$50M Wellcome Leap Program in Human Organs, Physiology, and Engineering (HOPE)

We need better models of human physiology –
the mouse is not a human and dialysis is not a kidney.

In the past 15 years, even as the number of new drug candidates in development nearly doubled, 90% of those that succeeded in preclinical animal studies failed in human trials. This high attrition rate – approximately 50% of failures for lack of efficacy and 30% for toxicity – raises the cost per new drug up to $2.8 billion according to recent estimates.[i,ii] In the current pandemic, mRNA vaccines went from virus sequence to first dosing in humans in an unprecedented 63 days – but the clinical trials will take more than a year. Even a single month saved in clinical trials would have meant trillions of dollars in global economic damage avoided.

For patients with organ failure, allograft donor transplant is often the only option. Worldwide, millions[iii] of patients on dialysis await a kidney transplant, for example. In the United States, alone, about 5,000 people die each year while waiting[iv], making kidney disease a top ten killer[v] – responsible for more deaths each year than breast cancer[vi] and more than $100B in Medicare costs. Of the more than 2 million patients with late-stage chronic kidney disease receiving dialysis replacement therapy,[vii] only 35% will survive the wait.[viii]

What do these challenges have in common? An inability to replicate human physiology; in particular, functional organs and immunological responses.
It's time to bioengineer a change.

Indeed, if we are to build new solutions that catalyze a Health Age, we need 10x, scalable, transformations in the underlying time, cost, and efficiency of finding new solutions. Historically, fundamental advances in these parameters have exploded the number of innovators and the pace of innovation in fields as diverse as semiconductors, software, and genome sequencing.

Program goals.

In this program, we aim to leverage the power of bioengineering to advance stem cells, organoids, and whole organ systems and connections that recapitulate human physiology *in vitro* and restore vital functions *in vivo*. We have two goals:

1. Bioengineer a multiorgan platform that recreates human immunological responses with sufficient fidelity to double the predictive value of a preclinical trial with respect to efficacy, toxicity and immunogenicity for therapeutic interventions targeting cancer, autoimmune and infectious diseases.
    - The platform should represent tissue-resident and lymphatic immune systems and recreate immunological mechanisms involved in pathologies and drug-induced reactions (including effects on target and non-target organs), as demonstrated in retrospective studies of a statistically relevant number of immunomodulator drugs.
    - The platform should predict the wanted immunogenic activity of vaccines and the unwanted immunogenic risks of therapeutics, especially to inform the design principles for an immunologically tolerated transplantable organ.
2. Demonstrate the advances necessary to restore organ functions using cultivated organs or biological/synthetic hybrid systems that would result in a doubling of the 5-year survival rate of patients on replacement therapy or awaiting organ transplantation and point to a fully transplantable, non-rejected, human organ within 10 years.
    - In the program we expect to produce the first hypo-immunogenic living functional unit at human scale, for example a kidney nephron with a filtration rate at least equivalent to moderate CKD stage and an immunogenicity profile in the range of an autologous graft.
    - Advances are expected to contribute to the physiological relevance and robustness of organ system platforms for *in vitro* applications such as drug testing.

Background.

Over the past decade, there have been significant advances in the development of *in vitro* systems demonstrating the viability and function of organotypic 3D tissue cultures. Progress has been made in at least four key areas: (1) advances in cell sourcing including the use of stem cells and iPSCs differentiated in single cell types or multi-cellular organoids; (2) the diversity of cell/tissue types cultured (representative of

heart, liver, kidney, lung, gut, brain, muscle, ovaries, and others); (3) replication of *in vivo*-like tissue structures and functions; (4) sustainment of 3D tissues/organoids viability for several months vice days; long enough for heart organoids to synchronize and beat in unison, for *in vitro* ovary tissue cultures to produce ovulation hormones after a month, and for brain organoids to demonstrate neural differentiation and self-assemble into long-range circuits.

At the same time, advances in bioengineering approaches, such as 3D bioprinting— across resolution, speed, volume and diversity of biological/ biocompatible materials— have demonstrated directed tissue construction, including vasculature within organoid clusters and structural collagen matrices for stem cell organization and maturation. Modular microfluidic systems with self-regulating pumps, in-line sensing & signal processing have been developed to demonstrate parallel arrays of 100s to 1,000s of individually controlled and interconnected cultivation chambers. Finally, continued shrinking of feature sizes in semiconductors presents the possibility to sense, compute, and take action at human cell dimensions.

Since the groundbreaking approvals of two CAR-T therapies in 2017, cell therapy research has pushed the boundaries of applied immunology, stem cell biology and genetic engineering towards broader capabilities such as augmented biology and universal cell sourcing. Moreover, the nascent field of immunoengineering has progressed at high speed – from applying materials-based approaches to create 2D and 3D immune structures to investigating dynamic 4D materials for the generation of immune-organ systems.[ix]

The time is right to foster synergies between organoids, bioengineering and immunoengineering technologies, and advance the state-of-the-art of *in vitro* human biology — much closer to how organs and immune cells function and interact in the human body — by building controllable, accessible and scalable systems.

Call for abstracts and proposals.

We are soliciting abstracts and proposals for work over 3 years (with a potential additional one-year option) in one or more of the following thrust areas. Proposers should clearly relate work in these thrust areas to one or more of the program goals.

Thrust Area 1: Human Cell Survival, Expansion and Identity.

Establish and engineer strategies for the expansion, haplotyping and gene-editing of human primary cells representative of the human immune cell repertoire – so as to ensure survival and proliferation of cell population covering at least 90% of the overall immune cell diversity (including blood, lymphoid and non-lymphoid organs) as

demonstrated by quantitative genotyping and immunophenotyping methods, and to reach log-scale increases in yield and throughput compared to standard lab methods.

Of interest are stem cell differentiation and genetic engineering approaches that would allow the generation of patient-derived and hypoimmunogenic systems, representation of population risk factors, enhancement of desired properties and insertion of traceable markers, as cell sources for constructing a human immune cell repertoire and tissue/organ building blocks.

Thrust Area 2: Immune System: Structure & Function.

Bioengineer a functional lymphatic organ system that can elicit a specific adaptive cellular and humoral response to foreign antigens – as demonstrated by a germinal center (GC)-like cell population capable of antigen presentation, clonal expansion and differentiation of naive lymphocytes into effector and memory T and B cells (as shown by quantitative immunophenotyping, cytokine profiling and systems immunology methods), and antibody production of high yield, affinity and neutralizing potential (equivalent to freshly isolated lymph node output, and/or benchmarked to clinically reported antigen/antibody profiles).

Platforms that recreate lymph node structures, vascularization, biophysical properties, cellular density and cytokine gradients (e.g. by use of printed design, biomaterials, stromal cells, organoids) to preserve biological fidelity (cellular abundance, diversity and function, e.g. equivalent to fresh tonsil explants) are of high interest.

Thrust Area 3: Tissue/Organ Maturation, Scalability & Standardization.

Develop processes for cultivation of functional organ units of high-fidelity and reproducibility with scalability and speed – as demonstrated by emerging *in vivo*-like organ properties (cellular composition, structure, function, vascularization), reduced batch-to-batch variability (e.g. < 10% divergence from a quality-controlled representative organoid), log-scale increases in functional unit size, yield and throughput compared to standard lab methods and platforms, and quality-controlled by use of molecular, cellular and functional profiling (e.g. transcriptomics, proteomics, high-content imaging, molecular probes).

We are particularly interested in unbiased approaches (e.g. with small molecules/growth factors/morphogens/genetic screens) to identify new experimental strategies that improve organoid maturation (so as to reach neo/postnatal stages as shown by scRNAseq analysis vs human tissue) and eliminate off-target cell populations.

Thrust Area 4: Tissue/Organ Vascularization and Resident Immunity.

Develop biological and engineering methods that induce neovascularization and recreate tissue-resident immunity – as evidenced by 1) formation of mature vascular

networks that support viability of bulk organs (i.e. absence of necrotic centers in organoids) and trafficking and infiltration of leukocytes, 2) representation of tissue-specific innate and adaptive immune cell populations (confirmed by immunophenotyping and benchmarked with human biopsies) capable of mounting immune responses (e.g. cytokine release after pathogenic invasion) and interact with circulating leukocytes (e.g. neutrophilic recruitment at site of tissue damages), and 3) emulation of biophysical properties of the tissue niche (e.g. by use of biomaterials, biomechanical forces, micropatterning, shear stress).

Understanding and leveraging the impact of vascularization and immunological cues on organoid development, maturation and function is key.

Thrust Area 5: System Demonstrations.

Integrated, system-level demonstrations of advances toward and against the program goals. Examples include, but are not restricted to, demonstrations such as:

A lymphoid organ model-based immunogenicity testing platform that 1) recapitulates historical clinical profiles (quantitatively and qualitatively) of vaccine-elicited and anti-drug antibody production for a statistically relevant number of vaccines and biologicals, and 2) demonstrates the spectrum of cellular and humoral immunological responses raised against bioengineered allogenic tissues and organs and their hypoimmunogenic derivatives (e.g. using gene-edited iPSC as cell source, and benchmarked against an autologous living control).

A multiorgan system comprised of interconnected lymph nodes, vascular networks and immunocompetent healthy and diseased tissues/organs (e.g. inflamed gut mucosa, tumoroid, cerebral tauopathies), that recreates, senses and integrates the immunological crosstalk (e.g. by use of biosensors and machine learning) necessary to recapitulate the pharmacology and toxicity profiles (e.g. cytokine release syndrome, vasculitis, organ injuries) of a statistically relevant number of immunomodulator drugs of known clinical outcome.

A vascularized organoid-based (or biological/synthetic hybrid) nephron system that demonstrates 1) *in vitro* blood flow and filtration capacity equivalent to 30% of normal human kidney (i.e. moderate CKD not requiring dialysis) at a scale corresponding to at least 10% of renal cortical volume, and/or 2) durable orthotopic engraftment and recovery of renal function output in an animal model of renal failure (e.g. increasing glomerular filtration rate by 50% in an ischemia-induced acute renal failure mouse model).

Proposers in this thrust area should have access to data, directly or through established partnerships, of historical results sufficient to complete retrospective studies.

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[iv] https://www.pennmedicine.org/news/news-releases/2019/august/many-kidneys-discarded-in-the-united-states-would-be-transplanted-in-france

[v] https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm

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[ix] Kim, S., Shah, S.B., Graney, P.L. *et al.* Multiscale engineering of immune cells and lymphoid organs. *Nat Rev Mater* **4,** 355–378 (2019). https://doi.org/10.1038/s41578-019-0100-9