9 Case studies

9.1 Metabolomics & Analytics Centre response to Covid

The Metabolomics and Analytics Centre has expertise in developing high throughput metabolomics methods, analyzing clinical samples, finding biomarkers for health and disease, and to support the development of personalized medicine strategies using omics integration, computational modeling and linking FAIR data with existing knowledge. Therefore, when the world was hit with the COVID-19 pandemic, we responded by using our resources and expertise to tackle this urgent societal problem.

We used four of our technologies to tackle different aspects of Covid-19 infection (Figure 1):

A. Discovery of biomarkers for disease progression from hospitalized patients
B. Use of organ-on-a-chip models for mechanistic studies and testing potential treatments drugs
C. Advanced AI modeling to understand biochemical links between metabolomic data, reported clinical and omics data, and effect of existing drugs or compounds
D. Development of an automated Rapid Covid Diagnostic Test using mass spectrometry

Clinical studies were initiated together with several clinical partners (LUMC; Erasmus MC; Amphia hospital, Breda). Plasma samples were collected as a time course from hospitalized patients and those who were moved to the intensive care units to understand why certain patients become seriously ill after a COVID infection. The first studies had patients who were not treated with drugs as none were available yet. We identified significant changes in mediating lipids, redox balance and metabolic demands of profilerating cells (Figure 1A; PMID: 35888743 & 35888742).

We used plasma from COVID patients in our organ-on-a-chip vascular model to investigate whether metabolites in circulation that cause vascular dysfunction could be detected. In addition, we tested addition of newly developed drugs to investigate whether they could reverse the damage caused by COVID related metabolites or proteins. We observed vascular leakage in our cell model as well as changes in eNOS and platelet activity. We conducted a successful proof-of-principle study revealing that drugs could counteract the destabilizing metabolites in circulation (Figure 1B).

The rapid COVID-19 diagnostic test was developed and evaluated together with the public health service (Dutch: GGD) and addressed the issues of lab capacity, limited resources, accuracy, sensitivity and speed. Together with DSM, we designed a new swab that could be mass produced locally with no chemical background to interfere with our test. We developed (and patented) a high throughput mass spectrometry-based screening test and validated this using the PCR results from a parallel GGD swab as reference. We demonstrated that we could detect all positive COVID subjects who had Ct values lower than 30 (Figure 1D). Our method was finally not implemented in the public test and trace facilities but can be easily adapted for other viruses and contributes to the pandemic readiness of the Netherlands. We are currently developing this test further to include the characterization of variants and host response to an infection within the public-private project ‘Staying ahead of the virus’.

The whole scientific community was active in learning as much as we could to fight the COVID-19 pandemic and sharing results quickly and securely without violating the privacy of the patients was a priority. We were members of many consortia making data FAIR and contributing data to share with the broader community (e.g. ‘Virus Outbreak Data Network’ and 'Trusted World of Corona'). We used knowledge graphs to tie our data to existing knowledge to understand the biochemistry behind the progression of COVID-19 infection and serious complications (Figure 1C). This experience of FAIR data sharing has become a major focus in our Metabolomics and Analytics Centre as we learned how enabling collaborative science is key to making impact on addressing societal issues.

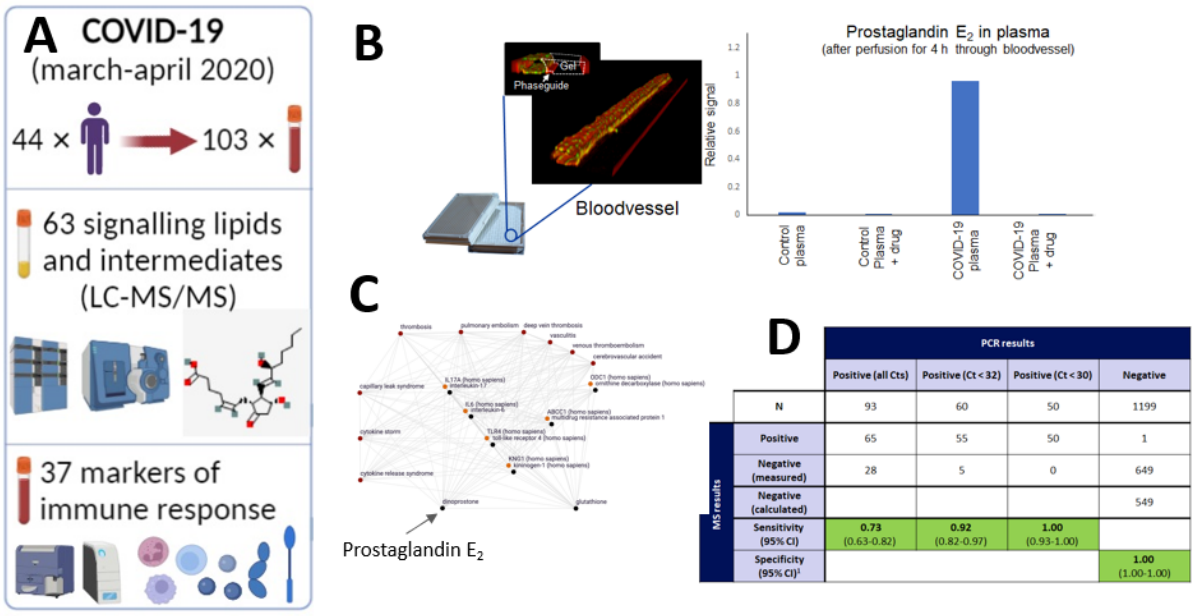

Figure 9.1. Approaches used by Metabolomics and Analytics Centre to address different aspects of COVID-19 infection. See main text for further details.

9.2 Advanced data analytics and innovative translational models to enable precision medicine strategies

Treatment failure and serious side effects due to 'one size fits all' drug treatment approaches have a major impact on patient quality-of-life and pose a high economic burden. In special patient populations such as neonates, elderly, (pregnant) women, obese patients or critically ill patients, selecting the optimal drug treatment strategy is complex. This can be attributed to the multifactorial heterogeneity of these patient populations, in which differences in co-morbidities, organ function(s), genetics, and drug-use modulate the pharmacokinetics (PK) and pharmacodynamics (PD) of drugs and hence affect treatment efficacy and safety. These special patient populations are moreover often not fully considering in pre-approval clinical trials. Thus, there exists a high unmet need for precision medicine approaches, which consider drug- and patient-specific factors driving the selection of the right drug and the right dose for each patient.

The Division of Systems Pharmacology and Pharmacy (SPP) aims to develop precision medicine approaches to characterize and predict variation in treatment response, by employing PK-PD and QSP modeling, analysis of clinical data through collaboration with hospitals and clinicians, and complementary translational experimental approaches.

Pharmacological modeling approaches

Characterization of PK-PD relationships is key to enable development of individualized drug treatment strategies. Such relationships can be derived from data obtained as part of clinical studies or routine patient care. Importantly, clinical data obtained during (routine) patient care and clinical studies is often highly variable in terms of the timing and number of samples. Moreover, there exist often ethical limitations in aforementioned special patient population in terms of the number of invasive measurements that can be obtained. The use of advanced population PK-PD, or nonlinear mixed effect modeling approaches enables us to analyze such sparse datasets and to derived optimized treatment schedules, in which the division of SPP has broad and extensive expertise.

Special patient populations

Neonates, and particularly preterm neonates, are a key special patient population where a high unmet need for precision medicine exists. Research in the last period has focused on further characterizing patient-associated

factors which explain age-associated variation in renal and hepatic drug clearance[1,2] and on the application of such approaches to rationally individualize drug treatments in neonates[3], with a primary focus on antimicrobials, analgesics and sedatives. In recent years, more attention within our division has been attributed to complex pharmacodynamic data in special patient populations, to for instance characterize complex multivariate real-life data from clinical rating scales for pain, sedation and withdrawal symptoms in neonates [4,5]. Results from this type of analyses have been incorporated into national dosing guidelines demonstrating the clinical relevance of this work.

Quantitative systems pharmacology

The division SPP has a strong track record in developing mechanism-based or physiological PK/PD models, also known as quantitative systems pharmacology (QSP). QSP approaches represent a powerful approach to enable scaling from preclinical models to humans, or to evaluate the impact of physiological factors that are challenging to measure directly in patients. Through QSP approaches we can integrate experimental *in vitro* and preclinical in vivo data to make informed predictions about expected clinical response, thereby QSP offers the opportunity to truly bridge bench to bedside. For example, within the Pharmacy group of SPP, we work on systematic mapping of the effect of inflammation (e.g. such as in critically ill patients or patients with auto-immune conditions) on cytochrome P450 activity[6] in patient-associated liver tissue samples, obtained as part of a close collaboration with the Leiden University Medical Center. In the field of CNS, innovative physiologically-based PK models have been established at our division [7], which have led to the coordinator role in the recently funded H2020 consortium QSpainRelief. This model is applied to research lines on pain [8], oncology [9], and neurodegenerative disorders [10]. In the past period, the of QSP associated approaches in infectious diseases has also expanded, which for instance included the use of QSP modeling to predict host-directed therapies against tuberculosis [11], the use of organ-on-chip models to study viral hemorrhagic diseases[12,13], and the design of treatment strategies to combat antimicrobial resistance[14].

Outlook: Towards real-world precision medicine

The increasing availability of large-scale real-world data from routine patient care offers unique opportunities to identify drivers of currently unexplained inter-patient variability and to derive strategies to overcome such variation in individualized treatment strategies. Here, we see important opportunities for further bridging established PKPD and QSP modeling approaches with machine learning/AI strategies. In addition, our division will further focus on expanding the use of translational pharmacological *in vitro*, *in vivo* and organ-on-chip models to further map and predict sources of variation between patients, which can be used as basis for predictive QSP models that can be used to support the prediction of drug treatment response in patients.

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9.3 Immune-targeting to prevent cardiovascular disease

Cardiovascular diseases (CVD) are the leading cause of global morbidity and mortality. Acute cardiovascular events, including myocardial infarction and stroke, mostly occur at advanced age. The main underlying pathology of these catastrophic clinical manifestations is atherosclerosis, which is characterized by the progressive accumulation of low-density lipoproteins (LDL) and immune cells in the arterial wall, resulting in the formation of atherosclerotic plaques. Current treatment of CVD by modification of risk factors, such as cholesterol levels, high blood pressure and lifestyle, are far from adequate and a high risk for cardiovascular complications remains. This is partially attributed to an ongoing inflammatory response in atherosclerotic plaques. At the division of BioTherapeutics we work on generating a personalized blueprint of the immune response in the atherosclerotic plaque and in the circulation, which can identify persons at risk for CVD. Moreover, this fundamental research will enable us to develop more specific anti-atherosclerosis immunotherapies, such as antigen specific tolerance induction or RNA based reprogramming of proinflammatory immune cell populations within the plaque.
With respect to antigen specific immune tolerance, we employ state-of-the-art technology, namely single cell RNA sequencing (Depuydt et al. Circ. Res. 2020), TCR/BCR repertoire analysis and mass-spectrometry-based identification of autoantigens (Benne et al. JCR 2018), on atherosclerotic plaque tissue and corresponding cardiovascular patient blood samples obtained from the Haaglanden Medical Centre in the Hague (Kritikou et al. Cells 2019). With access to unique population based cohorts spanning adults of 40-75 years of age, we gain insight into the health-to-disease transition and will establish the prognostic value of circulating pro-atherogenic immunity in (sub)clinical atherosclerosis. In collaboration with the LUMC we have used these patient cohorts to identified auto-antigens that can be used to detect T-cells that drive atherosclerosis and have prognostic value. While we are currently protecting IP, we have also started to use such antigens to induce antigen specific tolerance and study the therapeutic potential of vaccination in atherosclerosis.

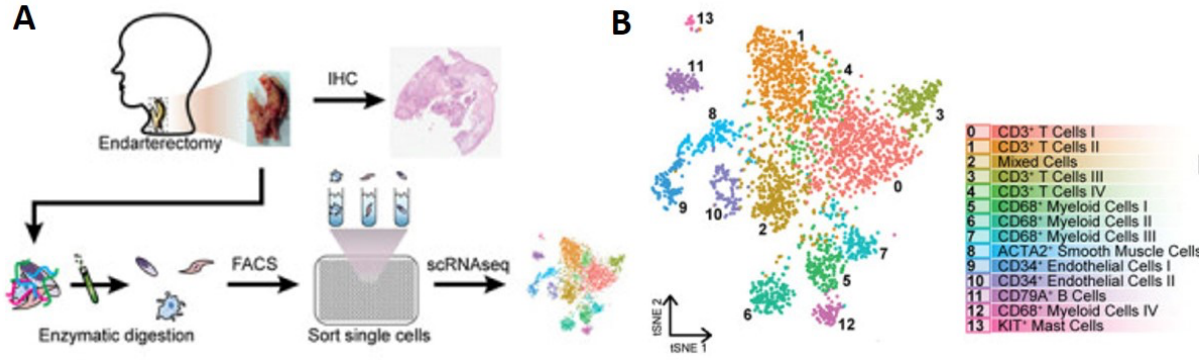

Figure 9.3: A) Processing of human atherosclerotic plaque material allows assessment of its contents by cutting edged techniques, incl. flowcytometry, masscytometry and scRNAseq. B) scRNAseq has allowed accurate assessment of cellular content in atherosclerotic lesions and has revealed new potential targets for interventions such as $GrzB^+$ CD4 T-cells, $Trem1^+$ macrophages and endothelial-mesenchymal transition (Depuydt et al. Circ. Res. 2020).

In the future we will further expend the therapeutic interventions further and employ reprogramming or immune cell populations using peptides/proteins or RNA based therapies using lipid formulation or polymeric

nanoparticles (Kappel et al. ACS Nano 2021). Besides we aim to establish therapies based on repurposed drugs (e.g. monoclonal antibodies to modulate immune checkpoints such as OX40L, BTLA and IgE) and drug delivery systems to lower pro-atherogenic inflammation side specifically.

9.4 Modeling and predicting adversity

Current evaluation of the safety of chemicals and drugs in development still primarily depends on animal tests. However, a gradual shift is both required and ongoing towards methods that involve responses of human cells directly and that are based on a detailed mechanistic understanding of the processes underlying adverse effects (such as drug-induced liver and kidney injury). The Division of Drug Discovery and Safety is contributing to this shift via participation in several national (SysBioToP-Moving and VHP4Safety) and international research programs (Horizon 2020 EU-ToxRisk and RISK-HUNT*3R*, Innovative Medicine Initiatives TransQST and eTRANSAFE, Horizon Europe PARC). In these systems toxicology projects, high-throughput imaging and omics approaches are applied in various cell systems, and computational modeling of resulting data is leveraged for mechanistic understanding and predictive purposes.

Various cellular stress pathways, such as the oxidative stress response and the unfolded protein response, have an important role in the development of adversity, or in protection against it. The Van de Water lab has established a multitude of reporter cell lines that allow to quantify activity of key proteins in these stress pathways and downstream effects (e.g., cell viability). Cells are exposed to (potential) toxicants in 2D or 3D *in vitro* set-ups and the response of specific proteins is monitored with time-lapse microscopy in high throughput. Moreover, a broad overview of cellular responses is established with transcriptomics measurements at various time points. Besides applying these techniques to human cell lines which may not be fully representative for *in vivo* behavior, such reporters have also been integrated in human induced pluripotent stem cells that are subsequently differentiated into various cell types, allowing for screening of compounds with physiologically-relevant cells.

The rich imaging and transcriptomics data are exploited using various sophisticated computational approaches applied within the Division of Drug Discovery and Safety. The Van de Water lab has established cell tracking and image analysis pipelines that allow quantification of imaging data, whereas bioinformatic approaches such as co-regulated gene network analysis provide an overview of adversity-relevant pathways that become active over time. Based on such analyses, novel biomarkers for specific adversities are under further investigation. The Van Westen lab has applied machine learning and cheminformatics to predict the adversity of compounds based on chemical structure as well as activity within stress pathways. In the Beltman lab, dynamical computational models have been developed and applied to the data for specific compounds (currently for oxidative stress, protein folding stress and DNA damage stress) to describe the temporal response within the corresponding stress pathways. Since these pathways are intertwined and compounds typically evoke activity within multiple pathways, the current focus is on connection of models while taking into account potential crosstalk and on modeling the relation to cellular adversity. The aim is to subsequently also connect to models predicting the kinetics of administered compounds in vivo (physiologically-based pharmacokinetic models), rendering detailed quantitative predictions that can be tested by comparison with existing *in vivo* data. In general such computational models represent (parts of) quantitative adverse outcome pathways (qAOPs). qAOPs are anticipated to play an important role in next-generation risk assessment in combination with appropriate toxicological assays.

9.5 Modeling and targeting the tumor microenvironment

Many pathologies, including fibrosis and cancer involve extensive remodeling of the affected tissue, which drives progression of the disease and negatively impacts on drug targeting and efficacy. In addition to rewiring of signaling networks between various cell types such as fibroblasts and immune cells, changes in the metabolic environment and altered tissue mechanics are involved (Br J Cancer, 124, 49–57).

The Danen lab in the division of Drug Discovery and Safety develops tissue culture models incorporating the complex interplay between tumors and the tumor microenvironment (TME) including extracellular matrix, fibroblasts, and different types of immune cells (Sci Rep, 6:22580). With Pharma/biotech, the models are used for testing of small molecule drugs in the context of (cancer) fibrosis and have been applied successfully to the screening of antibody drugs for cancer immunotherapy. The lab leads an NWO Science XL project seeking fundamental insight in the TME and providing new handles for rational design of future cancer therapies targeting principles shared across solid tumors. In an NWO Perspective program such models are translated into OoC designs allowing better capture of the intricate dynamics of pathological tissue remodeling.

A program has started to investigate the impact of tumor-associated mutations in GPCRs. Chemokine signaling through GPCRs controls tumor-immune interactions in the TME. Mutations are characterized using chemoinformatics (van Westen lab) and receptor pharmacological strategies (Heitman lab) and subsequently studied in complex tumor models (Danen lab) with the aim to unravel functional effects of mutations on tumor biology and response to clinically relevant antagonists.

The Barz lab in the division of Biotherapeutics develops strategies to modulate the TME. They have shown how nano- and macroscale materials can be employed to improve the outcome of conventional chemotherapy or cancer immune therapy (Chem Soc Rev, 48: 351-381). In addition, they have shown that the release of non-toxic concentrations of an adenylate cyclase (AC) inhibitor from PeptoMicelles, targets the increased cAMP production in monocytes caused by the acidic TME. In combination with selective, non-therapeutic immune activation, this led to a complete remission of established tumors in an animal model in hand with long term immunity (Nat Commun, 12; 1-9).