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A Supramolecular Nanocarrier for Delivery of Amiodarone Anti-Arrhythmic Therapy to the Heart

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Abstract

Amiodarone is an effective anti-arrhythmic drug used to treat and prevent different types of cardiac arrhythmias. However, amiodarone can have considerable side effects resulting from accumulation in off-target tissues. Cardiac macrophages are highly prevalent tissue-resident immune cells with importance in homeostatic functions, including immune response and modulation of cardiac conduction. We hypothesized that amiodarone could be more efficiently delivered to the heart via cardiac macrophages, an important step toward reducing overall dose and off-target tissue accumulation. Toward this goal, we synthesized a nanoparticle drug carrier composed of *L*-lysine cross-linked succinyl- β -cyclodextrin that demonstrates amiodarone binding through supramolecular host-guest interaction as well as a high macrophage affinity. Biodistribution analyses at the organ and single-cell level demonstrate accumulation of nanoparticle in the heart resulting from rapid uptake by cardiac macrophages. Nanoparticle assisted delivery of amiodarone resulted in a 250% enhancement in the selective delivery of the drug to cardiac tissue in part due to a concomitant decrease of pulmonary accumulation, the main source of off-target toxicity.

Graphical Abstract

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Keywords

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Introduction

Macrophages were originally believed to function solely as gatekeepers to prevent infection.¹ More recently, studies have shed light on the diverse functions of abundant tissue resident macrophages that far extend this original understanding. For example, macrophages have been shown to regulate thermogenesis in adipose tissue², synaptic pruning in the brain³, iron recycling in the spleen and liver⁴, wound healing^{5–8}, and promote or inhibit cancers depending on different functional subtypes.^{9–11} Furthermore, it has been recently demonstrated that tissue resident macrophages are highly abundant in normal cardiac tissues and actively participate in cardiac conduction.^{8,12–14} This observation was particularly unexpected, but emerged after macrophages were observed at a high local density in the atrioventricular (AV) node of the heart.¹⁴ Interestingly, when macrophages were ablated (e.g. through Cd11b^{DTR}), progressive AV block resulted.¹⁵ It was subsequently shown that these AV nodal macrophages facilitate electrical conduction in the heart via gap junction communication with conducting cells. Since cardiac macrophages are also abundant near conducting myocytes, we reasoned that targeting these cells would potentiate the delivery of anti-arrhythmic drugs. Indeed, macrophages have a propensity for nanoparticle phagocytosis.¹⁶ This feature has been effectively exploited to deliver therapeutic modalities to cancers^{17–19}, to re-educate macrophage sub-populations^{20,21}, and to decrease toxicity of small molecule drugs or to prolong drug availability through nanoparticle encapsulation.²² Given the demonstrated prevalence of cardiac macrophages and the intersection between macrophages and cardiac conductivity, we hypothesized that it should be feasible to more selectively deliver anti-arrhythmic drugs to cardiac macrophages via nanoparticle formulations.

Cardiac arrhythmia refers to an abnormal pattern of heart beat, and there are many different types including atrial and ventricular arrhythmias. Atrial fibrillation is a very common arrhythmia seen in many patients as they age or in co-morbid conditions such as heart failure, and it can result in various complications, including stroke. Amiodarone is a widely-used class III anti-arrhythmic used to treat different arrhythmias, such as atrial fibrillation, and it has adrenergic inhibition (alpha and beta-blocking) properties as well as effects on

potassium, sodium, and calcium channels. Unfortunately, the drug has considerable side effects in the lung, liver, and thyroid resulting from the prolonged circulation time and off-target tissue accumulation.²³ Indeed, all patients on chronic amiodarone therapy require continuous surveillance of lung, liver, and thyroid function. To mitigate these toxicities, experimental studies have been performed to modulate drug pharmacokinetics by encapsulating amiodarone into liposomes or lipid nanoparticles, resulting in some beneficial effects.^{24,25} However, directing amiodarone to cardiac macrophages more selectively has not been described and thus represents an alternative approach.

We hypothesized that such targeting could be achieved by specialized nanoparticle preparations. One such approach uses cyclodextrin nanoparticles, optimized for high payloads and ideal pharmacokinetics.²⁰ Cyclodextrin is a well established tool for drug delivery, enabling the delivery of small molecule drugs,^{26–28} plasmids or siRNA,^{29,30} and other therapeutics or imaging agents^{31,32} primarily through transient host-guest association of cyclodextrin and the molecule delivered. These and other supramolecular drug delivery strategies have been highly effective towards achieving localized delivery.^{33–36} Here, we describe the first use of a cyclodextrin nanoparticle as a vehicle for anti-arrhythmic drugs. We show that the approach results in delivery of amiodarone to the heart while decreasing drug concentrations in off-target tissues commonly associated with toxicity.

Results and Discussion

Characterization of Cyclodextrin Nanoparticles (CDNP) and Amiodarone Complexation.

Our lab has previously demonstrated the high affinity of polyglucose-derived nanoparticles for macrophages *in vivo*, motivating their use as imaging agents in the tumor environment and in cardiac applications.^{17,37,38} Recently, the application of polyglucose nanoparticles for macrophage targeting has been extended to include those derived from β -cyclodextrin to act as drug delivery agents for suitable hydrophobic drugs.²⁰ The utility of this material system is derived from both the host-guest interactions between the host macrocycles (β -cyclodextrin) and the encapsulated drug guest (amiodarone) and importantly, the affinity of the material towards macrophages (Figure 1). We sought to monopolize on these attributes to use CDNPs to facilitate the preferential delivery of amiodarone to macrophage-rich tissues — specifically the heart and the cardiac conduction system.

CDNPs were synthesized by methods previously described.²⁰ Briefly, CDNPs were prepared by cross-linking succinyl- β -cyclodextrin with *L*-lysine, mediated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) in buffered aqueous reaction conditions (Figure 1). Nanoparticles were prepared at a fixed ratio of *L*-lysine to carboxylic acid (0.5:1), which resulted in a particle size of 27.0 ± 4.6 nm, determined by DLS. Particle size was similarly when quantified from SEM images (27.0 ± 1.6 nm, with a range of 8 to 71 nm). Nanoparticles within this size range are well suited for delivery applications, as they are above the threshold hydrodynamic radius for renal excretion and small enough to allow both tissue penetration and cellular uptake.^{37,39}

Cyclodextrin is a common solubilizing agent for pharmaceutical drugs due to well characterized host-guest interactions. These bonds are transient hydrophobic associations

between the cyclodextrin (host) cavity and a drug (guest) of complementary size.^{33,40} The incorporation of amiodarone into the nanoparticle was accomplished by mixing in aqueous conditions to yield amiodarone loaded CDNP (CDNP-Amio). Drug loading resulted in a similar overall size and shape of the loaded nanoparticle (DLS: 34.3 ± 1.6 nm; Figure 2A,B). The nature of the interaction was probed via ^1H - ^1H ROESY NMR which demonstrated interaction between the benzofuran protons (1–4) of amiodarone with the hydrophobic protons of the cyclodextrin cavity (Figure 2C), indicative of hydrophobic association driving drug inclusion within the cyclodextrin cavity. This host-guest interaction allowed for amiodarone to be solubilized through the use of CDNP. Upon administration, a continual release of amiodarone may be expected due to the transient nature of the interaction.

Macrophage-Associated Distribution of β -Cyclodextrin Nanoparticles (CDNP).

Macrophages play a critical role in determining nanoparticle biodistribution through nanoparticle uptake.^{16,41} RAW 264.7 murine macrophages were used to interrogate the uptake kinetics and intracellular distribution of a fluorescently labeled derivative (CDNP-VT680) *in vitro* (Figure 3). Relatively uniform uptake of the nanoparticle across the cell population was observed which increased over time (Figure 3A). Individual cells were segmented, as illustrated in Figure 3B, allowing automated quantification of nanoparticle uptake. Rapid cellular uptake was observed (Figure 3C), approaching saturation within 4 hours ($90.0 \pm 4.0\%$ of maximum). To understand the fate of the CDNPs which were internalized by the macrophages, the cellular uptake after 4 hours was analyzed with lysosomal staining (anti-LAMP1) used to identify subcellular features (Figure 3D). A high degree of co-localization was observed between the lysosomal stain and CDNP-VT680 (Figure 3E; Pearson $R = 0.81$, Spearman $R = 0.84$). The high level of co-localization indicates that CDNP are thus primarily located within the lysosome of macrophages.

Ultimately, efficacy of amiodarone treatment depends on accumulation in cardiac tissue. To examine the ability of the CDNP to act as a therapeutic targeting agent, we scrutinized the cardiac accumulation of amiodarone loaded CDNP-VT680 24 hours after intravenous injection. To allow examination of cellular distribution, these studies were performed in a reporter mouse where macrophages are identified through expression of MerTK^{GFP/+}.⁴² Fluorescence reflectance imaging (Figure 4A) of the heart indicated accumulation of CDNP-VT680 which was unaffected by amiodarone loading. Uptake by resident cardiac macrophage was observed throughout the normal myocardium with drastically increased local accumulation within the area of the AV node, consistent with the demonstrated abundance of macrophages within the AV node.¹⁴

To examine the underlying cause of the observed distribution, tissue from the myocardium (Figure 4B) and AV node (Figure 4C) was examined by confocal fluorescence microscopy. Macrophages from both regions readily accumulated nanoparticle, as indicated by the co-localization of GFP and VT680 signals (Figure 4B,C). It is likely that increased accumulation within the AV node region is a result of increased macrophage density in this region. Recent findings have linked cardiac macrophages, particularly those within the AV node, to electrical conductivity of the tissue,¹⁴ and increased CDNP accumulation within

this tissue is a promising lead toward drug delivery for arrhythmia and AV node dysfunction in particular.

***In Vivo* Pharmacokinetics and Biodistribution.**

To study the *in vivo* pharmacokinetics and biodistribution, we again utilized the fluorescent CDNP-VT680 derivative. Vascular half-life following intravenous injection was determined through the use of time-lapse confocal fluorescence microscopy of vasculature within the ear of nu/nu mice. The half-life ($t_{1/2}$) was determined to be 53.7 ± 2.6 minutes (Figure 5A).

Subsequently, organ biodistribution of both CDNP and amiodarone was examined in C57BL/6 mice at 24 hours post-injection of CDNP-Amio. Nanoparticle distribution was quantified from fluorescence reflectance imaging (Figure 5B) and indicated an accumulation of nanoparticles in macrophage-rich tissue (i.e., liver, spleen, and heart) relative to other tissue (muscle and lung). The accumulation within macrophage-rich tissues is an observation in alignment with prior results for similarly composed nanomaterials,^{17,20,37} and are supported by the general consensus that macrophage uptake heavily influences nanoparticle biodistribution.

Tissue concentrations of amiodarone were determined by competitive ELISA following administration of CDNP-Amio or free amiodarone as a control. The percent difference in tissue concentration between these groups (Figure 5C) indicated that amiodarone concentrations closely mirror the distribution of the CDNP vehicle. Consequently, an increase in amiodarone concentrations was observed in the spleen ($72 \pm 9\%$) heart ($47 \pm 13\%$), and liver ($12 \pm 6\%$), while muscle and lung tissues showed minimal change. While amiodarone concentration in the spleen increased, splenic toxicity has not been associated with amiodarone. In contrast, a major limitation to amiodarone administration is the development of amiodarone induced pulmonary toxicity (APT).^{43,44} Amiodarone exhibited non-selective delivery to the heart when administered as the free drug, and tissue concentrations were therefore similar in both tissues. This is in contrast to administration of the CDNP-Amio formulation which considerably increased the concentration of amiodarone in cardiac tissue relative to the lung (Figure 5D). Notably, there is a smaller relative increase in liver delivery of CDNP-Amio compared to cardiac, which further suggests that a higher cardiac dose of amiodarone in the heart relative to both liver and lung can be achieved via CDNP formulation, potentially limiting the off-target toxicities for a given amiodarone dose. The lower concentration of amiodarone with CDNP in lung tissue suggests that some of the classical pulmonary toxicity associated with amiodarone might be mitigated. While the cellular fate of amiodarone following macrophage uptake has yet to be examined within the tissue, we are encouraged by the potential of macrophages to act as a local delivery reservoir for sustained delivery of accumulated drugs,⁴⁵ as well as the knowledge that the primary metabolite of amiodarone (N-disethylamiodarone) possesses clinically relevant anti-arrhythmic activity.⁴⁶ We speculate that local release of either amiodarone or its active metabolite from macrophages will drive improved efficacy in forthcoming models of atrial fibrillation.

Conclusion

We describe the rationale for targeting anti-arrhythmic drugs to cardiac macrophages, where such drug depots may result in normalization of conduction and myocardial contraction — an insight based on newly recognized biology.^{14,47} The developed supramolecular nanocarrier (CDNP) is suitable for encapsulation of anti-arrhythmic drug payloads, as the benzofuran interaction with cyclodextrin results in solubilization of the drug into the nanoparticle through host-guest interaction without substantial change to nanoparticle size or remaining insoluble drug in solution. The cyclodextrin nanoparticle loaded with amiodarone (CDNP-Amio) is amenable to targeted delivery of anti-arrhythmic drug payloads to cardiac macrophages. The nanoparticle was rapidly internalized by macrophages *in vitro* with near complete uptake 4 hours of treatment, a finding reflected in the blood half-life in *in vivo* studies, with a blood half-life of 53.7 ± 2.6 minutes. Multi-scale analysis of nanoparticle biodistribution *in vivo* demonstrate cardiac accumulation, and that cellular uptake within the heart is specific to cardiac macrophages — a property that enhanced accumulation in the macrophage-rich AV node region. Finally, biodistribution demonstrated high uptake of both nanoparticle and amiodarone in macrophage-rich tissues (particularly the spleen and heart), leading to a 250% increase in uptake of amiodarone in the heart relative to the lung. Looking forward, optimization of the nanoparticle size and charge may be beneficial toward further improving cardiac delivery of amiodarone once a mouse model to accurately depict atrial fibrillation is established and which unfortunately does not exist today.

Materials and Methods

Materials.

Unless noted, solvents and reagents were obtained from Sigma-Aldrich and used as received. Fluorescent dextran used in imaging studies was prepared from aminated dextran (500kDa, Thermo Fisher Scientific), labeled by Pacific Blue (40.1 ± 2.6 nM dye per mg dextran) as previously described.¹⁷ Amiodarone used throughout the study was of pharmaceutical grade (McKesson), purified by reverse phase chromatography using Biotage® SNAP Bio C18 300 Å as the stationary phase with a gradient of water (0.1% formic acid) and acetonitrile (0.1% formic acid) as the mobile phase, and stored a stock solution at 100 mM in DMSO at -20°C until use.

Nanoparticle synthesis.

Cyclodextrin nanoparticles (CDNPs) were prepared as previously described.²⁰ Briefly, succinyl- β -cyclodextrin (300 mg, 1.0 eq. carboxylate), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1778 mg, 10.0 eq. to carboxylate), and *N*-hydroxysuccinimide (660 mg, 5.0 eq. to carboxylate) were simultaneously dissolved in 6.0 mL of 50 mM MES buffer at pH 6.0 and stirred for 30 min. *L*-lysine (84 mg, 0.5 eq. to carboxylate) was dissolved and added in 3.0 mL MES buffer, and the reaction stirred overnight. The CDNP was recovered by addition of 200 μL of brine and precipitation from a tenfold excess of ice cold ethanol followed by repeated washing by water with concentration

in 10 kDa MWCO centrifugal filters (Amicon). The lyophilized nanoparticle was dissolved at 50 mg/mL in water and stored at -20°C until use.

Cyclodextrin Nanoparticles-Amiodarone (CDNP-Amio) Formulation.

A single dose of CDNP loaded with amiodarone was made by diluting amiodarone (1.55 μL , 0.1 mg/mouse) in DMSO (8.45 μL). This dilution was added to x1 PBS buffer (88.1 μL) with the subsequent addition of CDNP (1.875 μL) and mixed overnight at 50°C .

Nanoparticle Characterization.

Particle size was calculated by dynamic light scattering (Malvern, Zetasizer APS) at a concentration of 5 mg/mL in PBS buffer. Scanning electron microscopy was performed on samples prepared at 200 $\mu\text{g/mL}$ in water, spotted on silica wafers, freeze-dried and sputter coated prior to imaging. To examine the interaction of amiodarone with succinyl- β -cyclodextrin, 1.0 mg of amiodarone was dissolved in 100 μL DMSO, added to PBS buffer containing CDNP at a final concentration of 5.0 mg/mL and mixed overnight at 50°C . The sample was then freeze-dried to provide a white powder which was suspended in 1.0 mL of 1:9 DMSO- d_6 to D_2O . ROSEY spectra with solvent suppression was collected on a Bruker AC-400 MHz spectrometer.

Fluorescent Labeling of CDNP.

To enable imaging of cellular and systemic distribution of CDNP, fluorescent labeling was performed. The CDNP was dissolved at 20 mg/mL in 0.1 M carbonate buffer (pH 8.5) to which VivoTag 680 XL (Perkin Elmer, 1.0 mg/mL in DMSO) was added to achieve a final concentration of 50 μM . The reaction was allowed to proceed for two hours, and the final product recovered by buffer exchange into water against 10 kDa MWCO centrifugal filters (Amicon). A label concentration of 2.2 nmol/mg was determined from the absorbance at 668 nm (Nanodrop) by the Beer-Lambert equation. The fluorescent nanoparticle (CDNP-VT680) was stored at 50 mg/mL in water at -20°C until further use.

In vitro cell studies.

RAW 264.7 cells (ATCC) were maintained in Dulbecco's Modified Eagles Medium (DMEM, Invitrogen) supplemented with 10% fetal calf serum (Atlanta Biologicals), 100 IU penicillin (Invitrogen), 100 $\mu\text{g/mL}$ streptomycin (Invitrogen), and 200 mM *L*-glutamine (Invitrogen). For examination of nanoparticle uptake, cells were seeded at a density of 10×10^3 cells/well in optical-bottom 96-well plates (Ibidi, 89626) in phenol red free media. After 24 hours in culture, cells were incubated with CDNP-VT680 (50 $\mu\text{g/mL}$) for the specified time points, washed by PBS, and fixed with paraformaldehyde (4%, 30 min, 37°C). Cells were subsequently stained for cell membrane (5.0 $\mu\text{g/mL}$ Alexa Fluor 488 wheat germ agglutinin, Thermo Fisher) and nuclei (DAPI, Invitrogen) for 15 min at room temperature. For lysosome imaging, cells were similarly stained via DAPI and Alexa Fluor 555 wheat germ agglutinin, permeabilized by 0.2% Triton X-100 and 2.25% BSA in PBS, and incubated with anti-LAMP1 Alexa Fluor 488. Plates were washed and subsequently imaged on a custom high-content screening microscope (Olympus). Acquired images were imported into CellProfiler (Broad Institute) for automated segmentation and analysis of

nanoparticle uptake: quantified as integrated fluorescent intensity per cell, normalized to the mean intensity at 24 hours.

Animal models.

Animal studies were conducted in compliance with the Institutional Animal Care and Use Committees at Massachusetts General Hospital using female mice at 6 to 8 weeks of age. Pharmacokinetic analysis was performed in Nu/Nu mice (Jackson, #002019). Biodistribution analysis was performed in C57BL/6 mice (Jackson, #000664). Macrophage targeting of CDNP-VT680 in vivo was examined in NOD MerTK^{GFP/+} mice,⁴² crossed into NOD SCID mice (Jackson, #001303).

In vivo pharmacokinetic analysis.

Blood half-life of CDNP-VT680 was determined by time-lapse imaging of vessels in the ear by confocal fluorescence microscopy. Images were acquired continually over a four hour period immediately following intravenous injection of amiodarone loaded CDNP-VT680 and Pacific Blue labeled dextran.²⁰ Half-life was determined by identifying multiple regions of interest within the vasculature (Pacific Blue positive). The VT680 mean fluorescence intensity was determined over time, background subtracted relative to interstitial signal, and normalized to the maximum signal. Resulting data were fit as a bi-exponential decay.

In vivo biodistribution.

The distribution of both CDNP and amiodarone was examined at 24 hours following i.v. injection of amiodarone or amiodarone-loaded CDNP-VT680, each at a dose of 0.25 mg/mouse amiodarone. Under anesthesia, mice were exsanguinated prior to euthanasia. Tissues of interest were resected, briefly rinsed in cold saline, and massed prior to bright-field and fluorescent imaging (Olympus OV110; 10 s exposure time, λ_{ex} = 620–650 nm, λ_{em} = 680–710 nm). Distribution of CDNP-VT680 was quantified from the integrated fluorescence density of ROIs representing each individual organ (ImageJ), relative to standards as previously described.²⁰ Distribution of amiodarone was determined by competitive ELISA (MyBioSource) following manufacturer protocols. Both amiodarone and nanoparticle content are presented following normalization to tissue mass.

Intravital examination of amiodarone loaded CDNP-VT680 to cardiac macrophages was carried out in NOD MerTK^{GFP/+} mice. Mice received i.v. injection of amiodarone loaded CDNP-VT680 at 24 hr and Rhodamine-lectin at 20 min prior to tissue resection. Fresh tissues were examined via confocal microscopy (Olympus; FV1000MPE).

Statistical Analysis.

Co-localization analysis was performed in ImageJ. Statistical analyses were performed in GraphPad Prism 7. Data are presented as mean $\pm$ standard deviation unless otherwise indicated. For comparison of two groups, two tailed Student's t-test was used. Otherwise, statistical significance was determined by one-way ANOVA with post-hoc Tukey's honest significant difference test. Significance was assigned at $P < 0.05$.

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References

- (1). Cavaillon JM The Historical Milestones in the Understanding of Leukocyte Biology Initiated By Elie Metchnikoff. *J. Leukoc. Biol* 2011, 90, 413–424. [PubMed: 21628329]
- (2). Nguyen KD; Qiu Y; Cui X; Goh YPS; Mwangi J; David T; Mukundan L; Brombacher F; Locksley RM; Chawla A Alternatively Activated Macrophages Produce Catecholamines to Sustain Adaptive Thermogenesis. *Nature* 2011, 480, 104–108. [PubMed: 22101429]
- (3). Paolicelli RC; Bolasco G; Pagani F; Maggi L; Scianni M; Panzanelli P; Giustetto M; Ferreira TA; Guiducci E; Dumas L; Ragozzino D; Gross CT Synaptic Pruning By Microglia is Necessary for Normal Brain Development. *Science* 2011, 333, 1456–1458. [PubMed: 21778362]
- (4). Theurl I et al. On-Demand Erythrocyte Disposal and Iron Recycling Requires Transient Macrophages in the Liver. *Nat. Med* 2016, 22, 945–951. [PubMed: 27428900]
- (5). Zhang QZ; Su WR; Shi SH; Wilder-Smith P; Xiang AP; Wong A; Nguyen AL; Kwon CW; Le AD Human Gingiva-Derived Mesenchymal Stem Cells Elicit Polarization of M2 Macrophages and Enhance Cutaneous Wound Healing. *Stem Cells* 2010, 28, 1856–1868. [PubMed: 20734355]
- (6). Spiller KL; Anfang RR; Spiller KJ; Ng J; Nakazawa KR; Daulton JW; Vunjak-Novakovic G The Role of Macrophage Phenotype in Vascularization of Tissue Engineering Scaffolds. *Biomaterials* 2014, 35, 4477–4488. [PubMed: 24589361]
- (7). Ogle ME; Segar CE; Sridhar S; Botchwey EA Monocytes and Macrophages in Tissue Repair: Implications for Immunoregenerative Biomaterial Design. *Exp. Biol. Med. (Maywood)* 2016, 241, 1084–1097. [PubMed: 27229903]
- (8). King KR; Aguirre AD; Ye YX; Sun Y; Roh JD; Ng RP; Kohler RH; Arlauckas SP; Iwamoto Y; Savol A; Sadreyev RI; Kelly M; Fitzgibbons TP; Fitzgerald KA; Mitchison T; Libby P; Nahrendorf M; Weissleder R Ifr3 and Type I Interferons Fuel a Fatal Response to Myocardial Infarction. *Nat. Med* 2017, 23, 1481–1487. [PubMed: 29106401]
- (9). Bronte V; Murray PJ Understanding Local Macrophage Phenotypes in Disease: Modulating Macrophage Function to Treat Cancer. *Nat. Med* 2015, 21, 117–119. [PubMed: 25654601]
- (10). Engblom C; Pfirschke C; Pittet MJ The Role of Myeloid Cells in Cancer Therapies. *Nat. Rev. Cancer* 2016, 16, 447–462. [PubMed: 27339708]
- (11). Arlauckas SP; Garren SB; Garriss CS; Kohler RH; Oh J; Pittet MJ Weissleder R. Arg1 Expression Defines Immunosuppressive Subsets of Tumor-Associated Macrophages. *Theranostics* 2018, 8, 5842–5854. [PubMed: 30613266]
- (12). Pinto AR; Paolicelli R; Salimova E; Gospocic J; Slonimsky E; Bilbao-Cortes D; Godwin JW; Rosenthal NA An Abundant Tissue Macrophage Population in the Adult Murine Heart With a Distinct Alternatively-Activated Macrophage Profile. *PLoS One* 2012, 7, e36814. [PubMed: 22590615]
- (13). Epelman S; Lavine KJ; Beaudin AE; Sojka DK; Carrero JA; Calderon B; Brija T; Gautier EL; Ivanov S; Satpathy AT; Schilling JD; Schwendener R; Sergin I; Razani B; Forsberg EC; Yokoyama WM; Unanue ER; Colonna M; Randolph GJ; Mann DL Embryonic and Adult-Derived Resident Cardiac Macrophages Are Maintained Through Distinct Mechanisms At Steady State and During Inflammation. *Immunity* 2014, 40, 91–104. [PubMed: 24439267]
- (14). Hulsmans M et al. Macrophages Facilitate Electrical Conduction in the Heart. *Cell* 2017, 169, 510–522.e20. [PubMed: 28431249]

- (15). Duffield JS; Forbes SJ; Constandinou CM; Clay S; Partolina M; Vuthoori S; Wu S; Lang R; Iredale JP Selective Depletion of Macrophages Reveals Distinct, Opposing Roles During Liver Injury and Repair. *J. Clin. Invest* 2005, 115, 56–65. [PubMed: 15630444]
- (16). Weissleder R; Nahrendorf M; Pittet MJ Imaging Macrophages With Nanoparticles. *Nat. Mater* 2014, 13, 125–138. [PubMed: 24452356]
- (17). Miller MA; Gadde S; Pfirschke C; Engblom C; Sprachman MM; Kohler RH; Yang KS; Laughney AM; Wojtkiewicz G; Kamaly N; Bhonagiri S; Pittet MJ; Farokhzad OC; Weissleder R Predicting Therapeutic Nanomedicine Efficacy Using a Companion Magnetic Resonance Imaging Nanoparticle. *Sci. Transl. Med* 2015, 7, 314ra183.
- (18). Miller MA; Weissleder R Imaging the Pharmacology of Nanomaterials By Intravital Microscopy: Toward Understanding Their Biological Behavior. *Adv. Drug Deliv. Rev* 2017, 113, 61–86. [PubMed: 27266447]
- (19). Miller MA; Chandra R; Cuccarese MF; Pfirschke C; Engblom C; Stapleton S; Adhikary U; Kohler RH; Mohan JF; Pittet MJ; Weissleder R Radiation Therapy Primes Tumors for Nanotherapeutic Delivery Via Macrophage-Mediated Vascular Bursts. *Sci. Transl. Med* 2017, 9, eaal0225. [PubMed: 28566423]
- (20). Rodell CB; Arlauckas SP; Cuccarese MF; Garriss CS; Li R; Ahmed MS; Kohler RH; Pittet MJ; Weissleder R TLR7/8-Agonist-loaded Nanoparticles Promote the Polarization of Tumour-Associated Macrophages to Enhance Cancer Immunotherapy. *Nat. Biomed. Eng* 2018, 2, 578–588.
- (21). Kulkarni A; Chandrasekar V; Natarajan SK; Ramesh A; Pandey P; Nirgud J; Bhatnagar H; Ashok D; Ajay AK; Sengupta S A Designer Self-Assembled Supramolecule Amplifies Macrophage Immune Responses Against Aggressive Cancer. *Nat. Biomed. Eng* 2018, 2, 589–599. [PubMed: 30956894]
- (22). Zhang Y; Li N; Suh H; Irvine DJ Nanoparticle Anchoring Targets Immune Agonists to Tumors Enabling Anti-Cancer Immunity Without Systemic Toxicity. *Nat. Commun* 2018, 9, 6. [PubMed: 29295974]
- (23). Zhuge Y; Zheng ZF; Xie MQ; Li L; Wang F; Gao F Preparation of Liposomal Amiodarone and Investigation of Its Cardiomyocyte-Targeting Ability in Cardiac Radiofrequency Ablation Rat Model. *Int. J. Nanomedicine* 2016, 11, 2359–2367. [PubMed: 27313453]
- (24). Takahama H; Shigematsu H; Asai T; Matsuzaki T; Sanada S; Fu HY; Okuda K; Yamato M; Asanuma H; Asano Y; Asakura M; Oku N; Komuro I; Kitakaze M; Minamino T Liposomal Amiodarone Augments Anti-Arrhythmic Effects and Reduces Hemodynamic Adverse Effects in an Ischemia/reperfusion Rat Model. *Cardiovasc. Drugs Ther.* 2013, 27, 125–132. [PubMed: 23344929]
- (25). Nadimi AE; Ebrahimipour SY; Afshar EG; Falahati-Pour SK; Ahmadi Z; Mohammadinejad R; Mohamadi M Nano-Scale Drug Delivery Systems for Antiarrhythmic Agents. *Eur. J. Med. Chem* 2018, 157, 1153–1163. [PubMed: 30189397]
- (26). Mealy JE; Rodell CB; Burdick JA Sustained Small Molecule Delivery From Injectable Hyaluronic Acid Hydrogels Through Host–Guest Mediated Retention. *J. Mater. Chem. B* 2015, 3, 8010–8019. [PubMed: 26693019]
- (27). Rivera-Delgado E; Djuhadi A; Danda C; Kenyon J; Maia J; Caplan AI; von Recum HA Injectable Liquid Polymers Extend the Delivery of Corticosteroids for the Treatment of Osteoarthritis. *J. Control. Release* 2018,
- (28). Guimaraes PPG; Tan M; Tammela T; Wu K; Chung A; Oberli M; Wang K; Spektor R; Riley RS; Viana CTR Potent in Vivo Lung Cancer Wnt Signaling Inhibition Via Cyclodextrin-Lgk974 Inclusion Complexes. *J. Control. Release* 2018, 290, 75–87. [PubMed: 30290244]
- (29). Pun SH; Bellocq NC; Liu A; Jensen G; Machemer T; Quijano E; Schluep T; Wen S; Engler H; Heidel J; Davis ME Cyclodextrin-Modified Polyethylenimine Polymers for Gene Delivery. *Bioconjug. Chem* 2004, 15, 831–840. [PubMed: 15264871]
- (30). Wang LL; Chung JJ; Li EC; Uman S; Atluri P; Burdick JA Injectable and Protease-Degradable Hydrogel for SiRNA Sequestration and Triggered Delivery to the Heart. *J. Control. Release* 2018, 285, 152–161. [PubMed: 29981357]

- (31). Bellocq NC; Pun SH; Jensen GS; Davis ME Transferrin-Containing, Cyclodextrin Polymer-Based Particles for Tumor-Targeted Gene Delivery. *Bioconjug. Chem* 2003, 14, 1122–1132. [PubMed: 14624625]
- (32). Mondjinou YA; Loren BP; Collins CJ; Hyun SH; Demoret A; Skulsky J; Chaplain C; Badwaik V; Thompson DH Gd³⁺:dota-Modified 2-Hydroxypropyl- β -cyclodextrin/4-sulfobutyl Ether-B-cyclodextrin-based Polyrotaxanes as Long Circulating High Relaxivity Mri Contrast Agents. *Bioconjug. Chem* 2018, 3550–3560. [PubMed: 30403467]
- (33). Rodell CB; Mealy JE; Burdick JA Supramolecular Guest–Host Interactions for the Preparation of Biomedical Materials. *Bioconjug. Chem* 2015, 26, 2279–2289. [PubMed: 26439898]
- (34). Webber MJ; Langer R Drug Delivery By Supramolecular Design. *Chem. Soc. Rev* 2017, 46, 6600–6620. [PubMed: 28828455]
- (35). Li Y; Zou Q; Yuan C; Li S; Xing R; Yan X Amino Acid Coordination Driven Self-assembly for Enhancing Both the Biological Stability and Tumor Accumulation of Curcumin. *Angewandte Chemie* 2018, 57, 17084–17088. [PubMed: 30353638]
- (36). Purcell BP; Kim IL; Chuo V; Guenin T; Dorsey SM; Burdick JA Incorporation of Sulfated Hyaluronic Acid Macromers Into Degradable Hydrogel Scaffolds for Sustained Molecule Delivery. *Biomater. Sci* 2014, 2, 693–702. [PubMed: 24955239]
- (37). Keliher EJ et al. Polyglucose Nanoparticles With Renal Elimination and Macrophage Avidity Facilitate Pet Imaging in Ischaemic Heart Disease. *Nat. Commun* 2017, 8, 14064. [PubMed: 28091604]
- (38). Kim H-Y; Li R; Ng TSC; Courties G; Rodell CB; Prytyskach M; Kohler RH; Pittet M; Nahrendorf M; Weissleder R; Miller MA Quantitative Imaging of Tumor Associated Macrophages and Their Response to Therapy Using 64cu-Labeled Macrin. *ACS Nano* 2018, 12, 12015–12029. [PubMed: 30508377]
- (39). Behzadi S; Serpooshan V; Tao W; Hamaly MA; Alkawareek MY; Dreaden EC; Brown D; Alkilany AM; Farokhzad OC; Mahmoudi M Cellular Uptake of Nanoparticles: Journey Inside the Cell. *Chem. Soc. Rev* 2017, 46, 4218–4244. [PubMed: 28585944]
- (40). Szejtli J Introduction and General Overview of Cyclodextrin Chemistry. *Chem. Rev* 1998, 98, 1743–1754. [PubMed: 11848947]
- (41). Owens III DE; Peppas NA Opsonization, Biodistribution, and Pharmacokinetics of Polymeric Nanoparticles. *Int. J. Pharm* 2006, 307, 93–102. [PubMed: 16303268]
- (42). Mohan JF; Kohler RH; Hill JA; Weissleder R; Mathis D; Benoist C Imaging the Emergence and Natural Progression of Spontaneous Autoimmune Diabetes. *Proc. Natl. Acad. Sci. USA* 2017, 114, E7776–E7785. [PubMed: 28839093]
- (43). Kuhlman JE; Teigen C; Ren H; Hruban RH; Hutchins GM; Fishman EK Amiodarone Pulmonary Toxicity: Ct Findings in Symptomatic Patients. *Radiology* 1990, 177, 121–125. [PubMed: 2399310]
- (44). Wolkove N; Baltzan M Amiodarone Pulmonary Toxicity. *Can Respir J* 2009, 16, 43–48. [PubMed: 19399307]
- (45). Miller MA; Zheng Y-R; Gadde S; Pfirschke C; Zope H; Engblom C; Kohler RH; Iwamoto Y; Yang KS; Askevold B Tumour-Associated Macrophages Act as a Slow-Release Reservoir of Nano-Therapeutic Pt (Iv) Pro-Drug. *Nat. Commun* 2015, 6, 8692. [PubMed: 26503691]
- (46). Himmel HM; Dobrev D; Grossmann M; Ravens U N-Desethylamiodarone Modulates Intracellular Calcium Concentration in Endothelial Cells. *Naunyn-Schmiedeberg's archives of pharmacology* 2000, 362, 489–496.
- (47). Swirski FK; Nahrendorf M Cardioimmunology: The Immune System in Cardiac Homeostasis and Disease. *Nat. Rev. Immunol* 2018, 18, 733–744. [PubMed: 30228378]

Figure 1. Schematic overview of amiodarone encapsulation and delivery. Cyclodextrin nanoparticles (CDNP) are formed through cross-linking of succinyl- β -cyclodextrin by *L*-lysine and subsequently loaded with amiodarone through physical, supramolecular interactions prior to intravenous injection.

Figure 2. Nanoparticle characterization and drug interaction.

A. Dynamic light scattering measurement of hydrodynamic diameter of the cyclodextrin nanoparticle before (CDNP) and after (CDNP-Amio) drug loading. **B.** Scanning electron microscopy images of CDNP and CDNP-Amio. Scale bars: 100 nm, 25 nm (inset). **C.** ROESY spectra of succinyl- β -cyclodextrin interaction with amiodarone; expanded section highlights the interaction of benzofuran protons (1–4) with the hydrophobic protons of the CD cavity.

Figure 3. Cellular uptake and distribution.

A-C. The uptake of CDNP-VT680 was examined by fluorescence microscopy in RAW 264.7 murine macrophages. Fluorescence images (**A**) demonstrated rapid intracellular accumulation. Staining: DAPI (nuclei, red); WGA-AF488 (cell membrane, cyan); CDNP-VT680 (nanoparticle, yellow). Scale bar: 10 μ m. Individual cells, expanded in (**B**) for clarity, were segmented to quantify nanoparticle uptake. Cell outlines (white, right panel) illustrate quantified cellular regions. Scale bars: 10 μ m. Quantification of nanoparticle uptake as a function of time (**C**), normalized to cumulative uptake at 24 hours. Mean $\pm$ S.D.; N = 200 cells per condition. **D.** Confocal fluorescence microscopy of cells incubated with CDNP-VT680 (4hr). Staining: DAPI (nuclei, red); WGA-AF555 (cell membrane, cyan); anti-

LAMP1-AF488 (lysosome, magenta); CDNP-VT680 (nanoparticle, yellow). Scale bar: 10 μm . **E.** Co-localization analysis of nanoparticle and lysosomal staining.

Figure 4. Cardiac accumulation.

A. Fluorescence reflectance imaging of CDNP-VT680 accumulation in the heart at 24 hours following administration; $\lambda_{\text{ex}} = 620\text{--}650\text{ nm}$, $\lambda_{\text{em}} = 680\text{--}710\text{ nm}$. Localized regions of increased CDNP-VT680 accumulation (2) are indicated, relative to normal myocardium (1). Scale bar: 2.0 mm. **B-C.** The distribution of amiodarone loaded CDNP-VT680 was examined by confocal fluorescence microscopy in the myocardial tissue of MerTK^{GFP/+} mice, including in normal ventricular myocardium (**B**) and in the AV node region (**C**). Expanded views show nanoparticle accumulation in cardiac macrophages, outlined (white) for clarity. GFP (cyan, macrophage); CDNP-VT680 (nanoparticle, yellow). Scale bars: 50 μm (top), 20 μm (bottom).

Figure 5. In vivo pharmacokinetics and biodistribution of CDNP and amiodarone.

A. Quantification of nanoparticle vascular half-life in nu/nu mice by confocal fluorescence microscopy of CDNP-VT680. Mean $\pm$ S.D. (shaded); N = 3 regions of interest. **B.** Quantified biodistribution of CDNP-VT680, normalized to mass. **C.** Quantified amiodarone distributions following administration of CDNP-Amio, normalized to administration of amiodarone alone. **D.** Tissue uptake of amiodarone in the heart relative to lung. All biodistribution data represent the mean $\pm$ S.E.M., N = 3 mice.