ALTERNATIVES
RESEARCH & DEVELOPMENT
FOUNDATION

2022 ARDF GRANT RECIPIENTS

Established in 1993, the ARDF has been a mainstay of support for developing alternatives to animal-based methods in science. Through grant programs, achievement awards, and sponsorship of scientific conferences, ARDF advances high quality scientific research that aims to replace and reduce the use of animals.

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EUGEN DHIMOLEA, PHD
Albert Einstein College of Medicine, New York, NY

In vitro human-specific 3-dimensional co-cultures to substitute animal models of cancer drug efficacy

Millions of rodents are used every year in academia and the biopharmaceutical industry to test the efficacy of oncology drug candidates or/and validate mechanistic hypotheses generated in vitro. Despite this enormous sacrifice in animal lives, most therapeutic candidates fail to show efficacy in clinical trials. The key scientific premise for animal use in cancer research is that the drug sensitivity of human tumors growing in mice emulates the respective treatment responses in human patients. This postulation presumes that human cancer cells that grow in mouse-specific vs. human-specific tissue microenvironments have similar biological properties and therapeutic sensitivities. We propose to reduce animal use in cancer research by a) demonstrating that human-specific vs. mouse-specific tumor microenvironments can affect distinctively the drug sensitivity of human cancer cells, and b) providing an alternative to in vivo cancer drug testing, based on in vitro 3D co-culture systems developed in our laboratory which recapitulate the tumor-stroma interactions. This proposal will capitalize on our in vitro 3D spheroid/organoid models mimicking phenotypically and molecularly the drug response dynamics of clinical tumors. Our specific aims are to 1) Determine the species-specific effect of paracrine IL6 on the sensitivity of human cancer cells to pharmacological treatments, and 2) Identify the anti-cancer drug classes that are functionally affected by the human-specific tumor microenvironment. This project will generate phenotypic and mechanistic proof of concept data on the species-specific role of the tissue microenvironment on tumor response to various therapeutic classes. Furthermore, the alternative use of sophisticated human-specific 3D co-culture systems that recapitulate the architecture and composition of clinical tumors can significantly reduce the utilization of animals in cancer research and improve the success rates of preclinical approaches in the clinic.

2

HANG LIN, PHD
University of Pittsburgh, Pittsburgh, PA

Generation of "transgenic" microphysiological systems to study gene function in the pathogenesis of osteoarthritis: a pilot study

Osteoarthritis (OA) is a painful and disabling joint disease that affects more than 28 million Americans. Currently, there is no FDA-approved drug that can stop or reverse OA progression, which is primarily due to our insufficient understanding of molecular changes underlying OA pathogenesis. The conventional strategy to define the function of a gene/protein is to conduct knock-in and knock-out studies in cell culture and then use the same approach to suppress or overexpress the gene in rodents. These methods have helped us gain significant knowledge about OA, but the inherent deficiencies of these widely used experimental models have also been documented, which often result in so-called "translational failure." In addition, the current process of creating transgenic animals is costly and time-consuming. Recently, our team has successfully developed a human cell-derived microphysiological joint system (miniJoint) that integrates osteochondral, synovial, and adipose analogs. Given the potential of the miniJoint as a valuable complementary system to the current models, we hypothesize that we

can generate “transgenic” miniJoint through knock-out of gene(s) in one or more tissues, which will result in the changes of tissues similar, but not identical, to that have been previously observed in the animal models. In Aim 1, we will first test if we can generate a functional miniJoint using the chemically defined culture medium and biomaterial, which will completely avoid the use of animal products in this new system. In Aim 2, we will explore the feasibility of generating the “transgenic” miniJoint through knock-out of estrogen receptor-a, a protein that was shown to play a key role in OA pathogenesis in our recent study, and then examine the resultant changes in all tissues. The successful completion of this pilot study will prove the feasibility and usefulness of generating “transgenic” miniJoint, which potentially reduces the use of animals in research.

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EUGENE MURATOV, PHD
University of North Carolina at Chapel Hill, Chapel Hill, NC

OpenTox SSDM - A Novel Framework for Species Sensitivity Distribution Modelling

The major challenge in ecological risk assessment is to assess the hazard exposure in various trophic species residing in different environmental compartments. The species sensitivity distributions (SSDs) reflect the difference in observations of chemical sensitivity among different modelled species. The reliance on animals for ERA of environmental contaminants (ECs) is a heated topic of debate among various regulatory agencies. Thus, animal dependence should be minimized to the lowest possible threshold using intelligent testing strategies, including various in silico tools. Computational tools have long been used in ERA toxicity prediction and data gap filling. However, minimal effort has been made to develop SSD estimation models employing acute and chronic toxicity data of multiple species. Additionally, the available models hold a restricted application and can only be applied to assess SSDs of a few groups of species owing to their limited chemical, biological and taxonomic domain. The current project will not only address the limitations of all these existing models. Still, it will also propose several new models against various groups of environmental contaminants following strict OECD guidelines for model development. A “class-specific” read-across technique will be used to fill missing data points. In the second approach, the collected toxicity data against various groups of ECs will be used to check and identify the best performing read-across technique available for different chemical classes termed as “class-specific”. The “class-specific” analysis results will be used to propose a novel and better performing read-across technique validated on a large studied dataset. Finally, the resource outcomes of the project will be incorporated into a knowledge database and software tool, which will be made freely available on the cloud-based OpenTox web application.

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CRISTINA SCIELZO, PHD
Università Vita-Salute San Raffaele, Milan, Italy

Exploring 3D bioprinting and dynamic growth in bioreactors to recapitulate leukemia cell dissemination ex-vivo

Despite the advances in therapies several hematological cancers are still uncured, an example is Chronic Lymphocytic Leukemia (CLL). The disease is characterized by the expansion of B lymphocytes that recirculate between peripheral blood, bone marrow, and secondary lymphoid organs. It is well established that the tissue microenvironment plays a crucial role in the development and progression of CLL but also in the resistance to therapies. However, the currently available in vitro and in vivo models do not allow studying carefully the interactions occurring between leukemia cells and the tissue microenvironment. This is possibly linked to the objective difficulties in recapitulating in vitro the dissemination of leukemia cells in the various tissues in a humanized model. However, this aspect is crucial in particular for CLL, considering that most studies are focused on the leukemic cells circulating in the peripheral blood, thus hampering the possibility to study what is actually occurring in the lymphoid tissues which are the reservoir of the disease. To bridge this gap, we are currently developing a multi-organ system, by using 3D bioprinting and dynamic growth in bioreactors, where recreated human bone marrow and lymph node structures are interconnected through blood vessels. This will allow real-time analysis during each step of homing and trafficking of the leukemic cells before and during therapies and will reduce the use of animal models. In detail we specifically aim at: 1) developing a 3D-culture preclinical model, 2) studying CLL cells dynamics while disseminating and homing in the 3D tissues, 3) investigating the effects to current therapies on CLL cells ex-vivo. With this project, we expect to demonstrate the feasibility and advantages of a novel 3D culture model to study ex vivo leukemic cell dissemination. We will focus on the reduction or replacement of animal-derived reagents.

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MEENAKSHI UPRETI, PHD & PETER CHIARELLI, MD, DPHIL
Children’s Hospital Los Angeles, Los Angeles, CA

3D In-vitro platform integrating the Diffuse Intrinsic Pontine Glioma microenvironment

Diffuse intrinsic pontine glioma (DIPG) is a universally lethal pediatric brainstem tumor in children between 5-9 years of age, with median overall survival of less than one year after diagnosis. While traditional brain tumors are assessed through

direct biopsy, the precarious location and infiltrative pathology of DIPG makes acquisition of biopsies challenging, impeding progress in understanding the disease and treatment response. Animal models for DIPG have limitations in capturing interactions in the unique tumor microenvironment, hampering investigation and development of anticancer agents. Human xenograft animal models reported for DIPG, utilize rodents 6-8 weeks in age, equivalent to about 20-30 human years. This developmental discrepancy further contributes to the dismal prediction of clinical outcomes in pediatric patients. We propose the first transparent and reproducible in-vitro 3D microfluidic platform as an optimal preclinical model for DIPG, which will more faithfully capture phenotypic or morphometric modalities of the DIPG niche and therapeutic interventions through optical imaging in real time. Such an investment in miniaturization, throughput, reproducibility, and robust quantitative drug assessment with potential to alter clinical outcome, is warranted. The innovative 3D microfluidic culture platform aligns with the mission of the ARDF in utilizing non-animal methods for addressing a critical gap in modern investigations and will have immediate acceptance in academia, medicine, and the pharmaceutical industry for research in hard-to-treat cancers This approach will significantly reduce the testing of drugs and stressful invasive procedures in animals and find additional application in evaluation of tumor interactions with the host immune system. We have assembled a strong multidisciplinary team of cancer and neuro-oncology researchers, along with experts in imaging, to develop the proposed 3D platform and implement it in preclinical studies for DIPG.

6 NIGEL YARLETT, PHD
Pace University, New York, NY

Development of an in vitro culture system for *Cryptosporidium hominis* using a hollow fiber bioreactor

Cryptosporidium hominis causes pediatric diarrhea and is the second leading cause of death in children under three years of age in economically challenged countries. Currently the only way to propagate the parasite is using gnotobiotic piglets which significantly limits study of the basic biology of this parasite and hindering the development of chemotherapeutic agents to treat diseased children. We have recently developed a continuous in vitro culture system for a related parasite *Cryptosporidium parvum* using a hollow fiber bioreactor (HFB). This technology provides the parasite with a 3D-culture platform such that the host cells (human epithelial cell line) obtain nutrients from the basal surface (oxygenated growth media flowing through the hollow fibers), and the extra-capillary space contains a low oxygen, highly reducing growth medium that supports the parasite motile stages. We will utilize all synthetic or non-animal reagents for the culture system which will make this parasite available to laboratories without the use of animal models. This culture method has several advantages over the current one: (i) It permits access to all life cycle stages for studies involving the basic biology of the parasite; (ii) It permits drug studies to be performed in a time frame exceeding 48h which is currently the maximum time point for chemotherapeutic evaluation; (iii) It enables pharmacokinetic evaluation of potential chemotherapeutic agents which currently can only be achieved using animals. We will (a) develop an in vitro culture of *C. hominis* using the HFB; (b) Enumerate parasite stages (sporozoites, merozoites, oocysts); (c) sequence the genome. The availability of the bioreactor for the culture of *C. hominis* will replace the gnotobiotic piglet model for the study of this parasite which will facilitate expansion of research in this area by reducing the use of animals.

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