Chapter 7

Summary and Future Perspectives
Summary

Cardiovascular disease is still a leading cause of death.\(^1\) In order to improve cardiovascular outcome, development of novel drugs is warranted and the pharmaceutical enterprise is expected to provide an important contribution. In the last decades, many drugs were registered and authorized for clinical application. These drugs are targeted towards well-known cardiovascular risk factors like blood pressure or cholesterol, of which the reduction is assumed to be cardiovascular protective. To confirm that these drugs indeed provide cardiovascular protection, hard outcome trials are needed. These trials are commonly conducted at the end of a drug-development program or even post registration. In the latter case, the drug usually received marketing authorization based on surrogate outcome trials. The drug effect on the on-target risk factor is used to estimate the expected cardiovascular risk reduction, which determines whether conduction of a hard outcome trial is justified. This approach of estimating the potential cardiovascular protection by establishing the drug effect on the on-target risk factor is common in drug development and has led to the development of useful drugs.\(^2,3\)

However, during last years, a number of hard-outcome trials have been conducted after the drug entered the market, and in several cases these trials revealed that the drug effect on long-term cardiovascular outcome was not as expected based on its short-term effect on the on-target risk factor. The LIFE (Losartan Intervention For Endpoint reduction in hypertension study) trial tested the angiotensin receptor blocker losartan vs the beta blocker atenolol, both registered as blood pressure lowering drugs. The trial clearly showed that at prescribed dosages, losartan and atenolol had similar effects on blood pressure, while losartan was more effective in reducing cardiovascular morbidity and mortality.\(^4\) The IDNT (Irbesartan Diabetic Nephropathy Trial) trial showed that the angiotensin receptor blocker irbesartan had additional benefits on long-term cardiovascular outcome compared to the calcium channel blocker amlodipine at equal blood pressure control. This suggests that the ultimate effect of angiotensin receptor blockers on long-term cardiovascular outcome cannot be explained by its short-term effect on blood pressure alone.\(^5\) Post hoc studies conducted later-on have
demonstrated that angiotensin receptor blockers also have effects on albuminuria, conferring additional benefits on cardiovascular outcome, which could be considered as novel targets to provide cardiovascular protection.\textsuperscript{6} Given that RAAS blockade forms the mainstay therapy for cardiovascular protection and considering that dual agent RAAS inhibition causes more complete blockage of the RAAS, which should provide additional renal and cardiovascular protection, the ONTARGET (Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint) trial was conducted. The trial compared maximum doses of the combination of the angiotensin converting enzyme inhibitor ramipril and the angiotensin receptor blocker telmisartan vs the single agents in the other arms of the trial. Despite superior blood pressure reduction in the combination arm, no additional benefit was observed on long-term cardiovascular outcome and there was even a worsening in renal outcome.\textsuperscript{7,8} Consecutive studies have revealed that these unexpected findings could, at least partly, be attributed to either cases of excessive hyperkalemia, with hyperkalemia increasing cardiovascular risk and thereby offsetting the positive effects on blood pressure. Alternatively, or in addition, hypotensive effects may also have increased cardiovascular risk.\textsuperscript{9-10} These cases exemplify that a compound may affect multiple biomarkers and that the single-biomarker approach, as being the current paradigm in drug development, may result in serious misinterpretations of the actual long-term treatment effect. A more optimal implementation of biomarkers during the drug development process could provide a more reliable impression of the efficacy and safety of drugs. In the current thesis novel methodologies were explored to improve incorporation of biomarkers during late phases of drug development. Biomarkers have several aspects which may contribute to improved evaluation of drug efficacy and safety.

In \textbf{Chapter 2}, we have investigated whether biomarkers can aid to a more accurate identification of individuals who are at increased risk for development of cardiovascular complications, and would require protective treatment. Current guidelines prescribe that individuals in the general population should receive cardiovascular protective treatment in case their cardiovascular risks exceed a certain threshold, commonly 20%.\textsuperscript{11} To identify these individuals, current practice is
to assess traditional cardiovascular risk factors, including blood pressure, cholesterol, hba1c, etc. However, it may happen that an individual develops cardiovascular complications without (clear) evidence of any of the traditional cardiovascular risk factors. This implies that currently used cardiovascular risk factors do not sufficiently explain cardiovascular risk and that novel biomarkers may help to improve risk stratification. Several studies have revealed that the renal risk factors albuminuria and eGFR predict adverse cardiovascular outcome independently of the traditional cardiovascular risk factors in varying populations.

However, all these studies used an already diseased population which may have biased their results. We investigated whether the renal biomarkers have additive value on top of the traditional risk factors in a general population, by applying various methods to detect improved model performance. Based on statistically highly significant measures of improved model performance, we have demonstrated that albuminuria, but not eGFR, has additive value in cardiovascular risk stratification on top of traditional cardiovascular biomarkers in the general population. This implies that albuminuria aids in more appropriate identification of individuals at increased cardiovascular risk, while levels of the traditional cardiovascular risk factors are still in the normal range. This is not only important for cardiovascular risk stratification, but also for future clinical trials, because addition of albuminuria to inclusion criteria may help to create a larger target population, which can be included in the trial and may benefit from cardiovascular protective therapy.

Although it is of value that a biomarker enables more accurate identification of individuals at increased cardiovascular risk who require protective treatment, one has to realize that measuring biomarkers may not only be useful to determine the cardiovascular risk of an individual, but to use it as a guidance for intensity of protective treatment. More specifically, insights must be obtained what biomarkers are affected during protective treatment on short-term, and to what extent these changes in biomarkers are dictating the long-term cardiovascular outcome. In Chapter 3 we reviewed the current state with respect to studies investigating the short-term effects of various drugs intervening in the RAAS on biomarkers, and to what extent these short-term effects translate into improved
long-term cardiovascular outcome in patients with diabetes. Our literature research has revealed that, despite that agents intervening in the RAAS affect a panel of biomarkers including TGF-β, hemoglobin, C reactive protein, NT-proBNP, which predict cardiovascular complications, only a few studies have tested whether these treatment induced changes are associated with long-term cardiovascular protection. The trials that were included in the studies we reviewed, showed that superior prognostic abilities of biomarkers do not unambiguously imply that a treatment induced change will improve long-term outcome. However, these results were mainly obtained in small populations with limited duration of follow-up. The evidence that a short-term change in biomarker translates into long-term protection comes from large clinical trials with long duration of follow-up. RENAAL, for instance, included many patients with type 2 diabetes and nephropathy which were followed for about 4 years. This trial convincingly showed that ARB losartan lowers UACR and that each 50% reduction in UACR was associated with an 18% reduction in cardiovascular events.\textsuperscript{6} In Chapter 4, we report that angiotensin receptor blocker losartan has a significant serum uric acid lowering effect in the same trial. This effect is specific for losartan due to its affinity for the URAT-1 receptor in the renal proximal tubule, facilitating urine uric acid excretion, an effect which has not been observed for other angiotensin receptor blockers.\textsuperscript{18} High serum uric acid is associated with increased risk for cardiovascular morbidity and mortality, independently of traditional cardiovascular biomarkers, albuminuria, and low eGFR.\textsuperscript{19-21} In this study we aimed to assess whether reductions in serum uric acid during the initial 6 months of angiotensin receptor blocker therapy are associated with cardiovascular protection, by comparing the effects of the uric acid lowering angiotensin receptor blocker losartan with that angiotensin receptor blocker irbesartan, which does not result in lowering of uric acid. I found that reductions in serum uric acid during the initial 6 months were almost linearly associated with lower risk for cardiovascular complications. Furthermore, the reduction in serum uric acid explained a substantial part of the cardiovascular protective effect of losartan, which supports the hypothesis that serum uric acid is a modifiable risk factor for cardiovascular disease.
Because angiotensin receptor blockers influence multiple biomarkers, which may affect the long-term cardiovascular outcome in opposing ways, we hypothesized that incorporating all off-target effects in combination with the on-target effect would allow a more accurate estimation of the long-term outcome, as compared to focusing on the on-target effect alone. In Chapter 5 several off-target effects of angiotensin receptor blockers were identified, based on biomarker measurements in RENAAL and IDNT. Subsequently, a multiple parameter risk response (PRE)score was constructed, based on the short-term on-target and identified off-target effects, which was then compared with the observed treatment effect in both trials. In this study, I showed that incorporation of the short-term effect of angiotensin receptor blockers on multiple biomarkers results into an estimation of the long-term treatment effect, which does not significantly differ from the actually observed treatment effect. However, this study was conducted in a retrospective environment using data from already completed clinical trials. Therefore, the value of the PRE score for ongoing and planned clinical trials is not yet established and the PRE score should be validated prospectively. In Chapter 6, we validated the PRE score by predicting the long-term effect of aliskiren on top of conventional RAAS inhibiting agents. Whether aliskiren provides additional cardiovascular protection was to date not established on large scale, but under investigation in the ALTITUDE (Aliskiren Trial in Type 2 Diabetes using Cardiorenal Endpoints) trial.\(^\text{22}\) The ALTITUDE trial was prematurely terminated, because excessive cases of hyperkalemia, worsening of renal function, and stroke were reported in individuals assigned to aliskiren.\(^\text{23}\) By applying the PRE score, we estimated that treatment with aliskiren would indeed not result in the additional long-term cardiovascular protection, in contrast to what was expected based on results from previous studies that only evaluated on-target effects. By confirming the negative study results, as reported by the final efficacy evaluation of ALTITUDE, we show that PRE score applications may result in an accurate impression of the long-term drug effect, already in early stages of drug development.
Conclusion

In this thesis, novel methodologies were explored to improve biomarker use during late phases of drug development in order to facilitate drug efficacy evaluation. Results of this thesis have shown that biomarkers enable identification of individuals at elevated risk who require protective therapy. This implies that biomarkers may improve inclusion/exclusion criteria of clinical trials, facilitating drug efficacy evaluation in the appropriate group of patients. Furthermore, cardiovascular protective drugs exert multiple off-target effects on biomarkers, which may influence the long-term treatment effect in opposing ways. I have shown that the off-target effects of drugs should also be considered in monitoring cardiovascular protective therapy. Finally, incorporation of the on-target and off-target effects in a multiple parameter risk response score results in a more accurate estimation of the drug long-term treatment effect, compared to estimation based on the on-target effect alone. These conclusions have major implications for drug development, drug registration and risk estimation and treatment of individual patients, which requires revising of the current drug development process.

From a drug development perspective, a single drug may exert effects on multiple biomarkers, which in turn affect long-term outcome. Currently, during the drug development process, off-target effects are only considered as side-effects. This means that less systematic and less rigorous measurement and evaluation is pursued as compared to the parameter of interest. In many examples I have illustrated that this approach may result in serious misinterpretations of the long-term drug effect, potentially with market withdrawal as a consequence. These off-target effects should also be considered in drug efficacy evaluation and a drug should not be registered based on its effect on a single biomarker, but on its composite effect on multiple biomarkers. Furthermore, incorporation of the on-target and off-target effects enables to get prospective insight whether a drug has the potential to reach the market and will be successful after drug registration. This may prevent conduction of the costly hard outcome studies that are required to demonstrate that a drug improves long-term outcome in addition to earlier
demonstrated on-target effects. This may result in a reduction of the number of patients exposed to ineffective or even harmful drugs.

From a patient perspective, an integrated score including the on-target and off-target effects may offer the physician and the patient a more reliable tool to estimate and evaluate the overall prescribed drug effect on long-term outcomes. Changes in off-target effects may preclude adjusting or stopping treatment, despite absence of a substantial effect on the parameter of interest. This could be relevant for the patient-clinician dialogue.

Future perspectives

Although more optimal implementation of biomarkers during clinical trials has shown to improve accuracy of drug efficacy estimation, these results were only obtained using clinical trials investigating the effect of RAAS intervening drugs in patients with type 2 diabetes and nephropathy. Whether these results can therefore be extrapolated to drugs of other classes and to other populations needs to be considered in future research. More specifically, extrapolation of the PRE score to other antihypertensive drug classes, or other cardiovascular protective drugs, such as cholesterol lowering or blood glucose lowering drugs, are the first choice drug classes to validate the PRE score.

Involvement of different stakeholders in the drug development process is critical to implement the PRE score in practice. Regulatory agencies (such as the Food and Drug Administration and the European Medicine Agency) are aware of bottlenecks in drug development and have proposed several solutions, which are laid out in the Roadmap initiative and the Innovative Medicine Initiative. Although these agencies recognize the value of single valid surrogate endpoints as a basis for marketing authorization, assessment of drug effects on all biomarkers as a basis for long-term drug assessment and marketing authorization is a novel concept and not (yet) proposed. Clearly, close collaboration with pharmaceutical companies and regulatory agencies is critical to implement the PRE score in practice.
To assess usefulness of the PRE score for daily clinical practice and individual patients, the PRE score should be validated in a clinical trial in primary and secondary practice. To this end, health care providers should have access to the PRE score, for example via an internet tool, and receive PRE score estimates when drug regimen changes (either addition of new drugs or change in drug dose). To determine the additive value of the PRE score beyond care-as-usual, a clinical trial should be conducted in which primary health care providers are randomized to provide either the PRE score guided therapy, or the care-as-usual therapy. Widespread implantation of the PRE score would be supported, if patients assigned to the PRE score guided therapy have better survival compared to patients receiving care-as-usual. Availability of the PRE score to health care providers through an internet website should be the first step in implementation of the PRE score in clinical practice.

Application of the PRE score has the potential to facilitate drug development and drug registration more efficiently. However, prospective studies with different drugs from other classes in different populations are required to further validate the PRE score. If the promising results, obtained in this thesis, will be confirmed in such studies, it may support the implementation of the PRE score in drug development and registration.