B·DEBATE International Center for Scientific Debate BARCELONA

biocat | Obra Social "la Caixa"

SYNTHETIC BIOLOGY

ENGINEERING LIFE FOR THE MEDICINE OF THE FUTURE

June 13th and 14th, 2019

COSMOCAIXA BARCELONA. ISAAC NEWTON, 26. BARCELONA

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CO-ORGANIZED BY:

CRG Centre for Genomic Regulation | upf. Universitat Pompeu Fabra Barcelona

COLLABORATORS:

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SYNTHETIC BIOLOGY:
ENGINEERING LIFE FOR THE MEDICINE OF THE FUTURE

Short presentations:

- **Evolving new antimicrobials**
  Daniela A. Garcia-Soriano, Pompeu Fabra University, Barcelona

- **PepID technology combines multi-genome wide surface protein analyses with peptide high density arrays and mapping of expressed epitopes for vaccine development**
  Josef Maier, ATG:biosynthetics GmbH, Merzhausen, Germany

17:30 **End of the session**

Friday, July 14, 2019

9:30 **Registration & welcome coffee**

9:45 **SESSION 3. Synthetic biology for human therapy**

Chair: **Francesc Posas,** Pompeu Fabra University, Barcelona

Genome Editing for Human Therapeutics
Matthew Porteus, Stanford Medicine, US

Lentiviral Gene Therapy and Genome editing for the treatment of a Rare Hemolytic Disease, Pyruvate Kinase Deficiency
José Carlos Segovia, Centre for Energy, Environment and Technology Research, Madrid, Spain

Synthetic biology for human therapy: CAR T-cells against tumors
Manel Juan Otero, Hospital Clínic de Barcelona, Barcelona

11:30 **Coffee break and networking**

12:00 **Implementing Biological computation with Distributed Multicellular Consortia**
Francesc Posas, Pompeu Fabra University, Barcelona

Engineering E. coli bacteria for the injection of proteins into tumor cells
Luis Ángel Fernández, Spanish National Centre for Biotechnology, Madrid, Spain

13:15 **Lunch**

14:15 **SESSION 4 (round table). Translational directions of Synthetic biology**

Chair: **Luis Serrano,** Centre for Genomic Regulation (CRG), Spain

Laia Crespo, Sanofi Ventures, France
Sylvain Sachot, Asabys Partners, Barcelona
Lluís Pareras, Invivo Capital and Healthequity, Barcelona
John L. Collins, SynbiCITE, UK

15.45 **Coffe break and networking**

genome editing broadly accessible to the scientific community because of its ease of design, high activity and excellent specificity. We have developed a genome editing system using the S. pyogenes Cas9 system combined with AAV6 delivery of a donor molecule to achieve high frequencies of genome editing in primary therapeutically relevant human cells. This system is efficient to permit multiplexed targeted knock-ins. I will discuss our progress towards applying the system to develop genetically engineered cell based drugs for human disease. I will also briefly review the stringent criteria that the National Academy Study Committee on Human Genome Editing proposed should be adopted before germline editing might be attempted. These strict criteria, while not a direct call for a moratorium, would, if adopted, provide a functional moratorium to its use in humans.

José Carlos Segovia Sanz, Head of Division at **Center for Energy, Environment and Technology Research**, Madrid, Spain

Dr. José Carlos Segovia has focused his research on the study of hematopoietic stem cells (HSC), their interaction with viral pathogens, their ex vivo purification and manipulation and, on gene transfer of Hematopoietic Stem Cells, with the aim of developing gene therapy protocols for the treatment of genetic diseases with hematopoietic pathology. He is currently the Head of the Cellular Technology Division at the Center for Energy, Environmental and Technological Research (CIEMAT). He has published more than 100 scientific articles in journals of high impact in the areas of Gene Therapy and Cell Therapy, has participated in more than 45 projects, being a Principal Investigator of more than 10 and has obtained 7 patents, one of them already licensed. During the last years he has focused his research on the development of gene therapy protocols for Pyruvate Kinase Deficiency (PKD). He has achieved the Orphan Drug Designation by the European Agency and the American Agency for Medicines (EMA and FDA, respectively) for an addition gene therapy drug, which will be used in the first-in-human gene therapy clinical trial for PKD. He has coordinated meetings with PKD affected patients in Spain and Europe, to report on the progress being made with this new therapy. Recently, he has applied the new technologies of gene editing to the treatment of PKD. As a result of these studies, he has published 3 scientific papers and presented a patent that is in the process of being approved. He has directed three workshops of gene editing during the last years. Dr. Segovia is also a collaborator in master's degrees in biotechnology and biomedicine taught at various public and private universities and is the assistant coordinator of the Genetic Engineering module at the Biotechnology Master's Degree at the Francisco de Vitoria University. Finally he has been vocal and secretary of the Spanish Society of Gene and Cell Therapy and currently vice president of the Iberian Cytometry Society.

Lentiviral Gene Therapy and Genome editing for the treatment of a Rare Hemolytic Disease, Pyruvate Kinase Deficiency

The modification of the genome is increasingly becoming recognized as a safe and effective strategy to treat genetic diseases, many of which are life-threatening or associated with extensive morbidity. Some therapies have been already approved by the regulatory agencies, such as Strimvelis™, approved by the European Medical Agency (EMA) for the treatment of Adenosine Deaminase (ADA) inherited immunodeficiency. With the improvement of gene editing tools that allow precise integration of desired genetic sequences, the possibility of making this technology a clinical option is becoming a reality. We are working to develop all the aforementioned modalities for the treatment of Pyruvate Kinase Deficiency, a genetic hemolytic anemia with extensive morbidity and likely diminished life-expectancy.

Pyruvate kinase deficiency (PKD) is an autosomal recessive disorder caused by mutations in the PKLR gene leading to a reduction of the activity of erythroid pyruvate kinase (RPK) protein. This disease is associated with reticulocytosis, splenomegaly and iron overload, and may be life-threatening in severely affected patients. Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) has been shown to correct the disorder; however this is associated with extensive toxicity. Autologous HSCT of genetically corrected cells is intended to provide a durable and curative therapeutic option. Preclinical gene therapy studies conducted in pyruvate kinase deficient mice have shown safety and efficacy of a new PGK-coRPK-Wpre therapeutic lentiviral vector that has been granted orphan drug designation by the European Medicine Agency (EU/3/14/1330) and the US Food and Drug Administration (FDA#DRU-2016-5168).

A first-in-human PKD gene therapy clinical trial has been recently presented to the FDA and the Spanish Medical Agency and the trial is anticipated to start during the following months. Over the last years, gene editing has emerged as a promising gene therapy approach for blood cell disorders. To correct PKD in human HSPCs, we set up a knock-in gene editing strategy at the genomic starting site of the PKLR gene by combining RNP electroporation and adeno-associated viral vector (AAV6) carrying donor sequences. Specific gRNAs generating up to 60% indels at the RPK starting site in human Cord Blood CD34+ (CB-CD34+) were designed. Two different AAV6 constructions were produced to deliver a TurboGFP expression cassette or a promotor-less therapeutic codon optimized RPK cDNA (coRPK), flanked by specific homologous arms. Up to 40%

specific integration and stable expression of both donors was detected in colony forming units generated from gene edited CB-CD34+cells, without evident toxicity related to the procedure. Moreover, these edited CB-CD34+ cells engrafted efficiently in both primary and secondary NSG mice, demonstrating the gene editing of HSCs. Overall, gene therapy for PKD is approaching clinical-stage and may offer a potentially curative alternative for PKD patients.

Manel Juan Otero, Head of Immunotherapy Section at **Hospital Clínic**, Barcelona, Spain

Degree and Doctorate: Medicine and Surgery (1988 and 1994). Clinical specialty: Immunology (1994). CLINICAL WORK: Head of immunotherapy Section; Immunology Service (2007 -). Direct Responsibilities; - Cell immunotherapy + Cytometry coordination + 2 main approaches: DCs + CART. TEACHING: Assistant Professor (2015 -). Department of Medicine. Medical School, Universitat de Barcelona (UB). Assistant Professor of Immunology (1997- 2007). A3.3 level. Medical School. UAB. Coordinator in master of autoimmune diseases (UB); 9 directed doctoral theses (5 co-directed). RESEARCH (SUMMARY): 80 articles in journals with IF (3 national and 71 international); 105 papers in total. Total IF: 497.321. Publications in last 5 years: 16. Summatory impact factor: 152.952. Communications: 75 National + 87 International Congresses. 2 active projects + 10 previous projects as Principal Investigator + 16 projects as a research collaborator. 3 licenced patents (3 PCT).

Synthetic biology for human therapy: CAR T-cells against tumors

T-cells transduced with a Chimeric Antigen Receptor (CAR) is already one of the available treatments against CD19+ B-cell leukemias and lymphomas, but hundreds of clinical trials with other CARs are locating this immunotherapy as one of the most promising proposals. CART combines gene therapy tools (mainly lenti and retrovirus) in T-cells, with cell therapy processes to obtain by ex-vivo manipulation a cell product to be reinfused into the patient. Although in general CART is defined as an autologous drug, donor-obtained product (allogeneic drug) are also done with the aim to have an "off-the-sell" drugs. In any case there are still a lot of work to improve constructs, introduce gene editing for transduction, increasing efficacy of the current proposals, reducing adverse effects ... In fact, CRS and Neurotoxicity are severe adverse effects that should be improved.

It is quite clear that if we want to go one step forward, the improvement of CART antitumoral proposal should continue and probably only collaboration between pharma and academic proposals can asure this strong and continuous development. Our experience in Hospital Clinic of Barcelona with CART19-BE-001 CT is our first model for developing an Academic Clinical Trial in Spain, although we have additional proposals for the most near-future pipeline.

Francesc Posas, Director of Institute for Research in Biomedicine (IRB) Barcelona and Professor of Biochemistry and Molecular Biology at **Pompeu Fabra University**, Barcelona, Spain

Read bio in page 14.

Implementing Biological computation with Distributed Multicellular Consortia

Engineering approaches to synthetic biology have shown that there are a number of strategies allowing to build complex functional constructs with computational abilities. There are a number of efforts towards building artificial computational devices that could be used for a wide range of applications, including bioremediation, food production or biomedicine. Using yeast as a model organism we have been able to implement complex circuits by distributing computation within cellular consortia. This approach to biological computation has opened the possibility to develop a novel method of properly design general purpose which can be combined in multiple ways to create complex computational circuits. The potential use of this approach is demonstrated by implementation of complex logical functions responding to up to six inputs, the building of a synthetic biological memory switch or a circuit with an incoherent feed-forward loop architecture (FFL) to generate single pulse responses or implementing reprogrammable biological devices. Our results might serve as a blueprint for future development of biocomputing cellular devices.
