Network modules as novel molecular disease definitions for precision theranostics

Impact

Life expectancy has dramatically increased during the past century, mainly due to significant advances in medicine and overall improvements in life quality. People are living longer, and, consequently, the number of comorbid chronic patients have also raised, to the point that chronic diseases are the primary cause of death and disability worldwide. The number of people over 65 years old in the EU-27 is projected to increase from 90.5 million at the start of 2019 to 129.8 million by 2050. Thus, with the increasing ageing population, this situation will only worsen in the upcoming years. Moreover, current pharmacological treatments are not precise, leading to high numbers needed to treat (NNT) i.e., the number of people that need to take a drug to prevent a single adverse outcome. Statins are perhaps one of the clearest examples; up to 50 patients need to be treated so one benefits. However, this is far from an isolated case. In fact, the ten highest-grossing drugs in the US fail to improve the condition of most patients. Thus, precision medicine approaches that reduce NNTs, and simultaneously lower the economic impact and burden of the healthcare system, are of utmost importance, especially for chronic diseases.

To achieve precision medicine and NNT equal to one, current phenotypic and organ-based disease definitions need to be overcome. This dissertation proposes and introduces a shift to redefine disease phenotypes based on their underlying causal mechanism, an essential step towards more precise diagnosis, therapy, and most importantly prevention. In complex diseases, however, the causal mechanism is most likely a complex network or disease module, unknown in most cases. For instance, high blood pressure is diagnosed as hypertension in 95% of cases. Hypertensive patients are then treated with different blood vessel-dilating drugs targeting single mechanistically unrelated proteins until the symptom, elevated blood pressure, disappears, yet the cause remains unknown. Most of these patients will still suffer from adverse effects such as stroke and myocardial infarction.

Here, the first causal molecular mechanism of essential hypertension was identified. Through the action of the ROS-forming enzyme NADPH oxidase (NOX) 5, endothelial NO synthase (NOS) is converted to its uncoupled state, interrupting the NO-cGMP signalling pathway and leading to less vasodilation and thus higher blood pressure. This mechanism represents an endotype present

in 15 — 25% of all hypertensive patients. In such patients, a network pharmacology treatment that reverses NOX5/ROS-induced damage would turn into a first-in-class anti-hypertensive therapy that is mechanism-based, causal, and possibly curative. Moreover, the precision of this pharmacological intervention can be further enhanced by guiding the approach with predictive mechanistic biomarkers, easily obtained from the blood. Only those hypertensive patients with a defective ROS-cGMP (ROCG) signalling module would be selected for subsequent therapy, and are thus very likely to respond to and benefit from the intervention. The repercussions of a successful intervention are significant since, so far, non-responsive patients will have a treatment option and better analyses. Moreover, it also has substantial economical and societal benefits. By targetting the causal disease mechanism, the consequences of hypertension, e.g., stroke or heart attack, will also be prevented. Thus, fewer patients will suffer them, avoiding the treatment costs of these pathologies and reducing the strain on healthcare workers.

In ischemic stroke, a network pharmacology approach within the ROCG signalling module was validated in vivo. This therapy is composed of a combination of a NOS inhibitor, NOX inhibitor and a soluble guanylate cyclase activator, administered at subthreshold doses being neuroprotective in a highly synergistic manner while preventing diabetes-associated post-stroke hemorrhagic transformation. Indeed, the positive results yielded by the drug combination therapy in these disease phenotypes have also led to a patent for future commercial application. Moreover, the preclinical experimentation, shown effective in rodents, serves as an essential and regulatory required proof-of-concept for subsequent translation into the clinics. Indeed, a follow-up phase I study in acute stroke therapy is currently ongoing, followed by a phase II to be started in late 2021.

Network pharmacology approaches targeting disease signalling networks may turn into a top-tier mechanism-based causal therapy for chronic cerebro-cardiovascular disease phenotypes. The approach is also undoubtedly translatable to other disease phenotype clusters once their causal mechanism is fully understood. Hence, this dissertation aims to contribute to the concept of mechanism-based reclassification of diseases, paving the way for translation to other fields in medicine. For instance, network pharmacology treatments can be potentially applied in complex cancer disease networks to reduce, inhibit, and decrease tumour cell growth until the immune system can take over, similar to cancer immunotherapy. The safety of such therapies is also guaranteed, by focussing on repurposing safe, registered drugs and nutraceuticals, meaning (i) cheaper drugs, (ii) faster translation to patients,

and (iii) less toxicity. A drug becomes unsafe when given to a non-responding patient, who will not only not benefit but also maximally experience unwanted side effects. The strategy here proposed significantly reduces or even prevents this scenario by endotyping of diseases, mechanism-based patient stratification and curative therapy with safe registered drugs.