nanoparticle formulation in PBS was prepared at concentrations of 1–60 nM siRNA. Samples were then incubated for 24 h before gene expression analysis. Cells were washed with PBS and collected using 0.25% trypsin. RNA was extracted from cells using an Acturus PicoPure RNA isolation kit (Thermo Scientific). To study the in vitro uptake of NicheEC-15 by microscopy, bEnd.3 cells were plated in six-well plates (500,000 cells per well) and grown until confluence. NicheEC-15 containing Alexa Fluor 647 coupled luciferase siRNA was then added at a concentration of 100 nM RNA 2 h before imaging. At 15 min before imaging, 5 μM Syto 13 Green (Life Technologies) and 5 μM Syto 13 Green (Life Technologies) and 5 μM Syto 13 Green (Life Technologies) were added. During the last 5 min the cell cultures were washed repeatedly with PBS. The controls were treated identically, except that NicheEC-15 containing unlabelled luciferase siRNA was used. All steps except washing were done under humidified conditions at 37 °C and 5% CO2.

Mice. All animal procedures conducted at Massachusetts General Hospital and Massachusetts Institute of Technology were approved by the Institutional Animal Care and Use Committees and were in accordance with local, state and federal regulations. C57BL/6 (Jackson Laboratory, stock no. 000664), B6.129-Pdx1tm1J (Jackson Laboratory, stock no. 005582) and B6.129-Pdx1tm1J (Jackson Laboratory, stock no. 000214) mice were used at 11 to 13 weeks of age. To obtain hemizygous B6.129-Pdx1tm1J mice, hemozygous mice were crossed with C57BL/6J mice. Nanoparticles were administered by tail-vein injection. Myocardial infarction was induced by permanent coronary ligation in B6.129P2-Apoelip/Hemizygous mice (Jackson Laboratory, stock no. 002652) after 10 weeks of age. Echocardiography was performed on an echoscan (Eco 8C3, TDI, 8.8 MHz). The animals were sacrificed at 30 days after measurement or 8 weeks after injury.
branched DNA (bDNA) assay kit and QuantGene 2.0 probes against Tie2 and GAPDH (Thermo Fisher Scientific), according to the manufacturer's protocol. Luminex signals were measured using a Tecan Infinite 200 PRO plate reader (Tecan). Standard curves for femur tissues and each target gene were constructed using samples from untreated mice to ensure optimal dilutions for assay samples that avoid luminex signal saturation. Targeted gene sensing in treated mice was quantified by calculating the ratio of target gene luminescence to Gapdh gene luminescence, with all values normalized to target gene:Gapdh gene ratios from control mice.

Flow cytometry and sorting. Single-cell suspensions were obtained from peripheral blood, bone marrow, aorta, and lung. Briefly, blood was collected by eye bleusing using heparinized capillaries or, if larger volumes were needed, by cardiac puncture and addition of 10 mmol l−1 EDTA per 100 μl of blood. Red blood cells were lysed with (clone 8.1.1), Sca-1 (clone D7) and Ter119 (clone TER119). Antibodies were purchased from Biologend, R&D Biosciences or eBioscience. Monocytes were identified as CD19−CD14+PKH26+Ly-6C−Ly-6G−M1+M2+, and subdivided into M1 and M2 populations. Neutrophils were identified as CD11b+CD45+Gr-1+Ly-6G+Ly-6C−. Erythroid progenitor cells (MEP) and GMP were identified as CD45+CD34+CD117+Flk-1−, and CD45+CD34−CD117−Flk-1+ cells, respectively. Megakaryocytes were defined as CD45+CD41b+FcεRI−. Monocytes were identified as CD11b+CD17−CD19−CD54+CD45R+CD14+CD16+CD33+HLA-DR−. Each cell population was stained with appropriate antibodies, and labelled with a near-infrared fluorochrome. Main organs were then collected and imaged on a planar fluorescent reflectance imaging system (OV-110, Olympus) with an excitation wavelength of 630 nm and exposure times of 60–75 ms.

Fluorescence reflectance imaging. For biodistribution imaging, mice were euthanized after injection of 2 mg kg−1 encapsulated siRNA labelled with a near-infrared fluorochrome. We used software IDEAS version 6.2.64.0. A total of 1,000 event single-colour compensation controls were collected (w/o BF or SSC) and later merged to create a compensation matrix for analysis. Once the RIF files were merged and compensated, a gating strategy was set up to the flow cytometry gating of BMECs was used for consistency; a bivariate plot of CD45/Ter19 versus SSC was first generated. Events negative for CD45/Ter19 were then used as a parent population for generation of a bivariate plot of Sca-1 versus CD31. Double-positive events were gated, visually verified as endothelial cells and confirmed to contain nanoparticle AF647 by imager.

Drug-induced stem cell release assay. To compare the release of stem cells into the peripheral blood after siSdf1, AMD3100 and G-CSF treatment, four groups of mice were formed. The control group received no injections, the siSdf1 group was treated once daily with 2 mg kg−1 siSdf1 for three consecutive days then collected 24 h after the last injection. In the AMD3100 group, mice received an injection of 5 mg kg−1 AMD3100 (Bio generics) for five consecutive days with 250 μg kg−1 daily, and collected 12 h after the last injection. All mice were collected at the same time and 100 μl of peripheral blood was drawn for c.f.u. assay.

Competitive peripheral blood transplantation and secondary bone marrow transplantation. For the primary, competitive peripheral blood transplantation (CD45.1) mice received lethal irradiation (9.5 Gy) 24 h before transplantation. CD45.2 donor mice received consecutive injections of 2 mg kg−1 siSdf1 every 24 h for three days while another batch of CD45.1 control mice were injected with PBS. At 24 h after the last injection, the blood of the siSdf1–treated CD45.2 and the CD45.1 control mice was collected by cardiac puncture, red blood cells were lysed by adding red blood cell lysis (RBCL) buffer for 2 min, the cells were washed by adding 20 μl of cold PBS, spun down (5 min, 300 r.p.m.) and resuspended for counting. Nucleated blood cells (CD45+CD14−) from the first, control bone marrow cells were injected with 5×10 bone marrow cells from tail-vein injection. Six months after secondary transplantation, blood chimeraism was analysed by drawing 50 μl of blood for flow cytometry.

For the secondary transplantation, another batch of CD45.1 recipient mice were lethally irradiated (9.5 Gy) 24 h before transplantation. On the day of the transplantation, femoral bones of mice from the first, competitive blood transplantation experiment were collected. The bone marrow was flushed out with cold PBS, plunged through a 40-μm cell strainer and centrifuged (5 min, 300 rpm), then red blood cells were lysed for 2 min with RBCL buffer, centrifuged again and resuspended in PBS with 5% albumin for counting. Each lethally irradiated, secondary recipient CD45.1 recipient mouse was transplanted with 5×10 bone marrow cells by tail-vein injection. Fluorescence reflectance imaging was performed with a Nikon Eclipse Ti-S inverted microscope equipped with a Nikon DS-LU1 digital camera. Micronutrient (Methocult M3434, Stem Cell Technology) following the manufacturer's protocol; 100 μl of whole blood was processed following the protocol, plated on 35-mm plates in duplicates and incubated for 10 days. Colonies were counted using a low-magnification inverted microscope.

Intravital microscopy. To visualize the in vivo uptake of NicheEC-15 in bone marrow endothelial cells, we imaged the skull bone marrow as described previously. Osteosclerosis was injected i.v. 24 h before imaging. NicheEC-15 AF647-siRNA 2 h before and PE-labelled CD31 and Sca-1 antibodies 1 h before. Magnetic resonance imaging. ApoE−/− mice were kept on HFD for 10 weeks starting at age 8 weeks, before induction of MI by permanent ligation of the left anterior descending coronary artery. Mouse magnetic resonance imaging (MRI) was performed using a Bruker 4.7 Tesla Pharmascan magnetic resonance imaging system with a stack of contiguous short-axis slices covering the entire left ventricle. We used a cine intragate flash (FLASH) sequence with the following parameters: echo time, 2.945 ms; repetition time, 12.0 ms; flip angle, 45°; oversampling, 300; matrix size, 200×200; pixel size, 0.150×0.150 mm; slice thickness, 1 mm; number of movie frames per cycle, 16. The left ventricular myocardium and cavity were segmented manually at diastole and systole using Horos software to calculate the ejection fraction and volume of the left ventricle. In addition, the percentage of the left ventricle that was infarcted was also determined manually by segmenting the infarct area.

Histology. To investigate bone marrow vessel density, mice were euthanized and femurs were removed seven days after injection of siSdf1 (2 mg kg−1), siTie2 (2 mg kg−1) or PBS as control. The femurs were fixed in fresh 4% paraformaldehyde at 4°C overnight and then decalcified in 0.5 M EDTA solution (Sigma-Aldrich).

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for 14 days by exchanging the solution every two days. After decalcification, the femurs were paraffin-embedded and serial sections were prepared for immunohistochemistry. The sections were deparaffinized, dehydrated and blocked with 3% H2O2 in methanol for 30 min at room temperature before staining. An endomucin antibody (Abcam ab106100) was incubated at 4°C overnight and a biotinylated anti-rat IgG antibody (Dako) at room temperature for 30 min. A TSA Biotin System Kit (PerkinElmer) and DAB (Vector Laboratories) were used to visualize the staining, and the sections were counterstained with Fast Green (Sigma–Aldrich).

For histological evaluations of hearts after MI in ApoE−/− mice fed on HFD with siMcp1 (2 mg kg−1) or siLuc treatment before and after surgery, mice were euthanized and hearts were perfused thoroughly with PBS and harvested. The hearts were embedded in optimal cutting temperature compound (Sakura Finetek) and subsequently snap-frozen in a 2-methylbutane bath cooled with dry ice. For the scar area assessment, Masson’s trichrome staining was performed according to the manufacturer’s instructions (Sigma–Aldrich). For each individual mouse, three evenly spaced short-axis sections of the left ventricle were collected that sampled the infarct from basal to apical regions. The infarct size was calculated as (scar area slice 1 + scar area slice 2 + scar area slice 3) / (remote area slice 1 + remote area slice 2 + remote area slice 3) × 100%. For immunohistochemistry, 6-μm frozen sections were stained using antibodies: CD11b (BD Biosciences, clone M1/70), alpha-smooth muscle actin (αSMA, Abcam ab5694) and collagen I (Abcam ab21286). The appropriate biotinylated secondary antibodies followed by the VECTASTAIN ABC kit (Vector Laboratories) were applied and AEC (3-amino-9-ethylcarbazole substrate) (Dako) was used for colour development. The sections were counterstained with Harris haematoxylin (Sigma–Aldrich). All the slides were scanned using a digital scanner (NanoZoomer 2.0RS, Hamamatsu) and quantification was performed using Image. Three high-power fields per section per animal were analysed.

Enzyme-linked immunosorbent assay. ELISAs for Sdf1 and Mcp1 (both R&D) in the bone marrow were performed by spinning (11,000g; 2 min, 4°C) the bone marrow out of one femur and taking the supernatant for ELISAs, which were performed according to the manufacturer’s instructions.

Statistics. The statistics were performed as published previously. Data are expressed as mean ± s.e.m. Statistical analyses were performed using Prism (Version 7, GraphPad Software). Two-sided Student’s t-test was used when two groups were compared and the pre-test for normality (D’Agostino–Pearson normality test; significance level 0.05) was not rejected. For nonparametric data, the Mann–Whitney test was used. If more then two groups were compared, two-sided ANOVA tests followed by Bonferroni post-tests were applied. P values smaller than 0.05 indicate statistical significance.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this Article.

Data availability. The main data supporting the results in this study are available within this paper and its Supplementary Information. The raw and analysed datasets generated during the study are too large to be publicly shared, but they are available for research purposes from the corresponding authors on reasonable request.

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Author contributions
M.K.-G., M.J.M., M.J.S., D.G.A. and M.N. designed the experiments. M.J.S., O.F.K., G.C., P.P.G.G., S.C., Y.S., M.T., J.W., K.W., D.R., P.S.K., R.N., V.F., M.H., A.C., F.F.H., Y.I., S.P.S. and G.R.W. performed experiments and collected data. M.K.-G., M.J.M., F.K.S., R.L., D.G.A. and M.N. discussed the results and strategy. M.K.-G., M.J.M., D.G.A. and M.N. wrote the manuscript, which was edited by all co-authors. D.G.A. and M.N. supervised, directed and managed the study.

Competing interests
R.L. has served as an advisor to Alnylam Pharmaceuticals. For a list of entities with which R.L. is involved, compensated or uncompensated, see www.dropbox.com/s/yc3xqbj5ia9v47x/Rev%20Langer%20COI.pdf?dl=0. M.N. has received consulting fees unrelated to this work from Verseau, Gimv and IMF Therapeutics. The remaining authors declare no competing interests.

Additional information
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Reporting Summary

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- Clearly defined error bars
- State explicitly what error bars represent (e.g. SD, SE, CI)

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Software and code

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- **Data collection**
  - LSR II flow cytometer with BD FACSDiva software.
- **Data analysis**
  - FlowJo V9, PRISM V6, OsiriX DICOM Viewer V10, ImageJ V1.52, Horos V4.0

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample sizes were calculated on the basis of the predicted between-group difference and power needed to reach a p level of 0.05 (cutoff for statistical significance in this study). |
|-------------|--------------------------------------------------------------------------------------------------|
| Data exclusions | No data points were excluded. |
| Replication | Experiments were in general performed three times (two replications) to confirm the results. Experiments are only shown if replications were successful and all experimental results could be confirmed. |
| Randomization | Mice / cages were randomly allocated to the experimental groups. |
| Blinding | Given the need to properly formulate the nanoparticles, the researchers were not blinded to the components that were used to synthesize each formulation. Tie2 knockdown studies were conducted by an independent operator, who was unaware of the treatment conditions. ImageStream experiments were performed and analysed by a core facility unaware of the study rationale. MRI and biodistribution imaging were performed and analysed in a blinded fashion. Flow-cytometry experiments were performed and managed by only one researcher, which precluded blinding; but analysis, specifically FACS gating, was defined by negative controls and all FACS data were independently reviewed by M.N. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | ☑ Unique biological materials |
| ☐   | ☑ Antibodies |
| ☐   | ☑ Eukaryotic cell lines |
| ☑   | ☑ Palaeontology |
| ☐   | ☑ Animals and other organisms |
| ☑   | ☑ Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq |
| ☑   | Flow cytometry |
| ☑   | MRI-based neuroimaging |

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

All materials used are commercially available or made from commercially available materials (this applies to the nanoparticles). A detailed description of all the steps necessary to produce the nanoparticles is available in Methods.

Antibodies

Antibodies used

Information for the antibodies is listed as follows: fluorochrome; target; catalog #; supplier; clone; dilution

FITC; CD34; 553733; BD; RAM34; 1:100
FITC; CD45.2; 553772; BD; 104; 1:100
FITC; Ly6c; 553104; BD; AL 21; 1:100
FITC; Ly6g; 127606; BioLegend; 1A8; 1:100
PE; B220; 103208; BioLegend; RA3-6B2; 1:100
PE; CD31; 102508; BioLegend; MEC13.3; 1:100
PE; CD90.2; 140308; BioLegend; 53-2,1; 1:100
PE; NK-1.1; 108708; BioLegend; PK136; 1:100
Validation

All antibodies have been validated by the manufacturers for the applications used. The gating strategies used for identifying cell populations in this manuscript have been used and published by our group and others before.

CD34: Validated for use in mice as a stem cell marker https://wwwbdbiosciencescom/us/applications/research/stem-cell-research/cancer-research/mouse/fcy-rat-anti-mouse-cd34-ram34/p/553733 and is widely used for this application https://dx.doi.org/10.1016%2Fj.stem.2015.04.008

CD45: Validated for use in mice as a leukocyte marker https://wwwbdbiosciencescom/us/applications/research/stem-cell-research/cancer-research/mouse/fcy-rat-anti-mouse-cd452-104/p/553772 and is widely used for this application https://dx.doi.org/10.1161%2FIRCRESAHA.115.303567

Ly6c: Validated for use in mice and as a leukocyte marker https://wwwbdbiosciencescom/us/reagents/research/antibodies-buffers/immunology-reagents/anti-mouse-antibodies/cell-surface-antigens/fcy-rat-anti-mouse-ly-6c-al-21/p/553104 and is widely used for this application https://dx.doi.org/10.1161%2FIRCRESAHA.115.303567

B220: Validated for use in mice and as a leukocyte marker https://wwwbiolegendcom/en-us/products/pe-anti-mouse-human-cd45r-b220-antibody-447 and is widely used for this application https://dx.doi.org/10.1161%2FIRCRESAHA.115.303567

CD31: Validated for use in mice and as an endothelial cell marker https://wwwbiolegendcom/en-us/products/pe-anti-mouse-cd31-antibody-379 and is widely used for this application https://dx.doi.org/10.1084%2Fjem.20070885

CD90.2: Validated for use in mice as a leukocyte marker https://wwwbiolegendcom/fr-ch/products/pe-anti-mouse-cd90-2-thy-1-2-antibody-6867 and is widely used for this application https://dx.doi.org/10.1161%2FIRCRESAHA.115.303567

NK-1.1: Validated for use in mice and as a leukocyte marker https://wwwbiolegendcom/en-us/products/pe-anti-mouse-nk-1-1-antibody-431 and is widely used for this application https://dx.doi.org/10.1161%2FIRCRESAHA.115.303567

Sca1: Validated for use in mice and as a leukocyte and endothelial cell marker https://wwwthermofishercom/antibody/product/ly-6a-e-sca1-antibody-clone-d7-monoconal-12-5981-82 and is widely used for these applications https://dx.doi.org/10.1084%2Fjem.20070885 and https://dx.doi.org/10.1161%2FIRCRESAHA.115.303567

Ter119: Validated for use in mice and as a red blood cell marker https://wwwbdbiosciencescom/eu/applications/research/stem-cell-research/mesoderm-markers/mouse/pe-anti-rat-mouse-ter-119erythroid-cells-ter-119/p/553673 and is widely used for this application https://dx.doi.org/10.1161%2FIRCRESAHA.115.303567

CD41: Validated for use in mice as a stem cell marker https://wwwbiolegendcom/en-us/products/percp-cyamines5-5-anti-mouse-cd41-antibody-9696 and is widely used for this application https://dx.doi.org/10.1016%2Fj.stem.2015.04.008

CD51: Validated for use in mice https://wwwbiolegendcom/en-us/products/pe-anti-mouse-cd51-antibody-488 and used as an osteblast marker https://dx.doi.org/10.1038%2Fn.3589

CXCR1: Validated for use in mice and as a leukocyte marker https://wwwbiolegendcom/en-us/products/percp-cyamines5-5-anti-mouse-cxcr1-antibody-10461 and is widely used for this application https://dx.doi.org/10.1161%2FIRCRESAHA.115.303567

F4/80: Validated for use in mice and as a leukocyte marker https://wwwbiolegendcom/en-us/products/pe-cyanine7-anti-mouse-f4-80-antibody-4070 and is widely used for this application https://dx.doi.org/10.1161%2FIRCRESAHA.115.303567
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) | bEnd.3 (spelled BEND3 as well); manufacturer ATCC; catalog # CRL-2299
Authentication | The authentication was provided by the manufacturer.
Mycoplasma contamination | Mycoplasma free cells were purchased. No further testing for mycoplasma was done.
Commonly misidentified lines (See EURLAC register) | No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals | C57BL/6J (Jackson Laboratory, stock # 000664), B6.129P-Cx3cr1tm1Litt/J (Jackson Laboratory, stock # 005582), B6.SJL-PtprcaPepcb/BoyJ (Jackson Laboratory, stock # 002014) mice were used at 11 to 13 weeks of age. To receive hemizygous B6.129P-Cx3cr1tm1Litt/J mice, homozygous mice were crossed with C57BL/6J mice. B6.129P2-Apoetm1Unc/J were started on high fat diet at 8 weeks of age. Mice of both sexes were used.
Wild animals | The study did not involve wild animals.
Field-collected samples | The study did not involve samples collected from the field.

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation | Single-cell suspensions were obtained from peripheral blood, bone marrow, aorta and lung. Briefly, blood was collected by eye bleeding using heparinised capillaries or, if larger volumes were needed, by cardiac puncture and the addition of 50 mmol/L EDTA. Red-blood-cell lysis was achieved by adding 1x red-blood-cell lysis buffer (Biolegend) for 2 minutes. After blood collection, mice were perfused through the left ventricle with 30 mL ice-cold PBS. Bone marrow was harvested by flushing femurs in PBS with 0.5% bovine serum albumin (BSA) for leukocyte and HSPC staining or with HBSS containing 2% FBS, 2 mg/ml disease 2 and 1 mg/ml collagenase IV and incubated with gentle agitation for 30 minutes at 37°C for endothelial cells. Lungs were excised and minced with a fine scissors before digestion in DMEM w/o phenol red containing 2% FBS, 2 mg/ml dispase 2, 5 mg/ml collagenase I (Worthington) and 1 mg/ml DNase I (Sigma) at 37°C and 125 revolutions for 20 minutes. Tissues were then plunged thorough...
40-μm nylon mesh (BD Falcon), washed and centrifuged (8 minutes; 300g; 4°C). The obtained single-cell suspensions were stained at 4°C for 30 minutes and afterwards washed, centrifuged and resuspended.

| Instrument | LSR II flow cytometer with BD FACSDiva software. For cell sorting the populations were defined as described and sorted using a FACSARia II cell sorter. |
|------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Software   | FlowJo V9                                                                                                                                 |
| Cell population abundance | For qPCR experiments at least 10,000 cells were sorted. Purity for endothelial cells was confirmed by a second sorting step, PCR and microscopy. |
| Gating strategy | Monocytes were identified as CD90- CD19- NK1.1- Ly-6G- D45.2+ CD11bhighCD115+ F4/80low and separated into Ly-6Clow and a Ly-6Chigh populations. Neutrophils were identified as CD90- CD19- NK1.1- D45.2+ CD11bhigh Ly-6G+. Blood and bone marrow LSK were identified as (B220 CD3e CD4 CD8a CD11b CD11c CD19 CD90.2 GR1 IL7Ra NK1.1 Ter119)- c-kit+ Sca-1+, LKs as (B220 CD3e CD4 CD8a CD11b CD11c CD19 CD90.2 GR1 NK1.1 Ter119)- c-kit+ Sca-1- and subdivided into CMP, MEP and GMP by their CD16/32 and CD34 expression. Bone-marrow and lung endothelial cells were gated as CD45.2- CD41- Ter119- CD31+ Sca-1+ and Ter119- CD45+ CD31+, respectively. |

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.