Supplementary methods

Table 1 Details of antibodies and suppliers

Name	Type	Company	Cells	Usage
Goat anti-human FABP-4 antigen affinity-purified polyclonal antibody	Primary	(R&D systems)	Human (adipocytes)	IHC
Goat anti-human aggrecan antigen affinity-purified polyclonal antibody	Primary	(R&D systems)	Human (chondrocytes)	IHC
Mouse anti-human osteocalcin monoclonal antibody	Primary	(R&D systems)	Human (osteocytes)	IHC
Alpha smooth muscle actin (αSMA) (MAB1420)	Primary	(R&D systems)	Human MSCs	IHC
Phalloidin-Atto 633	Primary	Sigma-Aldrich	Any	IHC
DAPI	stain	Santa-Cruz Biotechnology	Nuclear stain	IHC/FC
Annexin V PE	Apoptotic cell marker	ThermoFisher	Phosphatidylserine on cell surface	FC

Northern Lights TM NL557-conjugated donkey anti-goat antibody (NL001)	Secondary	(R&D systems)	NA	IHC
Northern Lights TM NL557-conjugated donkey anti-mouse antibody (NL007)	Secondary	(R&D systems)	NA	IHC
CD90 FITC	Primary	BD Biosciences	Human MSCs	FC
CD90 APC	Primary	BD Biosciences	Human MSCs	FC
CD73 PE	Primary	BD Biosciences	Human MSCs	FC
CD105 PerCP-Cy 5.5	Primary	BD Biosciences	Human MSCs	FC
CD45 APC	Primary	BD Biosciences	Leukocytes	FC
CD19 APC	Primary	BD Biosciences	B-cells	FC
HLA-DR APC	Primary	BD Biosciences	Myeloid cells	FC
CD34 APC	Primary	BD Biosciences	Immature	FC
CD14 APC	Primary	BD Biosciences	Myeloid cells	FC
CellROX® Green Reagent	Stain	Thermofisher	NA	FC
Anti-CD19 antibody	Primary		Human B-cells	IHC
Anti-Nestin antibody	Primary	Abcam	Murine Stromal cells	IHC
Mouse Background Blocker	Background Blocker	Menarini Diagnostics	NA	IHC

Supplementary Methods Table 2

ELISA/cytokine bead assays

Sl. No.	Analyte	Company
1	CCL2/MCP-1	Invitrogen (88-7399-88)
2	CXCL1/Gro-α	Qiagen (SEH00696A)
3	CXCL2/Gro-β	R&D systems (DY276-05)
4	IL6	Invitrogen (88-7066-88)
5	IL8	Invitrogen (88-8086-88)

Supplementary Methods Table 3

Gene expression profile targets

#	Gene Symbol	Gene RefSeq #
1	*PDGFR-2*	NM_006206
2	*PDGFR-1*	NM_002609
3	*Nbla00170*	NM_006617
4	*FSP1*	NM_002961
5	*FAPA*	NM_004460
6	*IL-6*	NM_000600
7	*CD90*	NM_006288
8	*ACTA2*	NM_001613
9	*TN-C*	NM_002160
10	*FN*	NM_002026
11	*COL1A1*	NM_000088
12	*CXCL8*	NM_000584
13	*CXCL1*	NM_001511
14	*CXCL2*	NM_002089
15	*CCL2*	NM_002982
16	*MMP1*	NM_002421
17	*VEGF*	NM_003376
18	*FGF-2*	NM_002006

Supplementary Methods Table 4

Primers for mitochondrial DNA detection

Species	Genome	Forward Primer Seq 5'-3'	Reverse Primer Seq 5'-3'	Amplicon
Mouse	Mitochondria	CCC AGC TAC TAC CAT CAT TCA AGT	GAT GGT TTG GGA GAT TGG TTG ATG T	117
Human	Mitochondria	CCC CAC AAA CCC CAT TAC TAA ACC CA	TTT CAT CAT GCG GAG ATG TTG GAT GG	221
Mouse	Nuclear beta globin	CCA ATC TGC TCA CAC AGG ATA GAG AGG GCA GG	CCT TGA GGC TGT CCA AGT GAT TCA GGC CAT CG	494
Human	Nuclear beta globin	GAA GAG CCA AGG ACA GGT AC	GGA AAA TAG ACC AAT AGG CAG	408

Supplementary methods – *in-vivo* work

All animal experiments were performed according to UK Home Office approved protocols and institutional guidelines.

Disseminated NSG xenografts

Disseminated SEM xenografts were established in sixteen 8-10-week-old NSG (Non obese diabetic severe combined immunodeficiency gamma) male mice (Charles River, Margate, UK) by tail vein injection of 2 x 10^6 SEM cells expressing luciferase and blue fluorescent protein. One additional NSG male mouse not injected with SEM cells was used as a baseline control for imaging and subsequent analysis as a positive control for detection of murine mitochondrial DNA by PCR.

In vivo imaging

Seven days after tail vein injection of SEM luciferase expressing cells, engraftment was confirmed by bioluminescent imaging. Mice were shaved and injected i.p. with 200 µl of D-luciferin (Caliper Life Science, Cheshire, UK). They were then imaged under anaesthetic (Isofluorane) under IVIS 100 Lumina (Caliper Life Sciences, Chesire, UK). The results were analyzed using Living Image 3.2 software. The same protocol for in vivo imaging was used to assess leukemic burden at day +3 following treatment.

Chemotherapy treatment

Ten days following tail vein injection of SEM cells, the mice were split into four treatment groups of four mice each. The different groups of mice were treated with

either 200µl PBS (control), AraC 100mg/kg in 200µl PBS, VCR 0.25mg/kg in 200µl PBS or Nocodazole 9mg/kg in 200µl PBS i.p. daily for two days.

Sacrifice and Analysis

Two days following the final dose of treatment the mice were humanely sacrificed and bone marrow cells were extracted by isolation and crushing of the tibias and one femur. One femur per mouse was sent for histopathology. SEM cells were identified or flow sorted by BFP expression for subsequent analysis, including intracellular ROS (CellROX Green™, ThermoFisher C10444), mitochondrial mass (Green Mitotracker, ThermoFisher M7514) and DNA extraction for identification of murine mitochondria DNA by PCR. For isolation and expansion of murine stromal cells, residual cells post extraction of SEM cells were plated in 6 well plates at a density of 1-2 x 10^6 per ml in αMEM media supplemented with 10% FBS and Pen-Step-Glut. Seventy-two hours later the media was removed, washed with PBS and new media added. Two weeks later the adherent cells were removed with trypsin, washed with PBS and re-plated in a glass bottom plate for imaging. Following overnight culture to allow the stromal cells to adhere, the cells were fixed and stained with Phalloidin, DAPI and αSMA as previously described. The plates were then imaged using a fluorescent microscope as described previously in methods.

Immunohistochemistry of femur sections

Immediately following humane sacrifice of the mice, one femur was removed and fixed in 10% neutral buffered formalin. The samples were decalcified in 10% formic acid for 9-10 hours then processed and embedded. Following sectioning the samples were stained with the appropriate primary and secondary antibodies.

Supplementary figure legends

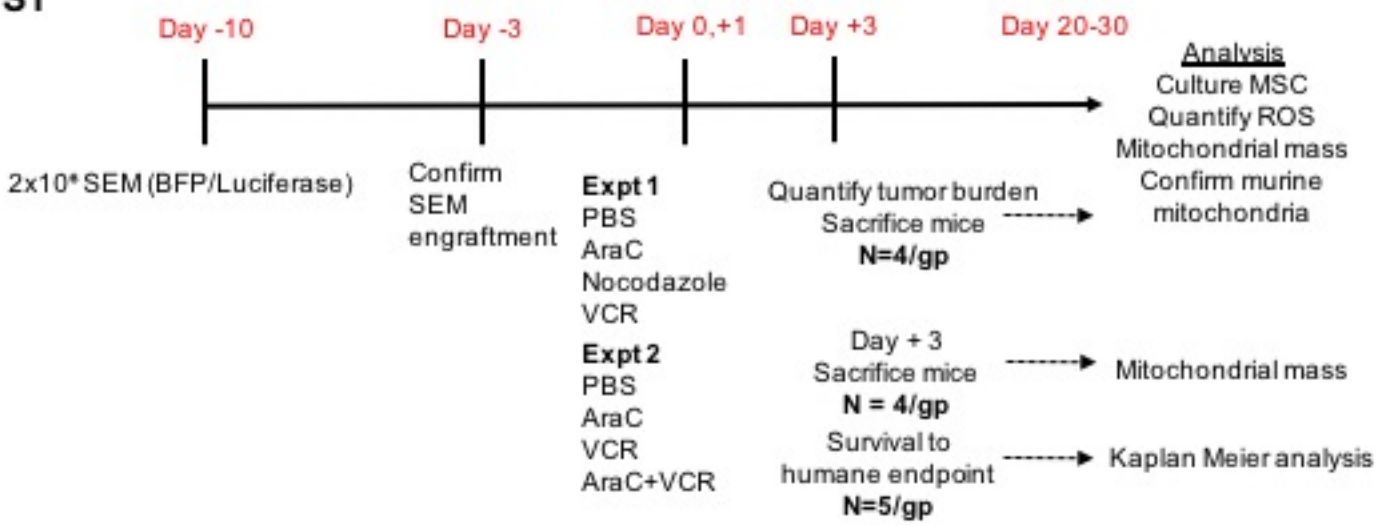
S1. Schema of in vivo experiments indicating timelines and experimental outputs from two independent experiments

S2. Agarose gel images showing PCR products from DNA extracted from human SEM cells xenografted into NSG mice. Murine nuclear and mitochondrial DNA and human mitochondrial DNA, is present as labelled. Lane 1, murine control bone marrow cells, Lane 2 Human SEM cell control, Lanes 3-6 SEM xenograft cells sorted on BFP after control treatment Lanes 7-10 SEM xenograft cells sorted on BFP after AraC treatment.

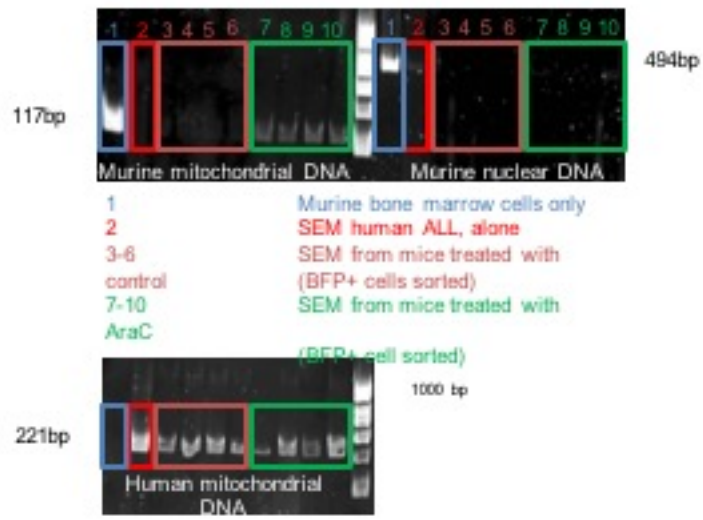
S3. Immunohistochemistry of sections of representative whole femora from each treatment group. Femora are single stained for human CD19 (brown) and murine nestin (pink) plus dual-stained for human CD19 and murine nestin. In the AraC but not the other conditions, CD19+ cells are seen closely associating with a nestin+ niche.

Supplementary figures

S1



S2



S3