Summary

Radiotherapy is an effective treatment of cancer, although it contributes to late toxicity in surrounding normal (non-cancer) tissue. Little is known about the underlying mechanisms and the contribution of microvascular damage to late cardiac toxicity after radiotherapy alone or in combination with chemotherapy or tyrosine kinase receptor inhibitors. In this thesis we aim to shed light on the underlying mechanisms of radiation and anthracycline-induced cardiovascular damage.

In **chapter 2**, a mouse model was used to investigate the histological and functional effect of low, intermediate and high dose (2, 8, 16 Gy) single irradiation to the heart at early (20 weeks) and late (40 and 60 weeks) time points. With this study we demonstrated that irradiation affects cardiac structure and microvascular function in a dose and time-dependent manner, with substantial damage after intermediate and high dose irradiation (8-16 Gy) and minor alterations after lower doses (2 Gy).

High cholesterol level in the blood is linked to age-related atherosclerosis, an event that is also induced by irradiation in *ApoE*$^{-/-}$mice, which have elevated levels of cholesterol, similar to humans on western-type diet. We therefore treated *ApoE*$^{-/-}$ mice in **chapter 3** with single irradiation to the heart of 2, 8 and 16 Gy, resulting in an early and pronounced inflammatory response and microvascular leakage in the hearts. These mice also developed atherosclerotic lesions in mid-sized coronary arteries.

The risk of cardiac toxicity after anthracyclines and radiotherapy is recognized, but little is known about the increased risk when these treatments are combined with inhibitors of epidermal growth factor receptor 2 (ErbB2). Thus, we investigated in **chapter 4** the effect of combined treatments on survival and growth of cardiomyocytes *in vitro*. We further studied histomorphology and microvascular damage of mice treated with radiation or anthracycline alone or in combination with ErbB2-inhibitor lapatinib. While radiation and anthracycline induced cardiac toxicity, we did not see any enhancement in structural and microvascular damage when blocking ErbB2.

Endothelial cells have been shown to be highly sensitive to radiation. Endoglin, co-receptor of TGF-β1, is essential for angiogenesis and predominantly expressed in proliferating vascular endothelial cells and therefore may play a crucial role in cell proliferation and thus revascularization in damaged cardiac microvasculature. **Chapter 5** demonstrates that radiation-induced endothelial cell damage was independent of endoglin expression levels. However, lower endoglin expression levels limits the early inflammatory response and fibrosis in our radiation-induced mouse model of cardiac damage.

A better understanding of the underlying mechanisms of radiation-induced cardiac damage allows for the development of intervention strategies. In the second part of this thesis we focused on intervention and strategies to overcome radiation-induced cardiovascular damage. Since inflammatory and fibrotic events are dominant features in radiation-induced heart damage, we tested whether an anti-inflammatory and anti-fibrotic agent thalidomide could prevent further fibrotic progression after irradiation. In **chapter 6** we have shown that radiation leads to inflammatory and fibrotic response and that these events could not be reduced by thalidomide.

Radiation induces endothelial cell loss, which results in decreased microvascular density. Perfusion defects have been identified in asymptomatic breast cancer patients shortly after radiotherapy. Vaculogenesis, stimulated by precursor cells that differentiate into endothelial cells, has been shown to be essential in tissue repair and remodeling during acute and chronic ischemic tissue damage. In **chapter 7** we used bone-marrow derived endothelial cells from either endoglin haplo-insufficient or endoglin profficient mice and transplanted them into our radiation-induced mouse models. We demonstrated that bone-marrow derived endothelial cells did reduce the amount of radiation-induced cardiac fibrosis. However, these cells were unable to restore microvascular damage.

We believe that our data on cardiac microvascular damage induced by radiation, give new insights into the underlying mechanisms and provide some crucial aspects for finding new strategies to overcome radiation-induced cardiac damage.