AdapTive Immune Response Models for Discovery and Repurposing in the COVID-19 Era

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Abstract

The pandemic caused by SARS-CoV-2 is expected to significantly alter the Global Health Burden, a measure of health-related impacts on world economies. Despite the availability of COVID-19 vaccines, newly emergent variants and long-term disease effects with immune system consequences pose challenges to the physical and mental health of patients. Resultant pathologies include not only Acute Respiratory Distress Syndrome (ARDS) and Cytokine Storm/Cytokine Release Syndrome, but a broad scope of immunological, cardiovascular, and neurological conditions. As the immune response is a central player in the recovery from, as well as exacerbation of, infectious disease, human-centric, *in vitro* assays and physiological models that capture relevant immune responses and inflamed tissue biology are critical to advance treatments for the sequelae of viral infection. In response to client needs in this space, Eurofins Discovery Phenotypic Center of Excellence has developed human primary cell-based AdapTive Immune Response Models with biomarker readouts specifically chosen for relevance to COVID-19, to inform on the immune response to approved and developmental therapies. Each system is a co-culture of pooled human primary PBMCs and one additional human primary tissue cell type stimulated with T Cell Receptor agonists to drive modeling of the adaptive immune response. With a comprehensive biomarker assessment that includes cell surface receptors, chemokines, cytokines and measures of cell health, these models serve a variety of program strategies from discovery to repurposing, and from individual to combination therapies. In the current case study we present data on the effects of dexamethasone (DEX) and hydroxychloroquine (HCQ), two broad spectrum anti-inflammatories highlighted in the early response to COVID-19. Biomarker readouts in the three tissue-specific systems show that in the B cell germinal center model, HCQ induced stronger immune inhibitory and anti-inflammatory effects than DEX, consistent with HCQ's clinical efficacy in treating lupus. In contrast, in the context of stromal and vascular tissues, the steroid DEX was more inhibitory on immune and inflammation response biomarkers than HCQ, this was most notable for TNFα. These results may explain, in part, how DEX is more active on systemic inflammation, consistent with its efficacy in acute treatment for COVID-19. The ability of these models to differentiate drug activity based on human phenotypic outcomes in distinct tissue-specific contexts can help inform on therapeutic potential prior to clinical trials.

Methods

For all assay systems, human primary cells were used at early passage (P4 or earlier) to minimize adaptation to culture conditions and to preserve physiological signaling networks and regulatory responses. Cells were pooled from multiple donors (minimum of three) to minimize inter-assay variance. Abbreviations as follows: human umbilical vein endothelial cell (HUVEC), peripheral blood mononuclear cell (PBMC), human neonatal dermal fibroblast (HDFn), T cell receptor (TCR) and B cell receptor (BCR). All drugs were tested at four concentrations in triplicate. Direct ELISA was used to measure biomarker levels of cell-associated and cell membrane proteins. Indirect multiplex electrochemiluminescence assays were used to quantify biomarkers from culture supernatants. Effects of drugs on cell proliferation and health (cytotoxicity) were detected by sulforhodamine B (SRB) staining for adherent cells, and alamarBlue® for cells in suspension. Each plate contained a drug control (n = 3), stimulation control (n = 3), and vehicle control (n ≥ 5). Data acceptance criteria depend on both plate performance (percent coefficient of variance (% CV) of vehicle control wells) and performance of a sentinel biomarker in the stimulated vs. unstimulated condition (Z' > 0.4).

AdapTive Immune Response Models with Tissue Context

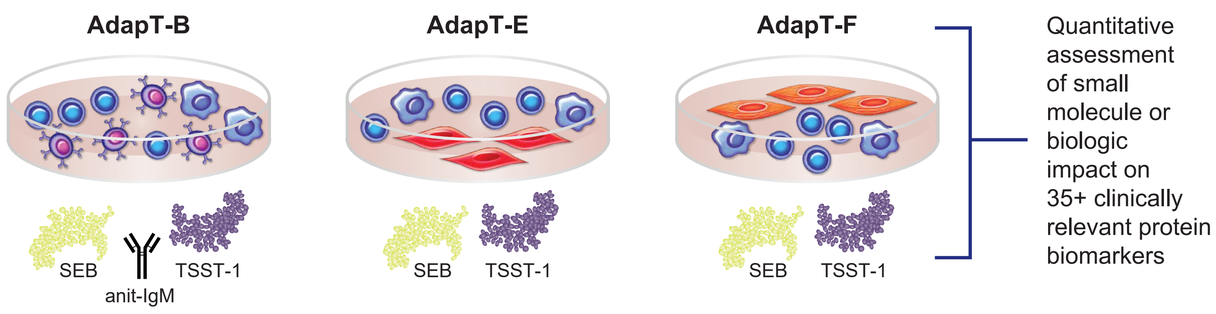


Figure 1. Overview of three available human primary cell-based adaptive immune response models with cell surface receptor, cell health, chemokine, and inflammatory cytokine biomarker readouts relevant for acute and chronic inflammation biology. Each system is a co-culture of human primary PBMCs together with one additional human primary cell type, stimulated with a T Cell Receptor agonist cocktail that includes staphylococcal enterotoxin B (SEB) plus toxic shock syndrome toxin-1 (TSST-1); AdapT-B is also stimulated with anti-IgM antibody. In each system drugs are added prior to stimulation.

Guided by Biology, Validated by Experience

System Name	Functional Model	Disease Biology Relevance
AdapT-B	Models T cell-dependent B cell proliferation and activation that occurs in the germinal centers of secondary lymphoid organs.	Secondary lymphoid tissue responses, including infection-mediated immune responses, autoimmune indications, respiratory inflammation, and allergy.
AdapT-E	Models inflammation driven by T cell effector responses in the context of activated vascular endothelium.	Systemic T cell-driven inflammatory conditions, including acute respiratory distress syndrome, gastrointestinal inflammatory diseases, and other responses to pathogens.
AdapT-F	Models inflammation driven by T cell effector responses in the context of stromal tissue.	Localized inflammatory conditions where T cell proximity to fibroblasts influences outcomes, including pulmonary fibrosis, airway remodeling, wound healing biology, and tissue inflammation.

Table 1. Activated T cell-driven inflammatory biology modeled in different human tissue contexts provides physiologically relevant data. Indicated are system, functional model, and disease biology relevance. Optimized for each system, biomarker readouts are determined from soluble levels measured in supernatants or from cell surface levels.

Differential Modulation of IL-6 Response with COVID-19-Relevent Therapeutics Depending on Inflamed Tissue Context

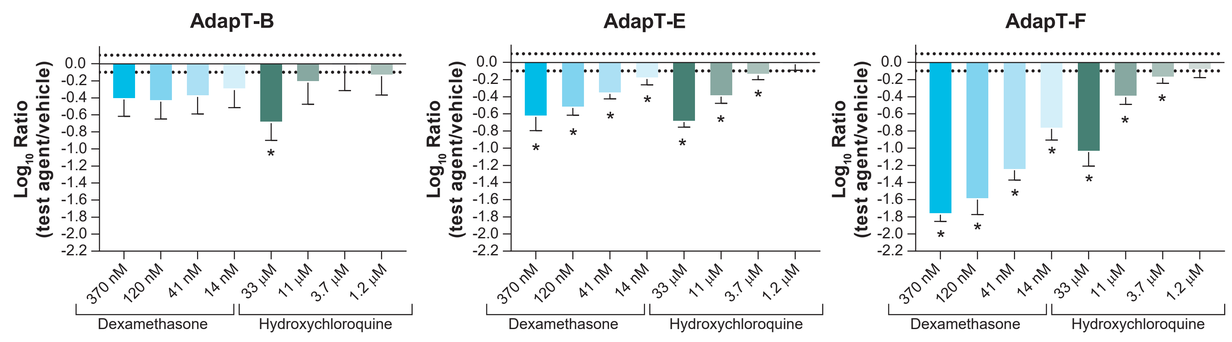
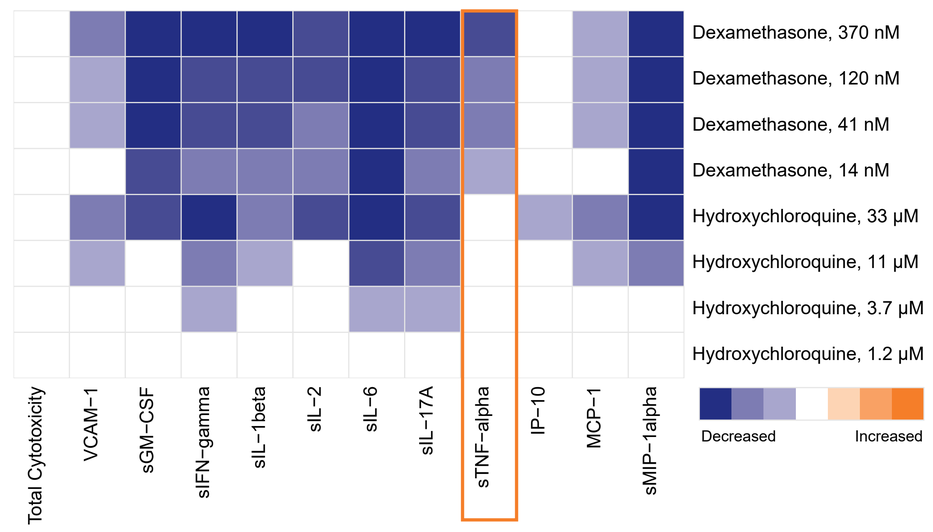


Figure 2. Concentration-dependent decrease in IL-6 in response to COVID-19 therapeutics dexamethasone (blue) and hydroxychloroquine (green) detected in AdapT-E and AdapT-F, but not AdapT-B models. Drugs dexamethasone (DEX) and hydroxychloroquine (HCQ) were added 1 hr prior to system stimulatory agents. IL-6 levels were determined as follows: AdapT-B (72 hr); AdapT-E (24 hr); AdapT-F (48 hr). Results are displayed as a $\log_{10}$ ratio of (averaged test agent replicates/averaged vehicle control replicates). Asterisk(*) indicates biomarker readout change meets both effect size and statistical significance criteria of $|\log_{10} ratio| > 0.1$ and p-value < 0.01. Dotted lines represent the 20% effect size threshold outside of which values are determined significant.

In a Model of Peripheral Tissue Inflammation, DEX and not HCQ, Inhibits TNFα Levels



- IL-17A induces chemokine and cytokine production at sites of infection to recruit immune cells; potential involvement in COVID-19-related thrombotic and vascular events
- Cell adhesion receptor VCAM-1 promotes adhesion of immune cells to fibroblasts

Figure 3. DEX and HCQ are broadly inhibitory on immune and inflammation response biomarkers in a stromal tissue setting. Heatmap visualization of biomarker readouts provides a head-to-head comparison of drug effects on multiple biomarkers and allows rapid identification of differentiating activities between drugs. DEX, but not HCQ, reduced sTNFα levels at all concentrations. DEX and HCQ were tested at ranges relevant to clinical exposures; neither was cytotoxic at tested concentrations. Modulated biomarkers are orange if protein levels are increased ($\log_{10}$ ratio > 0.1 and p-value < 0.01), blue if protein levels are decreased ($\log_{10}$ ratio < -0.1 and p-value < 0.01), and white if unchanged. Profiling Study; AdapT-F.

COVID Therapeutics: An Open Road for Immunomodulators

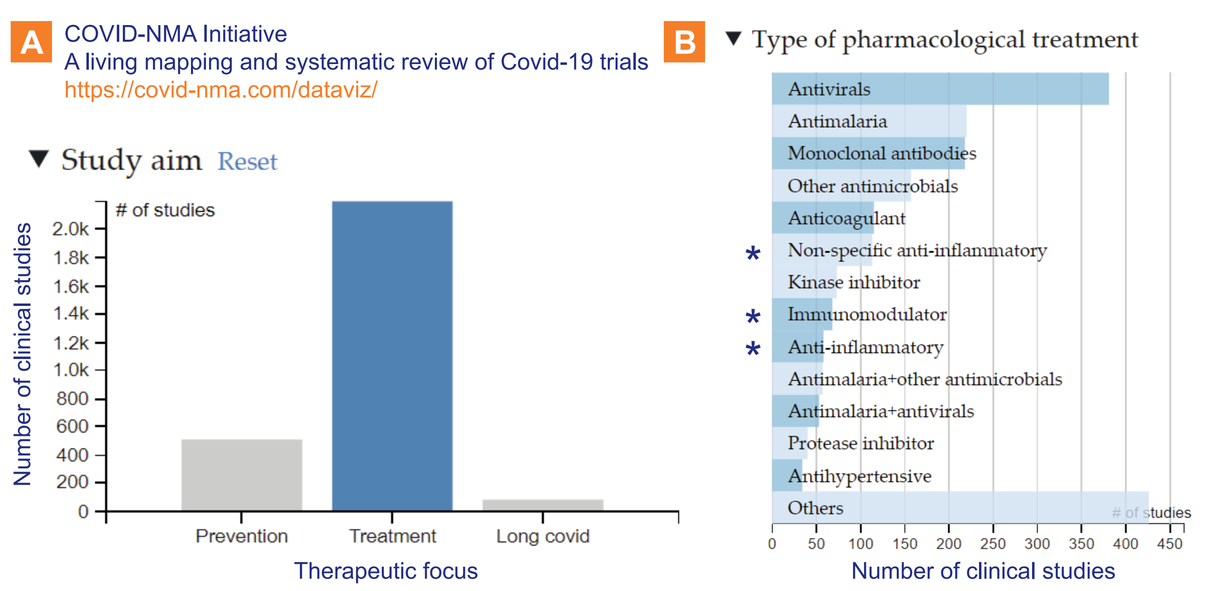


Figure 4. According to data collected by the COVID-NMA Initiative, anti-inflammatories and other treatments, rather than vaccines, have potential for greater growth in the years ahead. A. The number of ongoing clinical studies focused on different modalities of COVID treatment. B. Bar chart showing numbers of clinical studies underway for distinct treatment approaches for COVID-19. Asterisk indicates immune-focused treatments that could be addressed with the human phenotypic primary cell-based models presented in this poster.

Summary

- Despite the availability of COVID-19 vaccines, long-term disease outcomes involving immune system consequences are a likely reality for many infected patients
- There is a critical need for better COVID-19 treatments including newly discovered candidates or repurposed drugs
- Models that capture human immune/inflammation biology with activated T cell-driven responses are critical to prioritize high-potential candidates with increased chance of clinical success
- Models presented herein have been validated with clinical compounds to deliver reproducible data and actionable insights, and enable comprehensive assessment of immune biomarkers
- Human-centric *in vitro* assays increasingly are presented to, and accepted by, regulatory agencies for Investigational New Drug (IND) and Clinical Trial Application (CTA) filings
- Such innovations provide compliance with guidance & initiatives that advance non-animal alternatives in drug discovery & development, such as those indicated by the Organization for Economic Co-operation and Development (OECD), National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs), and National Center for Advancing Translational Sciences (NCATS)

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