Myocardial inflammation and fibrosis 40 weeks after cardiac irradiation: ApoE-/- > WT

Gabriels et al. R&O 2012
Seemann et al., R&O 2012
Darby et al. IJROBP 2010
Decreased microvascular density and impaired angiogenesis: ApoE-/- earlier than WT

Gabriels et al. R&O 2012

Hovings et al. IJRB 2013

Microvascular functional damage

Seemann et al., R&O 2012; Gabriels et al. R&O 2012

Vascular leakage (40 wks) WT & ApoE-/-

0 Gy  15-20%
2 Gy  30-50%
8 Gy  70-100%
16 Gy 90-100%

Less marked in ApoE-/-

Diffuse amyloidosis

Earlier in ApoE-/-
Importance of EC damage in radiation-heart disease

Specific EC deletion of p53 sensitized to cardiac death and functional damage

Radiation damage in Tie2Cre, p53^{FL-} mice results in cardiac ischemia, myocardial degeneration, fibrosis and vascular leakage

C-L Lee et al., Science Signaling 2012
Non-symptomatic perfusion defects from 6 months after RT for left sided breast cancer

Marks et al. IJROBP 2005

- Progressive increase in perfusion defects greatest when >5% LV in RT field
- Defects follow contour of RT field rather than coronary arteries
- Wall motion abnormalities in LV correlated with perfusion defects
Coronary artery and endocardial lesions: 
ApoE-/- mice

Atherosclerosis in CA of mid heart 20-40 weeks after 8-16 Gy

Endocardial foam cells 
20 weeks after 16 Gy

Endocardial collagen 
40 weeks after 8-16 Gy

Endocardial fibrin 40 weeks 
after 8-16 Gy

Gabriels et al., R&O 2012
Septal coronary artery lesions (lower branches) developing independently of valvular lesions

Gabriels, te Poele, Gasparini & Stewart, unpublished
Thrombotic aortic valve lesions:

16 Gy base and whole heart irradiation only

Intraplaque hemorrhage: 20 weeks

Fresh thrombus: 40 weeks

Gabriels, te Poele, Gasparini & Stewart, unpublished
Heart function: gated SPECT Tc-99m tetrofosmin (microvascular filling)

Orientation of LV
3D reconstructions of SPECT data
Analysis in diastole and systole give parameters of cardiac function

HLA  VLA  SLA

Diastole
Systole

Volume (ml) and Filling (ml/s)
Cardiac function 20-40 weeks: WT = ApoE-/-

Seemann et al., R&O 2012; Gabriels et al. R&O 2012

EDV (ml)

ESV (ml)
Cardiac function 20-40 weeks: WT = ApoE-/-

Seemann et al., R&O 2012; Gabriels et al. R&O 2012

Lethality in 38% WT mice only at 30-40 weeks after 16 Gy; strongly associated with vascular leakage and amyloidosis
Ingenuity pathway analysis of differentially regulated genes ApoE-/-

2Gy:

cellular growth and proliferation (MMP2), inflammation and heat shock genes, PI3K/Akt signaling (cardiomyocyte protection)

16 Gy:

Cell signaling (TIMP1), myocardial hypertrophy, fibrosis, collagen metabolism and ECM turnover, FN1 pathway (genes associated heart failure)

Gabriels et al., R&O 2012
Summary of cardiovascular damage after irradiation

• Doses > 2 Gy induce inflammatory and thrombotic changes (activated chemokine signaling between leukocytes/EC, activated ROS, increased type 1 MΦ activity)

• **Large arteries:** > 2 Gy initiates development atherosclerosis and predispose to inflammatory, unstable plaque

• **Heart:** >2 Gy causes capillary loss and damage, leading to perfusion defects, myocardial cell death and fibrosis

• Cardiac function remains near normal until sudden death after high doses (compensatory mechanisms?)
Possible mechanisms of cardiovascular damage after irradiation

**Local effects**
- Inflammatory /thrombotic changes in microvasculature
- Oxidative stress, cytokine cascades/imbalance
- Fibrotic responses
- Accelerated ageing (senescence) of microvasculature
- Accelerated atherosclerosis, thrombotic unstable phenotype

**Systemic effects**
- Elevated cholesterol levels
- Depressed immune system
- Hypertension/renal damage
- Metabolic changes
HER2 genes overexpressed in breast carcinoma

~ 20% of breast cancers HER2 +

- Increased cell proliferation
- Decreased apoptosis
- Enhanced cell motility

Treatment with humanized Ab trastuzumab or TK inhibitor lapatinib improves outcome

Adapted from Genentech BioOncology.com
HER2 agents increase risk of cardiac damage after anthracycline treatment

3-4% heart failure or death

Ongoing studies to investigate long term cardiac damage after RT and concurrent or adjuvant ErbB2 inhibition

Romond et al. NEJM 2005
Neuregulin-1 signaling in cardiac myocytes

**Stress**: oxidative stress by anthracyclines

**Ischemia**: restricted blood supply by radiation-induced microvascular damage

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*Sawyer et al. Circulation research 2012*
**Experimental setup**

**Concurrent**
- Irradiation 14 Gy
- Lapatinib diet
- Week -1, 0, 20, 40

**Sequential**
- Irradiation 14 Gy
- Lapatinib diet
- Week 0, 20, 40
Endothelial cell dysfunction and cell loss

Seemann et al. Breast Cancer Res Treat 2013

- Radiation-induced endothelial loss in microvessels not influenced by lapatinib

CD31

Alkaline phosphatase

Von-Willebrand-Faktor
Cardiac inflammation and fibrosis

Seemann et al. Breast Cancer Res Treat 2013

- Radiation induced inflammation in the epicardium decreased by lapatinib

- Radiation induced fibrosis not increased by lapatinib
Conclusion

Neither concurrent nor sequential treatment with HER2-blocking agent lapatinib enhanced cardiac toxicity up to 40 weeks after irradiation
Last words of caution!!!

Vasculature

Concurrent

Sequential

H&E score

Dilation of capillaries

Degeneration of the coronary arterioles

Myocardium

H&E score

Degeneration

Hypertrophy