VALORIZATION ADDENDUM
In the Netherlands alone, cardiovascular diseases account for roughly 1000 hospitalizations a day. The Dutch heart foundation calculated that in total 8.3 billion euro was spent on cardiovascular disease care in 2011, thereby illustrating the magnitude of the burden that cardiovascular diseases place on the national economy. The majority of this amount was spent on hospital care and care from medical specialists. However, despite the rise in life expectancy during the last decades, which can in part be ascribed to novel, often interventional technologies, these technologies are typically applied in advanced stages of disease and are therefore cost-ineffective. Early diagnosis and early treatment or even prevention of heart and peripheral vascular disease, will allow better risk stratification of patients with disease-stage-adapted-therapy instead of escalating to the most aggressive and costly therapy. However, from clinical trials that have been executed it has become clear that clinical readout parameters such as peak walking distance in peripheral vascular disease trials or exercise tolerance test in coronary artery disease trials are not sufficiently sensitive and reproducible to document incremental improvements in function and perfusion. In addition, gathering indirect evidence for neovascularization (i.e. increased perfusion or function) through angiography, SPECT, PET or MRI often takes months before any improvement can be detected.

The work described in this dissertation contributes to the translation of non-invasive neovascularization imaging to the clinic, thereby ultimately providing patients with tailored therapy programs as well as reducing healthcare costs. All of our scientific endeavors were performed within the framework of a Center for Translational Molecular Medicine (CTMM), project Eminence. Within this project we collaborated closely with industry partners from MILabs and PIE Medical Imaging.

As is often the case for preclinical endeavors, the scientific community is the first to benefit from the novel findings described in this dissertation. Through online publication the majority of the scientific work we describe here is globally available. Nevertheless, how these new insights are eventually translated into value beyond the scientific domain is unfortunately difficult to measure. Still, we can speculate on the potential value creation for the technologies that we have investigated.

TOWARDS BETTER DIAGNOSIS AND CLINICAL NEOVASCULARIZATION THERAPY

Candidate agents for non-invasive arteriogenesis imaging

In chapter 2 and 3 we described pioneering work in the field of nuclear arteriogenesis imaging. To our knowledge no studies have been published describing nuclear arteriogenesis imaging or the development of a nuclear arteriogenesis tracer. The knowledge we generated on this topic can serve as a starting point for future studies investigating non-invasive arteriogenesis imaging. We narrowed the window of potential
candidate arteriogenesis tracers down by eliminating the most obvious candidates. This prevents scientists to pursue avenues that are unfruitful and costly.

**Preclinical testbed**

Over the past decades numerous hind limb ischemia animal models have been developed to mimic the situation of peripheral vascular disease and to study neovascularization stimulating agents. In these models, the blood flow in one (or more) arteries of the hind limb is obstructed, thereby creating a drop in blood perfusion throughout the whole limb. This drop in perfusion is temporary since the body is able to (fully) restore the perfusion by means of neovascularization. The time until perfusion restoration is completed is often called the therapeutic window (i.e. the time in which it is beneficial to stimulate neovascularization). In the past, numerous studies have employed the therapeutic windows, created in these hind limb ischemia models, to test neovascularization stimulating agents thereby using laser Doppler perfusion imaging (LDPI) as a readout. In chapter 4 we used SPECT perfusion imaging to show the inaccuracy of LDPI as a readout in these models. The latter still indicates the need for perfusion recovery while SPECT imaging indicated that the relevant perfusion recovery over the total volume of the hind limb has already taken place. Using LDPI it is easy to overestimate the therapeutic window, which in turn can lead to the misinterpretation of neovascularization stimulating agents or application at the wrong time point. This is an important finding for cardiovascular disease patients since almost all clinical studies employing neovascularization stimulating agents have failed to capitalize on the positive outcomes of their preclinical predecessors. Interpretation of stimulating agents in the correct time window (determined by SPECT perfusion imaging) can lead to important new insights about the mechanism of action and impact of such agents thereby eventually leading to different therapeutic regimens for cardiovascular disease patients that can be tested in clinical trials and are hopefully more predictive.

SPECT offers the opportunity to perform multi-isotope imaging thereby making it an attractive option for studying the often multifactorial nature of cardiovascular disease. In chapter 5 we showed that ECG-gated SPECT can deliver reliable cardiac functional measurements while simultaneously indicating myocardial perfusion in a mouse myocardial infarction model. Valuable input from our colleagues at MILabs helped us extract the most out of our SPECT data. During this joint project a strong relationship MILabs was established that provides both parties with new opinions, insights and tools for refining the technology.

In chapter 7 we built on insights we gained from the study described in chapter 6. Three new SPECT tracers for angiogenesis imaging were developed and tested in a mouse myocardial infarction model. In chapter 6 we demonstrated specific angiogenesis imaging in the infarcted myocardium using a disulfide cyclized cNGR angiogenesis tracer.
Considering the rather low uptake of this tracer we set out to improve this by altering the nature and the valency of our tracer. With the two new tracer constructs that resulted from these efforts we made a valiant step forward with respect to uptake in infarcted areas. Both new tracers showed significantly higher uptake in infarcted areas. Considering the high specific uptake in infarcted myocardium in combination with low uptake in remote organs we expect these tracers to eventually make the step to clinical trials. However, before these tracers can be tested in randomized controlled trials in humans several additional steps have to be taken. Large scale animal studies have to be conducted in different species (including toxicity studies) to provide sufficient proof of efficacy before production of the tracer under good manufacturing practice (GMP) conditions can be considered. Thereafter, the true benefit of clinical angiogenesis imaging has to be proven against traditional endpoints or indirect effects such as clinical state and tissue perfusion or function. In case clinical studies indicate positive results, these tracers will become available for everyday myocardial infarction patient care.

I believe the time efforts and costs that were made during my PhD trajectory are all justified by the work presented in this dissertation. Big things have small beginnings and like with all preclinical research, a lot of work still has to be performed before cardiovascular disease patients will benefit from the knowledge generated by our endeavors.