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Meeting Report

R2N and the Use of Alternative Methods in COVID-19 Research

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The recent SARS-CoV-2 pandemic represents a global health emergency. In the urgent race to understand the molecular mechanisms of the disease and develop lifesaving treatments and vaccine candidates, many researchers are embracing *in vitro* and *ex vivo* approaches. Alternatives to animal experimentation are both time- and cost-efficient, and if human-derived cells are employed, experiments can be more human-predictive, thereby maximizing the quality and relevance of the investigations. In the battle against time, driven by the growing incidence of disease, the German research unit R2N (Replace and Reduce in Lower Saxony, https://r2n.eu/home-2) is ideally positioned to deliver a repertoire of physiologically-relevant alterna-

tive model systems to replace or reduce the need for animal experimentation in SARS-CoV-2 research. Accordingly, on July 2, 2020, the Steering Committee of R2N met to discuss the ongoing COVID-19-related research projects within the consortium.

Nasal mucosa explants, air-liquid-interface cultures of airway epithelial cells, and precision-cut lung slices are now being used to investigate host-specificity and the pathogenic mechanisms of SARS-CoV-2. The Baumgärtner group (University of Veterinary Medicine Hannover, TiHo) has established a well-equipped toolbox for the generation and characterization of *ex vivo* and *in vitro* models of the upper and lower respiratory tract of various animal species. The use of human *ex vivo* lung tissue is well integrated into several R2N research groups studying respiratory infections and pulmonary fibrosis. Experiments using human *ex vivo* lung tissue increase the predictive validity of animal disease models by considering interspecies differences for comparative pharmacology, and most importantly by providing a reference point associated and correlated with clinical pathology. Through the use of whole genome analysis, the R2N-associated Sewald group (Fraunhofer ITEM) is analyzing how human lung tissue responds to *ex vivo* SARS-CoV-2 infection. They aim to identify gene signatures relevant for COVID-19 elicited in tissue derived from the human lower respiratory tract. Treatment with viral inhibitors will reveal how well novel therapeutics block virus-induced pathology.

To overcome some of the limitations of immortalized cell lines and primary cells, the Olmer laboratory (Hannover Medical School) is utilizing human induced pluripotent stem cell (hiPSC)-derived respiratory epithelial cultures as an innovative organotypic *in vitro* SARS-CoV-2 infection model. Based on recent advances, hiPSCs can be genetically modified, easily expanded, and differentiated into many different cell lineages including respiratory epithelial cells. Targeted gene editing to knock out genes or overexpress endogenous factors in hiPSCs aims to identify key antiviral responses and cell protection pathways that could serve as new drug targets. This project was recently awarded funding from the German Federal Ministry of Education and Research.

A significant proportion of COVID-19 patients have gastrointestinal symptoms and suffer from severe oxygen deficiency (hypoxia). Recent studies have shown the intestine to be an important SARS-CoV-2 target organ. The von Köckritz-Blickwede, Naim, and Seeger groups (TiHo) are collaborating on a project to determine the effects of both SARS-CoV-2 exposure and hypoxia using intestinal cell lines, primary cells, and organoids of different species. These experimental models will be important to understand SARS-CoV-2 biology and infectivity of the gut.

R2N research groups are also active in crucial *in vitro* experiments elucidating pathogenic mechanisms of SARS-CoV-2 and screening new potential drugs and neutralizing antibodies. The Osterhaus group (TiHo) is involved in generating panels of human monoclonal antibodies against COVID-19 for therapy and prevention – the first of which is already being produced by a pharma company for large-scale clinical trials in humans. The German Primate Center is an R2N partner in the research alliance for the development of alternatives to animal experimentation whenever possible. Using cell culture, they have revealed key insights into how the SARS-CoV-2 spike protein – activated by host cell proteases – mediates entry and how this process can be inhibited by a clinically-proven protease inhibitor. Currently, they are *in vitro* screening viral inhibition potency of liposome-derived compounds as potential drugs for COVID-19 treatment. The R2N-associated Hust and Dübel groups (Technische Universität Braunschweig) specialize in animal-free methods for antibody development. Using phage display of human antibody gene libraries from healthy donors and COVID-19 patients, they have generated more than 350 human monoclonal antibodies against SARS-CoV-2. The best virus-neutralizing antibodies are already in GMP production for clinical testing (http://corat-therapeutics.com/). Furthermore, animal-free, highly-specific SARS-CoV-2 antibodies were generated for diagnostic use and made available to the community (https://abcalis.com/). These results underline the excellent quality of animal-free antibodies, which were generated in less than four weeks – something that could not have been achieved with animal-based methods.

The R2N members within the Centre for Ethics and Law in the Life Sciences (CELLS) at the University of Hannover examine ethical and juridical aspects related to the acceptance and implementation of alternative methods in research and regulation. CELLS' expertise has been in high demand as part of the public communication of science activities in relation to triage guidelines and the allocation of scarce resources during pandemics. They are providing ethical and legal assessments of epistemological aspects of evidence-based COVID-19-related health policy.

Therefore, the R2N consortium members are making significant contributions to COVID-19 research, with groups utilizing a wide repertoire of alternative methods that save both time and the lives of experimental animals. In this way, the R2N is well-suited to meet future challenges in the understanding, treatment, and prevention of SARS-CoV-2 infection.

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