

Supplementary Table S1: Pre-clinical, proof of concept studies for ionizing radiation for cardiac tachydysrhythmias

Study	Study Size	Radiation dose	Radiation Time	Results	Complications/Safety	Pathology
Pulmonary Vein Isolation						
Perez-Castellano <i>et al.</i> 2006	10 mini swine	Purposefully non-circumferential ablation of right superior pulmonary artery trunk at 60 Gy using brachytherapy catheter	359±157 sec	Successful delivery of radiation with necrosis and fibrosis visualized in the pulmonary vein. Decreased local electrogram voltage and increased pacing threshold at ablated site at 81±27 days. No change in bidirectional PV-LA conduction with noncircumferential ablation. Burst pacing in PV induced nonsustained AF in 80% of animals at baseline and in 20% of animals after radiation.	None reported	N = 2 (immediately post ablation) Acute effects of endothelial denudation covered by white thrombus, elastic lamina disruption, and PV sleeve necrosis. N = 8 (81±27 days) Late effects of neointimal hyperplasia and elastic lamina thickening.
Sharma <i>et al.</i> 2010	2 mini swine - LAA 3 mini swine – left pulmonary vein	32-80 Gy (LAA) / 38-40 Gy (PV) using X-ray beam irradiation	60 minutes	<u>Left Atrial Appendage:</u> Decreased local electrogram voltage at 38 Gy at 33 days. <u>Left Pulmonary Vein:</u> Decreased local electrogram voltage at PV-LA junction from 89-196 days (low frequency and low amplitude far field signal) and no local activation seen	no pulmonary vein stenosis at post-mortem; no pulmonary HTN or changes on echo; normal histology of trachea and esophagus	well-circumscribed lesion with transmural loss of myocyte architecture and increased fibrin
Gardner <i>et al.</i> 2012	3 canine 1 swine	35 Gy (canine) 25 Gy (swine) Delivered to the PV using x-rays (Cyberknife)	not reported	In-vivo dose measurement using surgically implanted dosimeters. Measurement of radiation delivery to cardiac structures for PV vein isolation. Dose-to-volume plans for cardiac target of 10%	Lower measured radiation exposure to the esophagus and epicardium of up to 25%.	Not reported
Blanck <i>et al.</i> , 2014	9 mini-swine	17.5 to 35 Gy Dose escalation study X-ray (LINAC) to the right superior pulmonary vein	not reported	Transmural scarring of cardiac muscle noted with doses >32.5 Gy. Not complete circumferential scarring of the pulmonary vein.	No superficial radiation damage and normal cardiac function.	Fibrosis noted at doses >30 Gy increasing with radiation dose. Transmural scarring at doses >32.5 Gy. Fat tissue necrosis at doses >20 Gy.
Lehmann <i>et al.</i> 2016	3 swine	40-50 Gy to the right superior PV and LA junction using carbon ion irradiation	23.4±8.1	Decreased local atrial electrogram voltage with disappearance of local atrial electrograms in one animal at 24 weeks	preserved LVEF at 6 months	RSPV-LA junction at 6 months showed local hemorrhage, inflammation, and fibrosis
Zein <i>et al.</i> 2018	17 canines 2 adult swine	15, 20, 25 and 35 Gy to the right superior PV/LA junction using X-ray beam irradiation	69.5±42.3 minutes	11/11 treated with 25 or 35 Gy; 3/5 treated with 20 Gy; 0/2 treated with 15 Gy had full treatment effect (PV/LA junction entrance and exit block). 1/5 treated with 20 Gy; 1/2 treated with 15 Gy had partial treatment effect (low voltage local electrogram voltage and/or partial PV/LA junction block)	At 6 months: mild LV hypokinesia (3/19), trace MR (12/19), PV diameter decreased 30-35% (3/19), pericarditis (1/19), T wave changes (3/19)	circumferential transmural scar at the PV ablation site; LV infarct (1/19); no damage of pericardium, valves, coronary arteries, esophagus, phrenic nerves, lung parenchyma
Cavo-tricuspid Isthmus						
Sharma <i>et al.</i> 2010	9 mini swine	32-80 Gy using X-ray beam irradiation	60 minutes	decreased local atrial electrogram voltage at all doses at 35-108 days; bidirectional block seen at 40 Gy in one animal at 30 days	normal histology of trachea and esophagus	well-circumscribed lesion with transmural loss of myocyte architecture and fibrosis
AV Node						
Sharma <i>et al.</i> 2010	2 mini swine	40-70 Gy using X-ray beam irradiation	60 minutes	third degree AV block at 49 days in one animal, the other sacrificed at 15 days	one animal euthanized early (15 days) secondary to pacemaker pocket infection	well-circumscribed lesion with transmural loss of myocyte architecture and fibrosis
Lehmann <i>et al.</i> 2015	4 swine (explanted hearts) Langendorff perfused	70, 90, 160 Gy using Heavy Ion ¹² C	Not reported	130 Gy caused disturbance of AV conduction and transitioned into complete heart block	Ex-planted hearts, no reported complications.	No macroscopically visible damage to the triangle of Koch or parts of the AV septum. Hyper eosinophilia is present.
Lehmann <i>et al.</i> 2016	8 mini swine	25, 40, 55 Gy using carbon ion irradiation	25 Gy – 15.7 min 40 Gy – 7.3 min 55 Gy – 10.8 min	Irradiation with 40 and 55 Gy led to complete heart block in 2/6 appearing up to 17 weeks but one swine treated with 40 Gy re-established AV conduction; irradiation with 25 Gy was not effective in any animal	no damage observed in tissues of the coronary arteries, esophagus, trachea or phrenic nerves at 6 months	macroscopic lesion was seen in animals treated with 55 and 40 Gy
Lehmann <i>et al.</i> 2017	7 swine	25, 40, 50, 55 Gy using photon beam irradiation	25 Gy – 9.9 min 40 Gy – 14.7 min 50 Gy - 19.9 min 55 Gy – 25.7 min	6/6 developed third degree AV block at median 11.2 weeks; remaining animal died early of pacemaker infection; the one animal treated with 25 Gy only developed AV block during final catheter EP study.	no short term side effects were observed and no damage to the esophagus, phrenic nerve or trachea	histology revealed dense fibrosis at 3 months
Refaat <i>et al.</i> 2017	5 swine	35-40 Gy using LINAC	15 min	Development of complete AV block from 55-235 days in all 5 swine.	No evidence of damage to surrounding tissue by histology.	Histology of AV node – severe architectural disruption with loss of smooth cellular organization.
LV Free wall						
Amino <i>et al.</i> 2006 Amino <i>et al.</i> 2010	24 rabbits	15 Gy heavy ion beam	Not reported	Increase of connexin43 mRNA and protein levels. Epicardial potential mapping of the free wall with reduced conduction velocity.	1 year follow up, no significant changes in ECGs and echocardiogram. No evidence of late radiation injury	Significant upregulation of connexin43 protein and mRNA
Lehmann <i>et al.</i> 2016	3 mini swine	40 Gy using carbon ion irradiation	22.9±8.6 min	epicardial mapping revealed clustering of fragmented electrogram potentials without significant voltage loss	echo displayed a circumscribed area of hyperchoic myocardium at the LV free wall	irradiated tissue displayed target fibrosis, cardiomyocyte disarray and hemorrhage
Amino <i>et al.</i> 2017	4 canines	15 Gy ¹² C heavy ion beam	Not reported	Normalization of QRS duration compared in canine's with myocardial infarction treated with radiation. Decreased inducibility of VT/VF by 25%. Immunostaining of connexin43 significantly increased at 1 year.	Skin reactions n=2, desquamation reaction and skin ulcers with alopecia. No echo changes or death	Immunostaining of connexin43 significantly increased at 1 year.
Hohmann <i>et al.</i> 2019	20 swine	Proton beam 40 Gy at 1 site n = 8 30 Gy at 2 sites n = 4 40 Gy at 3 sites n = 8 (Anterior, inferior, apex)	9.2 ± 2.4 min	High dose multi site radiation resulted in LV dilation and impairment of LV function. Changes seen up to 3 months.	6 died during follow-up from 40 Gy 3 site treatment (>60 days). No apparent cause on pathology. Reduction in LV function and LV dilation.	16 weeks – increased collagen reflecting fibrosis with necrotic tissue, vacuolar degeneration and myocytolysis