

Hypoxia in experimental atherosclerosis

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VALORIZATION

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Valorization is the process of creating value from knowledge. This includes (I) making knowledge available and suitable for economic and societal exploitation and (II) translating this knowledge into products, services, processes or even new businesses (1).

In general a drawback and at the same time strong point of basic academic research is that acquiring new knowledge should be independent of a commercial ulterior motive and based on general curiosity and a hypothesis derived from prior data. This presents a drawback, when trying to communicate the impact of results or research ideas with the general public or third parties with a commercial interest. As a strong point, however, unbiased academic research opens up the opportunity of unforeseen findings and translations. In this chapter, I would like to discuss how the knowledge gained from this thesis has been utilized until now and what potential ways of valorization remain to be explored. For all arguments presented, the reader should keep in mind the extensive 15 year time line from basic research findings presented in this dissertation to an actual applicable drug in humans.

Atherosclerosis, as part of cardiovascular diseases, presents an enormous economic burden in the western world nowadays. More than 70 million adults in the United States have one or more cardiovascular diseases, with hypertension, coronary heart disease and stroke being the most prominent ones. Cardiovascular disease direct and indirect costs were estimated to exceed \$400 billion in 2006 (2). When studying only acute coronary syndromes, these diseases accounted an annual cost of approximately \$75 billion (3). These costs are attributable to hospitalization and professional care, medication, home healthcare and indirect costs. Indirect costs are generally defined as reduction in work productivity of the patient itself and the associated economic burden (4). While all this evidence clearly justifies research on atherosclerosis, the challenge is to find novel drug targets to reduce this disease and economic burden.

(I) MAKING KNOWLEDGE AVAILABE AND SUITABLE FOR ECONOMIC AND SOCIETAL EXPLOITATION

In this dissertation, we explored the role of hypoxia in atherosclerosis development and progression. All data presented here have been communicated on various (inter-) national conferences and chapters 2 to 5 have been published in peer-reviewed journals. Thereby, the knowledge is available to experts in the field and can be used as starting point and reference for future research. Also, it will minimize chances of repetition of already performed research. This is particularly true for “negative data”. In chapter 4,

we showed a compensatory downregulation of plasma nitrate, nitrite and nitric oxide levels upon prolonged dietary nitrate supplementation. This makes future research using prolonged dietary nitrate in similar setups redundant. In line with that argument, we obtained an open access publication grant from ZonMW, more knowledge with less animals (MKMD), funding publication of neutral data to reduce use of animals. By publishing and communicating those data, we potentially prevented unnecessary animal experiments.

Besides academic and professional knowledge exchange, the data presented in chapters 2 to 5 were too preliminary to communicate with the general public. Nevertheless, I have gained experience in giving laymen talks on my research topics (TED-talk Prize, Vascular Biology, PhD-student course, Dutch Heart Foundation, The Netherlands) and have followed public writing courses (UM journal “Observant”) to improve communication of research results in the future.

(II) TRANSLATING KNOWLEDGE INTO PRODUCTS, SERVICES, PROCESSES AND EVEN NEW BUSINESSES

Current therapies for atherosclerosis involve surgical removal of the plaque as well as risk factor management. In particular LDL-C lowering strategies, including statins and PCSK-9 inhibiting agents, have proven effective in reducing cardiovascular risk (5,6). Anti-inflammatory agents are also undergoing clinical trials at the moment, however multiple agents have already failed to show a decrease in cardiovascular risk (7–10).

In this dissertation, we could show that reducing intra-plaque hypoxia by breathing of carbogen gas reduced atherosclerotic plaque burden and necrotic core expansion in a murine atherosclerosis model. Carbogen gas has already been successfully applied in hearing loss therapy (11) and hypoxia modification during radiotherapy (12) and has recently been tested in brain oxygenation for subsequent application to alleviate cerebral ischemia (13). Also in atherosclerosis, we could show a potential applicability of carbogen gas, as prolonged breathing reduced atherosclerotic plaque necrotic core expansion in mice. Though a translation of carbogen gas to human atherosclerosis remains to be shown, reversal of chronic intermittent hypoxia (CIH) in apnea patients using continuous positive airway pressure (cPAP) could improve cardiac symptoms and hemodynamic parameters (14,15). cPAP can increase daytime partial oxygen pressure by substantially reducing the number and severity of respiratory events in patients suffering from CIH (16). This knowledge suggests that methods to reduce intermittent hypoxia or atherosclerotic plaque hypoxia can indeed improve patient outcome.

Considering the epidemic “atherosclerosis”, oxygen therapy is however not feasible. In turn, downstream mechanisms affected by re-oxygenation might present a valid therapeutic target. In chapter 3, we identified defective efferocytosis capacity via receptor MerTK downregulation in hypoxia. Restoring MerTK function might thus be able to reduce atherosclerosis progression. However, these results are highly preliminary and remain to be confirmed in animal models, before proceeding to the human situation. This knowledge might forward research into MerTK targeting therapies in atherosclerosis management.

As an alternative for oxygen therapy, we aimed to reduce cellular oxygen consumption via multiple approaches. None of the approaches used could identify a clear-cut role for hypoxia in atherosclerosis progression, suggesting a more modulatory function of plaque hypoxia. Interestingly, noninvasive imaging of hypoxia has recently been successfully used and correlated with inflammation in human atherosclerotic plaques (17). The potential of hypoxia imaging as diagnostic or prognostic marker remains to be shown.

More promising as an intervention for atherosclerosis and dyslipidemia, we established a new role of oxygen sensor PHD1 in cholesterol metabolism in this dissertation. PHD1 deficiency resulted in profound plasma cholesterol level lowering, predominantly in the VLDL and LDL fraction. Epidemiological evidence inevitably link high LDL-C levels with increased cardiovascular risk and vice versa, lowering LDL-C using statins has been shown to reduce cardiovascular risk. The identified new player, PHD1, thus likely acts independent of statins and also PCSK-9, as it targets a different route in cholesterol processing, namely trans-intestinal cholesterol efflux. This makes PHD1 inhibition an attractive target as adjuvant therapy for statin-resistant patients, which we are exploring at the moment (chapter 7). This project allowed us to obtain a grant-4-targets grant from Bayer (10.000€, Co-PI), already showing the interest of the pharmaceutical industry to collaborate.

Pan-PHD inhibitors, selective for PHD2, are currently undergoing clinical trials as erythropoiesis-stimulating agents in the treatment of anemia (www.clinicaltrials.org). This already shows the drugability and short-term safety of interfering with PHDs systemically. Throughout this dissertation, we could show PHD isoform- and cell type-specific effects on atherosclerosis development and cholesterol metabolism. In line, PHD2 and PHD3 but not PHD1 deficiency affected erythropoiesis. Thus, we aim to make a specific PHD1 inhibitor, not affecting erythropoiesis along with cholesterol lowering. We aim to use the resultant inhibitor for possible testing in clinical trials. On

top of that, isoform-specific inhibitors might present a valuable research tool. In fact, making specific PHD inhibitors might present a feasible ground for starting up a spin-off company.

In conclusion, the findings of this dissertation show a minor role of hypoxia in atherosclerosis, which should be considered in future translational approaches. Alternatively, we identified a valid translational target in cholesterol metabolism, which we are further exploring at the moment. Future efforts include generating PHD isoform-specific inhibitors for clinical and basic research application.

A time line of the potential generation of an inhibitor is presented below (Figure 1). Upon discovery of a target, a drug needs to be developed, with subsequent *in vitro* and *in vivo* preclinical validation of specificity and drug activity. Subsequently, the drug must be optimized and screened for toxicological effects and formulated and manufactured for subsequent testing in clinical trials.

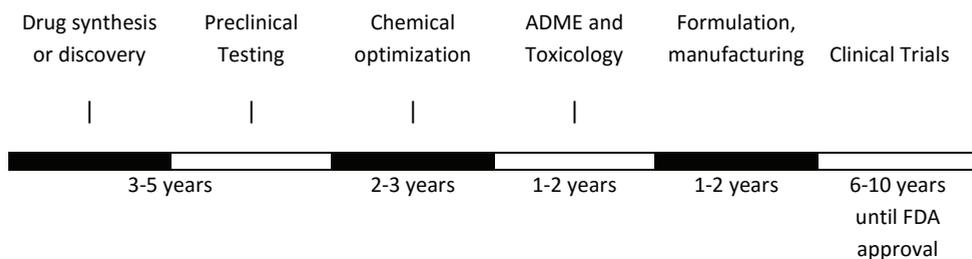


Figure 1: Drug discovery and development timeline, adjusted from (18).

*ADME = abbreviation for pharmacokinetics and pharmacology for "absorption, distribution, metabolism, and excretion."

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