**Cardiac Arrhythmia Ablation Using High-Energy Heavy Ions**

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**Abstract**

**Background** ─ High-energy ion beams are successfully used in tumor therapy, precisely delivering high doses of ionizing radiation to deep-seated target volumes. This study sought to ablate cardiac locations using scanned carbon (12C) beams in an intact sham-controlled animal model.

**Methods and Results** ─ Seventeen pigs were randomized to irradiation of atrioventricular junction (AVJ; 25, 40, and 55 Gy), left atrial right superior pulmonary vein junction (40 Gy), freewall left ventricle (LV; 40 Gy), and sham. Electroanatomical (EA) mapping and pacemaker implantation were performed. Cardiac gated CTs were obtained during breath-hold at expiration. Targets were contoured and case-specifically expanded for motion coverage. 12C ions were delivered using rescanned pencil-beams. Animals were followed for up to 24 weeks.

Fourteen pigs (mean weight 33.8 ± 3.5 kg) were irradiated using a horizontal beam line. For AVJ a mean volume of 1.8 ± 0.1 cc was irradiated. For PVI and LV, mean volumes were 14.9 ± 1.8, and 2.4 ± 0.3 cc, respectively. Risk structures were spared. In-beam positron-emission-tomography confirmed 12C range and beam position. Complete atrioventricular block developed over the course of four months after 40 and 55 Gy. Transmural lesions were also present at the PV-LA junction and in the LV freewall. Histology revealed strong target fibrosis. Apoptosis was found as one of the mechanisms of cell death, being present after 3, but not 6 months.

**Conclusion** ─ High-energy ion beams are effective in cardiac arrhythmia elimination without any invasive procedural access to the body.

**Keywords:** Ablation, Atrial Fibrillation, Atrioventricular Node, 12C Beam, Particle Therapy, Ventricular Tachycardia.

**Introduction**

Catheter ablation, which commonly uses radiofrequency energy or cryothermal technology, has also evolved into a powerful treatment option for atrial fibrillation[1](#_ENREF_1) and ventricular tachycardia.[2](#_ENREF_2) Yet, success rates of catheter ablation in these diseases are still limited. This is because the source often cannot be eliminated applying these presently used energy sources to endo- and epicardial surfaces of the heart.[3](#_ENREF_3) Furthermore, catheter ablation procedures are linked to several complications, including interventional risks such as silent embolic events,[4](#_ENREF_4) vessel or cardiac wall perforation,[1](#_ENREF_1) and atrial-esophageal fistula formation.[5](#_ENREF_5)

These pitfalls could be overcome by the noninvasive use of charged particle beams. In fact, accelerated protons (H+) and heavier ions (generally carbon [12C] ions) deliver most of their energy in the distal region of their path in the tissues (Bragg peak).[6](#_ENREF_6) Beam penetration depth is controlled by the initial particle energy in the accelerator. Pencil-beam scanning by magnetic deflection can deliver the radiation dose conformal to arbitrarily shaped targets.[7](#_ENREF_7) The favorable physical and biological characteristics of charged particles compared to X-rays justify their use in cancer therapy, where they are considered a cutting-edge technology.[6](#_ENREF_6) Irradiation with 12C can be monitored by Positron (β+) Emission Tomography (PET) as nuclear fragments of target and projectile yield β+-emitters with half-lives of up to about 20 min.[8](#_ENREF_8)

We have recently provided feasibility-data to ablate cardiac arrhythmias with 12C in isolated Langendorff-perfused heart preparations.[9](#_ENREF_9) Here, we demonstrate for the first time ablation with chronic interruption of impulse propagation of several cardiac locations by using pencil-beam scanned 12C in a sham-controlled, large animal model without required procedural access to the body.

# General Methods

An expanded Methods section can be found in the online data supplement.

All animal procedures and irradiation were approved by the regional board of the state of Baden-Württemberg, Karlsruhe, Germany (approval number G-7/14) as well as the committees of the Helmholtz Centre for Heavy Ion Research (GSI), Darmstadt, Germany. All animal procedures were carried out in accordance with the ‘German Law for Animal Research’ (Tierschutzgesetz) and with the guidelines established by Mayo Foundation’s Animal Care and Use Committee.

## Animals and Randomization to Target and Dose Groups

Seventeen pigs (*sus scrofa domestica*)of either sex were included at 10 weeks of age. Three animals were randomized to sham-procedures. Eight animals were randomized to irradiation of the atrioventricular junction (AVJ). Here, in addition, randomization to dose (25 [n = 2], 40 [n = 3], and 55 Gy [n = 3]) was performed. Three pigs were randomized to irradiation of the right superior pulmonary (RSPV) vein left atrial (LA) junction (40 Gy). Three animals underwent left ventricular (LV) freewall irradiation (40 Gy).

**Specific Methods**

A baseline study, assessing pertinent cardiac parameters was conducted for each animal. No information from this study was integrated and used for beam delivery, relying solely on the full-cardiac cycle computed tomography (CT).

**Anesthesia for Invasive Electrophysiological Baseline and Follow-up Studies**

Anesthesia was induced using an intramuscular injection of ketamine (100 mg/kg, Roche, Grenzach-Wyhlen, Germany), azaperone (2 mg/kg, Janssen Pharmaceutica, Beerse, Belgium), and midazolam (0.5 mg/kg, Roche, Grenzach-Wyhlen, Germany). Animals were maintained on 1-3% inhaled isoflurane (Baxter, Unterschleißheim, Germany). ECG was monitored using four surface electrodes. Invasive arterial blood pressure, digital pulse oximetry, and end-tidal CO2 were monitored. For post-operative analgesia morphine was used (sc., 1 mg/kg, Pfizer, NY, USA).

## Baseline Electrophysiological Study

Twelve-lead ECGs were obtained. Transthoracic echocardiography (Philips Healthcare Sonos 5500, Hamburg, Germany) was performed in a supine position for evaluation of LVEF and transmitral valve flow. Skin was prepped using Braunol® (B. Braun, Melsungen, Germany). For vascular access, cut-downs with vessel preparation for placement of introducer sheaths in the left/right external jugular vein and the right/left femoral arteries and veins were performed. A 7 Fr decapolar catheter was advanced into the coronary sinus (CS) from the external jugular vein for electrogram recordings. Catheterization was performed under fluoroscopic guidance (Siremobil Combact L, Siemens Healthcare, Forchheim, Germany). For intracardiac echocardiography (ICE) a 10 Fr 5.5-10 MHz probe was used (Acuson, Cypress, Mountain View, CA, USA). For LA and LV access, a transseptal puncture was performed under ICE guidance using a BRKTM needle (St. Jude Medical, St. Paul, MN, USA) through a SL1 sheath. Electroanatomical (EA) mapping was performed using a mapping system (Carto XP, Biosense Webster, Inc., Diamond Bar, CA, USA). Angiograms of RCA and LCA were performed with an AL-1 catheter (Cordis, Johnson and Johnson®, USA). Intracardiac fiducials were implanted for biplane X-ray positioning before irradiation (Quick Clip 2, 8mm x 2mm, Olympus, Shinjuku, Japan).

**Sedation and Ventilation during CT and Carbon Ion Irradiation**

## Sedation was induced as described above. Maintenance during CT and irradiation was achieved *via* infusion of propofol (iv, 10 mg/cc; 0.25-0.30 mg/kg/min, Diprivan, Fresenius, Bad Hoburg, Germany). No paralytic was required for ventilator and breath-hold compliance. Oxygenation was maintained through ventilation (IPPV, Evita XL, Draeger, Lübeck, Germany).

## Four-Dimensional CT Acquisition

Animals were immobilized using a vacuum mattress and a thermoplastic mask for reproducible positioning (**supplementary Fig. 1**). The CT reference point was marked by radio-opaque markers and also tattooed on the skin. Cardiac gated (4-dimensional [D]), native and contrast enhanced CT scans (1 mm voxel and 1 mm slice spacing) were acquired using a multidetector 64 row Siemens Somatom Definition Flash scanner (Siemens Healthcare, Forchheim, Germany). Scans were acquired at end-expiration using breath-hold of the respirator controlled through a Labview interface (National Instruments, Austin, TX, USA). Contrast-enhanced scans were obtained after injection of 50 cc contrast agent (4 cc/sec, 8-10 seconds delay, Omnipaque 350 mg I/cc, GE Healthcare, USA; [cannula in branch of caudal auricular vein]). Ten sequential cardiac phases were reconstructed using a field of view of 400 mm for skin-to-skin images, required in radiotherapy planning.

## Target Definition and CT Contouring

All contouring of specific structures was performed using Syngo® PRT Planning (VC11B, Siemens AG, Erlangen, Germany) and EclipseTM (Varian medical, Palo Alto, CA, USA) treatment planning software.[10](#_ENREF_10) A 5 mm sphere targeting the AVJ was contoured as ablation lesion, forming the clinical target volume (CTV). RSPV-LA junction and LV freewall ablation lesions were also defined by polygons in transverse CT slices. Besides target volumes, organs at risk (OAR) such as the esophagus, trachea, aorta, and coronary arteries, were demarked.

## Four-Dimensional Treatment Planning

Treatment planning was conducted using GSI’s treatment planning software TRiP4D[11](#_ENREF_11) as it was recently described for cardiac targets.[12](#_ENREF_12) Briefly, a treatment plan for scanned charged particles consists of sets of pencil beams (iso-energy slices, IES). Pencil beams were arranged on a regular grid with 2 mm lateral distance. Deformable image registration was carried out (Plastimatch version 1.15.17). Contoured volumes were propagated to each cardiac phase accordingly. AVJ and RSPV-LA junction contours were isotropically (x, y, z) expanded by 5 mm to account for setup errors. For LV freewall irradiation, 2mm+2% range margin (target depth uncertainty) was added. Considering the beam range to the target volume in all motion phases, the planning target volume was constructed as a union of all phases (range ITV).[13](#_ENREF_13) Subsequently, 12C beam treatment planning (e.g. accelerator energies, beam weights) was conducted.

Charged Particle irradiation

Irradiation was carried out at GSI Helmholtz Centre for Heavy Ion Research, Darmstadt, Germany where a single horizontal beam line is used. Pigs were immobilized as described above. Positional concordance was ensured using repeated matching of two orthogonal X-ray images to CT-derived digitally reconstructed radiographs with adjustment of the treatment table (four degrees of freedom). Pencil beam scanning followed the raster-scanning technique.[14](#_ENREF_14) Slice-by-slice rescanning was used, stochastically homogenizing the target dose.[15](#_ENREF_15) All beams were delivered at end-expiration. The average pencil beam width in the isocenter was 6.5 mm (full width at half maximum).

## In-Beam Positron Emission Tomography (PET) Imaging

For depiction of beam deposition, detection of β+-activity (fragmentation of target and projectile nuclei) was performed. GSI has a dedicated two-head, in-beam PET camera (CTI PET Systems Inc., Knoxville, TN, USA).[16](#_ENREF_16) PET measurements were conducted in expiration only for the first delivered field.[16](#_ENREF_16) Presented PET images display activity deriving from half of the total dose. PET activity was superimposed on reconstructed contrast-enhanced CTs for visualization of cardiac anatomy.

## Follow-up after Irradiation

Animals were followed for up to 6 months after irradiation. Twelve-lead ECGs and device interrogations were performed after 4, 8, 13, and 24 weeks. Skin biopsies of the beam entrance (in-field) and control samples (out-field) were obtained at baseline and during each follow-up. At termination of follow-up, animals underwent repeat electrophysiological study as described above. Animals were euthanized through intracardiac potassium injection.

## Pathological Examination

Heart, lungs, trachea, phrenic nerves, and esophagus were removed *en* block with the pericardium intact. Pericardial fluid was drained and collected. Gross pathological findings were assessed and documented. Triphenyltetrazolium chloride was used to delineate the ablation lesions.

## Histological Examination

For histological analysis, samples were fixed in 4% formaldehyde and processed as described.[17](#_ENREF_17) After fixation, samples were wax embedded and cut with a microtome. Cut sections (5μm) were stained with Hematoxylin & Eosin as well as Mallory’s Trichrome and evaluated using light-microscopy.

## Protein Extraction and Western Blotting

Protein lysation and Western blotting were conducted according to standardized protocols.[17](#_ENREF_17),[18](#_ENREF_18) Antibodies used were anti-caspase 3, anti-tubuline (Sigma Aldrich), and horseradish peroxidase-conjugated secondary antibodies (GE Healthcare Life Sciences). Protein expression was visualized using enhanced chemiluminescence (ECL, Amersham Biosciences) and detected on films (ECL-Hyperfilm, Amersham Biosciences).

## Statistical Analysis

Statistical analyses were performed using IBM’s SPSS (version 23.0). No animals and no data were excluded from analysis. Three animals deceased from device-related infection; follow-up data of these animals was included until the available date of death. Continuous variables are presented as mean ± standard deviation or in case of skewed distribution as median and range. Comparison of electroanatomical voltage mapping data for each animal before and after irradiation was performed using a two-sided t-test (Table 2) or Mann-Whitney’s U test. A *p-*value of less than 0.05 was considered to indicate statistical significance.

**Results**

Study Overview

General baseline characteristics and relevant contouring and targeting parameters are depicted in **Table 1**. The mean LVEF at baseline was 73±4%. Mean weight was 33.8±3.5 kg. Mean duration of follow-up was 20.3 weeks. All animals of all assigned dose and target groups stayed in sinus rhythm during irradiation.

## Atrioventricular Junction Ablation

**Ion Beam Treatment Planning and In-Beam PET:** An exemplary planned 4D-dose deposition including beam rescanning for ablation of the contoured AVJ with 55 Gy is shown in **Figure 1a**. To ensure delivery of >95% of the prescribed dose to the target, isotropic and anisotropic margins were added, also extending into the blood (see **Table 1**). Online PET-imaging during irradiation with 55 Gy revealed strong β+-activityin the atrioventricular septum (**Fig. 1b**) and a lower signal along the beam’s entry channel. Deposition of 40 and 25 Gy resulted in lower but evaluable PET responses. **Figure 1c** depicts the fast β+-signal reduction due to radioactive decay and biological washout over a time-course of 6 min.

**Surface ECG and Decremental Pacing Outcomes:** An overview of the electrophysiological effects of irradiation is given in **Figure 1d-e**. In two out of six animals, irradiation with 55 and 40 Gy led to complete AV block with presence of a junctional escape rhythm (**Fig. 1d**), which developed up to 17 weeks after irradiation (#1, 4; **Table 2**). One other animal of the 55 Gy dose group did not show significant change in atrioventricular conduction at termination of follow-up (#2; **Table 2**). In the animal that developed complete atrioventricular block following 40 Gy (#4), block was not persistent until the end of follow-up at six months. However, an increased Wenckebach-point in relation to the animal’s own baseline (#4, baseline: 260 ms *versus* post-irradiation: 570 ms, **Table 2**) was present. Over the course of six months of follow-up, no electrophysiological effect appeared in the two animals irradiated with 25 Gy (#7, 8; **Table 2**) and in sham-animals.

**Electroanatomical Mapping and Correlation to Macroscopic Lesion Outcome:** To describe extent of the lesion that led to atrioventricular block, electroanatomical voltage mapping was conducted after twenty-four weeks of follow-up. In the animal that developed atrioventricular block following 55 Gy, voltage mapping revealed a lesion area of 2.7 cm2 *versus* an area of 1.4 cm2 for the 40 Gy animal (**Fig. 1e**); concordant with macroscopically endocardial measured lesion dimensions in two planes (#1, 4; **Table 2**). Compared to the sham-procedure group, 40 and 55 Gy of irradiation in these two animals reduced the mean bipolar voltage amplitude of all target location tag-points at the AVJ by 2.0 mV (#1; p<0.0001) and 2.4 mV (#4; p<0.0001), respectively. In the animal of the 55 Gy group without apparent effect, the lesion was misplaced into the posterior left ventricular outflow tract.

## Left Atrial Pulmonary Vein Junction Ablation

**Treatment Planning and In-Beam PET:** Anexemplarycoronal plane of a treatment plan for irradiation of the RSPV-left atrial junction is depicted in **Figure 2a**. Despite the contoured lesion being located on the RSPV-LA junction, high-dose isodose lines extended into the RSPV muscular sleeve (**Fig. 2a**). During irradiation, PET showed only weak β+-activity (**supplementary Fig. 2d**), likely due to the large volume of blood included in the target volume that is quickly washed out.

**Multielectrode Catheter Mapping: Figure 2b** displays a representative image of the circular multielectrode catheter positioned at the RSPV’s ostium. In one of the three studied animals (#12; **Table 2**), irradiation with 40 Gy led to almost complete disappearance of local atrial electrograms in the contoured target area.

**Electroanatomical Mapping: Figure 2c** displays a voltage map of the LA and RSPV obtained before irradiation. In this animal (#12), voltage mapping revealed a large change in local voltage tag points of the LA myocardium close to the RSPV ostium (mean ∆: -1.7 mV; p<0.0001) with a small posterior-inferior gap at the carina to the inferior pulmonary vein (**Fig. 2d**). Extent of the LA ablation lesion over the roof and anterior mitral isthmus led to change in the myocardial activation sequence with late LA activation. In the other two animals, there was a statistically significant decrease in RSPV-LA junction voltage compared to baseline present (#13-14; **Table 2**), however, this was notably smaller.

**Macroscopic Lesion Outcome: Figure 2e** shows the macroscopic lesion outcome six months after irradiation (#12). The main portion of the ablation lesion was located in the intended area. Nevertheless, the lesion extended into myocardium of the PV muscle sleeve, mitral annulus, LA roof, and LAA, reflecting areas included into the beam deposition margin (white contour in **Fig. 2a**) added to the target contour. In the two other cases (#13, 14), there was no macroscopically visible lesion at the RSPV-LA junction present.

## Outcomes in Ventricular Tissue – Freewall Left Ventricle

**Ion Beam Treatment Planning and In-Beam PET:** Target contours were located at the LV freewall; a treatment plan, depicting transverse and coronal planes, with two lateral beam entry fields is shown in **Figure 3a**. **Table** **1** shows the mean target volume including margins to compensate for contractile motion. Irradiation of the LV freewall induced strong β+-activities captured in PET scanning in all animals (**Fig. 3b**) with pronounced washout due to myocardial perfusion (**supplementary Fig. 3**).

**Electroanatomical Mapping and Intracardiac Echocardiography:** All three irradiated animals showed some beam effects in the LV target location. **Table** **2** (#15-17)displays outcomes in LV tissue at two time-points (after 13, 25 weeks) of follow-up. In two cases, examined after thirteen weeks (#15, 16), ICE displayed a hyperechoic myocardium at the LV freewall (**Fig. 3c**). Here, endocardial mapping revealed no significant decrease in mean LV target voltage compared to baseline (#15, 16; **Table 2**). Epicardial mapping, however, showed clustering of fragmented potentials within the target location in one of these animals (#15; **Table 2**). In the animal followed until six months after irradiation (#17), lesion appearance on ICE was similar compared to three months. Endocardial mapping revealed decreased voltage within the target location (mean baseline: 4.0±2.3 *versus* post-irradiation:0.6 ± 0.3; p<0.0001; **Fig. 3d**).

**Macroscopic Lesion Outcome:** Macroscopic epi- and endocardial lesion outcomeis depicted in **Figure 3e, f**. A cross section of the LV ablation lesion outcome is shown in **supplementary Figure 4**.

## Lesion Histology (H&E, Mallory Trichrome) and Markers of Apoptosis

**Lesion Morphology:** Compared to sham-animals, target fibrosis along with hemorrhage (**Fig. 4b**) was present in all animals with present macroscopically induced lesions (**Table 2**). Three months after irradiation, ablation lesions were marked by a higher degree of hemorrhage, inflammation, and early fibrosis than lesions six months after irradiation (**Fig. 4b, c**).

**Dose Dependency:** A comparison for the three different doses applied to the AVJ, namely 25, 40, and 55 Gy, is shown in **Figure 4d-f**. Twenty-five Gy lead to minor fibrotic changes but, nevertheless, clearly led to manifold changes in the target tissue (cardiomyocyte disarray, apoptosis) while both 40 and 55 Gy led to a strong fibrotic response as described above.

**Tissue Apoptosis:** Cleaved caspase 3 was only present in irradiated tissue after three months of follow-up, whereas lesions were negative for cleaved caspase 3 after six months of follow-up (**Fig. 4g, h**), suggesting no ongoing apoptosis in cardiac tissue.

## Irradiation Toxicity during Follow-up

No damage was observed in the tissues of the esophagus, trachea, or phrenic nerves. Coronary arteries did not show a reaction during six months of follow-up. There was no statistical significant change in LVEF between irradiated animals of all groups and sham-animals (mean ∆ irradiation group: 2.4±8.3 *versus* mean ∆ sham-group: 3.3±6.6; p=0.81) or between sham-animals and LV irradiation group present (**Table 1**). No reaction in the beam’s entry channel was present in any animal.

**Discussion**

**Main findings**

These preclinical studies indicate that: i.) Heavy ion beams delivered by raster scanning can be successfully used for ablation of myocardium with chronic interruption of impulse propagation. ii.) In-beam PET is an accurate means for online verification of beam range and dose-deposition of these small, moving, and highly perfused cardiac targets. iii.) Target doses of 40-55 Gy induce complete blockage of impulse propagation; such blockage is to be expected between 13 to 17 weeks after irradiation. iv.) Ultrasound imaging can be used to depict created ablation lesions. v.) Target fibrosis is the main mediator of interruption of cardiac impulse propagation, while multiple non-destructive structural and subcellular changes present at lower doses also affect arrhythmogenesis.[19](#_ENREF_19)

**Charged Particle Heavy Ion Beams**

Despite application of charged particles in non-cancer diseases being completely novel,[20](#_ENREF_20) 12C ion beams to date have been applied as cutting-edge technology for treatment of malignant tumors in more than 15,000 patients worldwide.[21](#_ENREF_21) Physical properties of charged particle beams – inverted dose-profile with major energy release at a specific tissue depth at the end of the particle range (Bragg peak) – enable irradiation of deep seated target volumes with significant sparing of surrounding structures.[22](#_ENREF_22) In case of catheter-free arrhythmia ablation, this translates into decrease in dose-exposure of myocardium as well as mediastinal structures.[23](#_ENREF_23) In addition to irradiation of very large myocardial volumes, studies applying photon beams for catheter-free isolation of the RSPV have created accidental heart block and fistulas of mediastinal organs.[24](#_ENREF_24)

**Time to Effect using Heavy Ion Beams and Required Doses for Cardiac Tissue Ablation**

In this study, it took between 13-17 weeks for the electrophysiological endpoint to occur. There was no difference in that time-course between animals irradiated with 40 Gy and animals irradiated with 55 Gy; this time to atrioventricular conduction block was longer than previously described with photon beams.[25](#_ENREF_25),[26](#_ENREF_26) The irradiated tissue volume with charged particles is notably smaller and conformity to target volume is better than with photon beams, providing a biophysical rationale for different cellular responses. Although our group has shown in isolated hearts that an acute ectrophysiological effect may be seen with high dosing of 12C,[9](#_ENREF_9) the present study doesn’t show acute beam effects on cardiac conduction, but does show chronic effects within the applied dose range.

Single fraction deliveries of 40 and 55 Gy were effective in interrupting atrioventricular conduction chronically. Fifty-five Gy created durable complete atrioventricular block, while 40 Gy led to persistent conduction slowing. At the RSPV-LA junction, 40 Gy led to extensive blockage in atrial myocardium with a small gap present. Lesions in the LV histologically affected the whole wall, but fibrosis did neither homogeneously nor continuously cover the wall.

**Acute Verification of Ion Beam Range and Position**

In-beam PET-imaging successfully verified ion beam range and position. However, in this setup no corrections to either parameter were possible. PET-imaging right after application of a relatively small amount of dose could make early adjustments to ion beam position and/or ion range possible.[27](#_ENREF_27) Strong washout from myocardial perfusion was observed, underlining favorable properties of online in-beam PET for this application. Combination of PET with CT or MRI as it is done immediately following treatment of different tumors[28](#_ENREF_28) provides real-time high-resolution anatomy, but at expense of increased washout and loss of decays with shorter half-life.

**Mechanism of Cardiac Tissue Ablation**

Apoptotic markers were found positive after three months, but negative after six months. Activation of the apoptotic cascade, particularly of endothelial cells, is a known response to ionizing radiation in tumorous tissue.[29](#_ENREF_29) In the heart, ionizing radiation lesions have also been found to start with endothelial vascular damage, ultimately leading to myocardial fibrosis.[30](#_ENREF_30) To our knowledge, there is no previous data on the role and time-course of activation of the apoptotic cascade as a response to ionizing radiation of the *in situ* heart. However, the decisive role of apoptosis in myocardial infarction[31](#_ENREF_31) and subsequent scar remodeling is quite well established.[32](#_ENREF_32), [33](#_ENREF_33)

**Study limitations**

First, included number of animals is limited. Secondly, even though the pig is considered an adequate animal model for arrhythmia ablation, different responses to ionizing radiation compared to human tissue are conceivable in this form of tissue ablation and hence, required doses in human tissue might be different. Thirdly, outcomes in this study are not concordant within each dose and target group; three animals in the AVJ ablation group died of device-related infection. In these, effects could not be as thoroughly evaluated. In cases without electrophysiological effect, there was no macroscopic lesion present or the lesion was misplaced. The reason for this is likely to be multifactorial. State-of-the-art imaging for matching at the irradiation site, such as cone beam CT are becoming clinically available for heavy ion therapy and would have facilitated matching of the treatment planning CT.[34](#_ENREF_34) Positioning was also restricted through a four degrees-of-freedom, instead of six degrees-of-freedom-robotic positioning treatment table, available in centers using latest technology. Lastly, motion compensation for scanned particles is challenging;[35](#_ENREF_35) even though considerable care was taken to suppress effects of cardiac motion on beam delivery, residual dosing errors cannot be fully excluded.

**Clinical Implications and Perspectives**

Catheter-free arrhythmia ablation has been tried with less focused energy sources[25](#_ENREF_25), [36](#_ENREF_36) and has tremendous clinical implications. It would eliminate side effects and risks from introducing and ablating with catheters in the central circulation, including formation of blood clots, embolization, and perforation. Ablation for ventricular arrhythmias often fails because of limited accessibility of the arrhythmia substrate in the ventricles using standard techniques.[37](#_ENREF_37), [38](#_ENREF_38) Ionizing irradiation does have long-term side effects that need to be carefully evaluated in further studies and risk modeling. Toxicity data for high-dose irradiation of small cardiac volumes is scarce. Theoretically, physical properties of ion beams will allow localized, volume-conformal irradiation of arrhythmogenic sources at any given myocardial depth. This is not limited to 12C, but should also be valid for lighter ions with similar focusing capabilities that can be even accelerated using cyclotrons. Integrating particle beam technologies with cardiac MRI, CT, and body surface arrhythmia mapping[39](#_ENREF_39) would equip us with the necessary technology to achieve focused, completely noninvasive arrhythmia ablation.

**Conclusions**

Our preclinical, fully translational study is the first to demonstrate in an intact animal model that extracorporally applied high-energy ion beams can be successfully used for ablation of myocardium with chronic interruption of cardiac impulse propagation. Doses ≥40 Gy of 12C interrupted impulse propagation mainly *via* tissue fibrosis. In-beam PET can be used to verify correct range of ion beams applied to the beating heart. Ultrasound imaging technologies can be used for depiction of the time-course of lesion creation. High-energy particle beams have potential as new and precise means for arrhythmia elimination without any required procedural access to the body and without intrinsic restriction of tissue penetration.

**Author contributions:** D.L.P. conceived the study hypothesis. H.I.L., C.G., C.B., and D.L.P. conceived the study and wrote the study protocol. H.I.L. and D.L.P. wrote the manuscript. H.I.L., C.G., P.L., and D.T. obtained Federal Animal Care and Use Committee approval. H.I.L., M.T., P.S., P.L., C.G., A.C., A.E., R.K., R.R., A.K.R., D.R., M.P., F.F., S.H., N.E., and C.B. conducted the experiments. H.I.L, C.G., P.S., C.F., M.D., S.H., F.F., and D.L.P analyzed and interpreted data. H.IL, D.L.P., C.B., C.G., C.F., and M.D., secured funding. S.J.A., J.D., C.F., S.B.J., K.D.P., D.T., H.A.K., and M.D. helped to design and review experiments. All authors edited and approved the manuscript.

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**Figure Legends**

**Figure 1 – Application of Carbon Ions for Atrioventricular Junction Ablation.** a.) Coronal view treatment plan for AVJ ablation. Dose depicted as color-wash. Target contour in black, enlarged target contour white. 100% corresponds to prescribed dose of 55 Gy. b.) PET-image after 55 Gy of 12C, projected over coronal plane of contrast-enhanced CT; target contour in black. c.) Decay of β+-signal over course of six minutes. d.) Surface ECG (25 mm/sec) before and seventeen weeks after irradiation. e.) RAO projection of RA electroanatomical voltage maps. Local voltage >1.0 mV magenta. Voltage <0.5 mV red. Other colors mark voltages in-between. f.) Right lateral views of lesion outcomes at tricuspid annulus; dashed lines mark lesion. AoR=Aortic root; CS=Ostium of coronary sinus; FO=Fossa ovalis; LAA=Left atrial appendage; LV=Left ventricle; PA=Pulmonary artery; RA=Right atrium; RV=Right ventricle; SL=Septal leaflet of the tricuspid valve.

**Figure 2 – Impact of Carbon Ions on the Right Superior Pulmonary Vein Left Atrial Junction.** a.) Coronal view of treatment plan for RSPV-LA junction irradiation. Details as described for Fig. 1a. b.) RAO of RSPV venography and with circumferential mapping catheter. Dashed lines mark projection of RSPV. c.) Right-lateral projection of baseline LA endocardial voltage map and RSPV. d.) Right-lateral projection of LA and RSPV voltage map six months after irradiation (40 Gy). Voltage as in legend; voltages >0.7 mV in magenta, voltage <0.2 mV red. Other colors mark voltages in-between. d.) Macroscopic lesion outcome RSPV-LA junction. RSPV opened at 12 o’clock. Appreciate macroscopic lesion with hemorrhage and fibrosis. CS=multielectrode catheter in coronary sinus. Circ.=circumferential mapping catheter. Turquois dots=double potential. White dots=fragmented signals/area of slow conduction. FO=Transseptal puncture side in the fossa ovalis; LAA=left atrial appendage; MVA=Mitral valve annulus; RSPV=right superior pulmonary vein.

**Figure 3 – Outcomes after Left Ventricular (LV) Freewall Irradiation**. a.) Transverse and coronal plane of treatment plan with two 12C beams (40 Gy). Details as in Fig. 1a. b.) Anteroposterior view of by-product β+-activity *via* PET, superimposed on coronal plane of a contrast-enhanced CT scan. Black contour=target, β+-activity as color-wash. c.) ICE image of LV freewall after irradiation, hyperechoic lesion marked by dotted lines. d.) LAO of an endocardial voltage map of the LV six months after irradiation. Voltage >1. 0 mV depicted in magenta. Voltage <0.5 mV red. Other colors mark voltages in-between. White dots =fragmented potentials. e.) Macroscopic LV epicardial lesion outcome six months after irradiation, dashed line marks target zone. f.) Endocardial lesion outcome after six months. Ao=Descending aorta; F =Freewall; LAA=Left atrial appendage; LAD=Left anterior descending coronary artery; LIPV=Left inferior pulmonary vein; LVOT=Left ventricular outflow tract behind the mitral valve leaflet; LL=Left lung; IVC=Inferior vena cava; LV=Left ventricle; LV-A=Left ventricular apex; RIPV=Right inferior pulmonary vein; RL=Right lung; RV=Right ventricle; S=Septal site.

**Figure 4 – Mallory Trichrome Staining of Ablation Lesions, and Apoptosis Outcomes**. a.) Sham-control b.) Target tissue three month after irradiation (40 Gy); marked hemorrhage, inflammation, and early stage fibrosis, c.) Target tissue six months after irradiation; later stage fibrosis. d.-f.) Comparison of lesion outcomes for 25, 40, and 55 Gy six months after irradiation. g.) Western blot for cleaved caspase 3–marker for apoptosis; signals positive in myocardium three months after irradiation, no signals after six months. h.). Bz=borderzone; ep=Epicardium; IRR=Irradiated tissue; LV=Left ventricle; PC: positive control HaCaT (Lysats of HaCaT cells five days after irradiation with 10 Gy of X-ray), I=infield; Out=Outfield; RV=Right ventricle.

# Tables

**Table 1:** Study population general metrics, results for global cardiac function and treatment planning parameters for all dose groups and targets. The mean planning target volume corresponds to the irradiated volume. AVJ=atrioventricular junction ablation; LA-SPV junction=left atrial superior pulmonary vein junction; LVEF=Left Ventricular Ejection Fraction; MeV=Megaelectronvolt; min=minutes. ¥=Data only available for one animal. † Data not available. Data are depicted as mean ± standard deviation. ¶Median (range).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Study Group** | | | | | | |
|  |  | **All Pigs**  **(n = 17)** | **Sham-control**  **(n = 3)** | **AVJ 25**  **(n = 2)** | **AVJ 40**  **(n = 3)** | **AVJ 55**  **(n = 3)** | **RSPV-LA junction**  **(n = 3)** | **Freewall LV**  **(n = 3)** |
| **General Metrics** | Male sex, n (%) | 13 (76) | 3 (100) | 2 (100) | 1 (33) | 3 (100) | 2 (66) | 2 (33) |
| Mean weight at imaging, kg | 33.8±3.4 | 32.5±4.6 | 35.1±3.1 | 35.5±1.2 | 31.6±1.7 | 36.5±2.6 | 33.6±2.9 |
| Mean weight at irradiation, kg | 34.2±3.2 |  | 30.2 | 36.4±1.7 | 32.0±1.7 | 35.0±3.0 | 34.1±3.9 |
| Mean weight gain at 6 months, kg | 39.8±14.0 | 36¥ | 54.3±8 | 32.8¥ | 50.3±1.0 | 35.0±2.1 | 47¥ |
| Mean duration of follow-up, weeks | 20.3±5.7 | 18.7±5.6 | 24.4±0.1 | 17.3±5.3 | 20.3±5.6 | 24.5±0.1 | 16.6±5.6 |
|  | Mean time from CT to Irradiation, days | 10.9±3.4 |  | 13.5±1.5 | 16.0±1.4 | 14.0±2.5 | 8±0 | 9.3±0.5 |
| **Cardiac Function** | Baseline LVEF (%) | 73±4 | 70 ± 2 | 77±6 | 72±4 | 78±1 | 74±1 | 73±5 |
| LVEF (%) 6 months after irradiation | 76±6 | 73 ± 8 | 75±4 | 79¥ | 79±1 | 72±2 | 78±7 |
| Baseline E/A wave ratio | 0.9±0.5 | 0.66 ± 0.02 | 0.54±0.01 | 1.0±0.5 | 0.7±0.2 | 1.1±0.2 | 1.3±0.8 |
| E/A wave ratio 6 months after irradiation | 0.8±0.2 | 0.74 ± 0.1 | 1.2±0.1 | 0.6¥ | 0.6±0.01 | 0.6±0.04 | 1.0±0.3 |
| **Irradiation Parameters** | Mean clinical target volume (CTV, cc) |  |  | 0.5 | 0.5 | 0.5 | 0.9 (0.8–1.1)¶ | 2.3±0.3 |
| Mean clinical target volume +5 mm in x, y, z (cc) | 1.8±0.1 | 1.8±0.1 | 1.8±0.1 | 14.9±1.8 |  |
| Mean planning target volume (PTV, cc) | 3.7±0.3 | 3.4±0.5 | 3.9±1.0 | 23.2±1.7 | 8.9±1.8 |
| Iso-energy slices, n (IES) | 11.5±1.5 | 9±1.4 | 8.3±0.5 | 20±1 | 14 |
| Range of used acceleration energies, MeV | 172–211 | 175–208 | 183–212 | 129–233 | 112–186 |
| Mean treatment time (min. incl. breath-holds) | 15.7 | 7.3 | 10.8 | 23.4±8.1 | 23.9±8.6 |

**Table 2:** Upper row:Ablation outcome for animalsirradiated at AVJ with lesion outcome. Lower row: Ablation outcome for animals irradiated at LV freewall. n.a. 3º AVB=not applicable as complete atrioventricular block at end of follow-up present. Irrad.=Irradiation. TTE=transthoracic echocardiography; ICE=intracardiac echocardiography; †=Deceased from device-related infection; ¥=Complete atrioventricular block 17 weeks after irradiation. ¶Lesion misplaced into the posterior LV outflow tract. ‡Lesion in whole wall; fibrosis not necessarily continuous in whole wall.Two-sided t-test for comparison of mean baseline voltage in target *versus* mean at follow-up date. \*p<0.05; \*\*\*p<0.0001.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Animal #** | **AV junction ablation Dose (Gy)** | **Mapping Outcome (cm2) – tag points with voltage ≤0.3 mV** | **Macroscopic Lesion Dimension Surface (cm2)** | **Macroscopic Transmural Lesion in Septum** | **∆ Wenckebach end Follow-up (ms)** | **Follow-up (weeks)** |
| 1 | 55¥ | 2.9 | 2.5 | Yes | n.a. 3ºAVB | 24.3 |
| 2 | 55 | 0 | 1.7¶ | No | 10 | 24.2 |
| 3 | 55 | † | 0 | No | † | 12.4 |
| 4 | 40¥ | 1.3 | 1.0 | Yes | 310 | 24.3 |
| 5 | 40 | † | 0 | No | † | 11.6 |
| 6 | 40 | † | 0 | No | † | 16.0 |
| 7 | 25 | 0 | 0 | No | 10 | 24.4 |
| 8 | 25 | 0 | 0.6 | No | 50 | 24.3 |
| 9 | Sham-group | 0 | 0 | No | 10 | 14.7 |
| 10 | Sham-group | 0 | 0 | No | 10 | 14.7 |
| 11 | Sham-group | 0 | 0 | No | 10 | 26.7 |
|  | **RSPV-LA junction Dose (Gy)** | **∆ Voltage (mV) Endocardial Tag Points RSPV-LA junction** | **Macroscopic Transmural Lesion** | **Hyperechoic ∆ Wenckebach end**  **on TTE/ICE Follow-up (ms)** | | **Follow-up (weeks)** |
| 12 | 40 | -1.7\*\*\* | Yes | Yes -5 | | 24.4 |
| 13 | 40 | -0.8\* | No | Yes 10 | | 24.4 |
| 14 | 30 | -0.6\* | No | Yes 5 | | 24.6 |
|  | **LV freewall**  **Dose (Gy)** | **∆ Mean LV-Target Endocardial Voltage (mV)** | **Post Irrad.: Abnormal or Double Potentials Endo-/Epicardial (#tags)** | **Histology Transmural Lesion‡** | **Hyperechoic on TTE/ICE** | **Follow-up (weeks)** |
| 15 | 40 | -0.5 | -/10 | Yes | Yes | 12.6 |
| 16 | 40 | -0.6 | -/- | Yes | Yes | 12.6 |
| 17 | 40 | -3.4\*\*\* | 19/- | Yes | Yes | 24.6 |

**Figures**

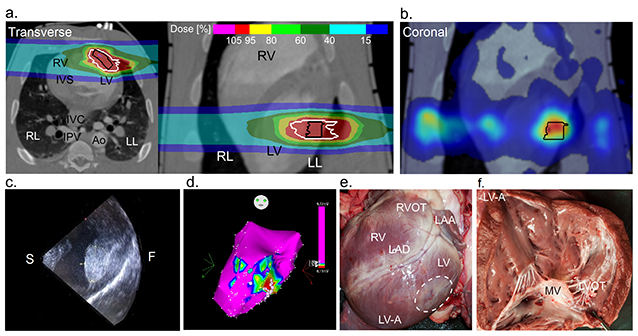
**Figure 1**

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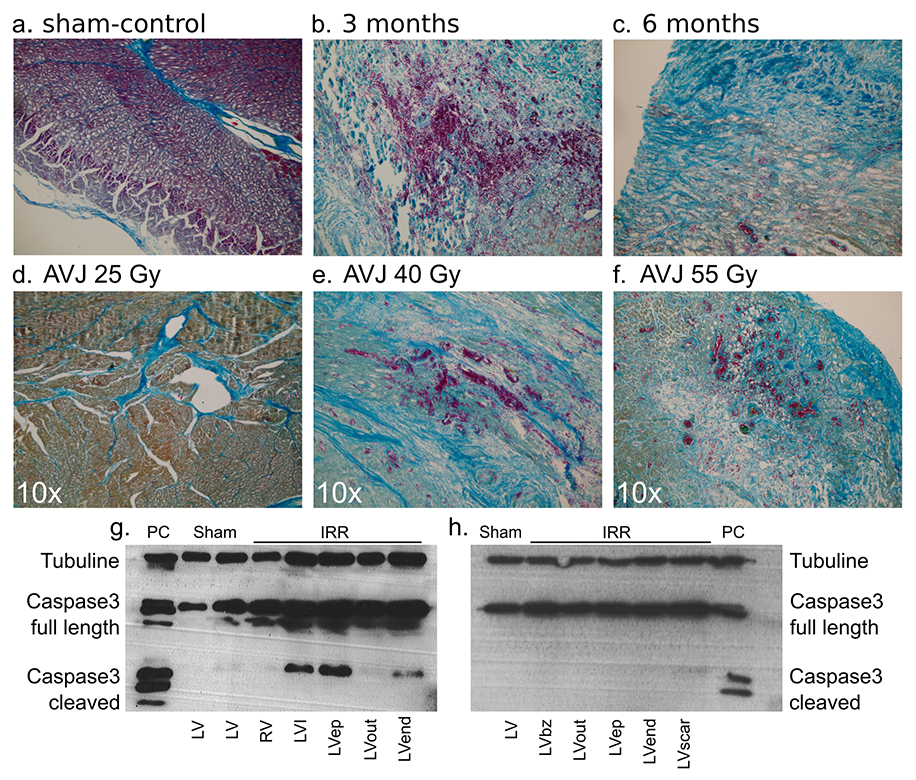
**Figure 2**

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**Figure 3**

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**Figure 4**

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