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Summary

- Positive, results-driven drug developer with experience in RNA therapeutics and gene therapy: mRNA therapeutics, CRISPR, RNA-binding proteins, microRNAs, RNA-editing, oligonucleotides, and viral and non-viral delivery systems
- Drug discovery experience in the rare genetic disease space (metabolic, lung, CNS)
- Extensive experience in inhaled drug delivery (nucleic acid formulations/small molecules)
- 20 years of research experience in the industry and academia, and formal training in RNA Biology, Immunology, Virology, Molecular and Cell Biology and Biochemistry
- Led drug discovery projects from early discovery to safe and efficacious clinical candidates and provided evidence for IND submissions
- Late stage toxicology experience in GLP *in vitro* and *in vivo* studies
- Proficiency in building and leading interdisciplinary biology, bioinformatics, protein engineering, screening, analytical chemistry, formulations, pharmacology, program management, operations and toxicology teams and labs
- Hands-on experience through the stages of preclinical drug discovery: target identification & validation, mechanism of action, screening, toxicology, analytical, formulation, delivery and preclinical development
- Proficiency in establishing drug discovery strategies to identify and derisk candidates, transferring and developing methodologies, and establishing SOPs
- Extensive background in establishing biochemical, *in vitro, ex vivo* and *in vivo* systems to assess target engagement, safety, dosing, efficacy and biomarkers
- Demonstrated experience in assay development for SAR and compound optimization, formulations (Lipid Nanoparticles, Polyplexes) and delivery systems
- Experience with managing internal and external resources, and establishing relationships with academic collaborators and KOLs

Experience – Research

LOCANABIO, RNA-TARGETING GENE THERAPY BIOTECH San Diego, CA

VP, Head of RNA Biology & Drug Discovery 12/2020-present

- Leading drug discovery efforts from early discovery to *in vivo* candidates for all projects based on CRISPR systems and engineered RNA-binding proteins
- Leading the major pipeline project targeting C9orf72 for ALS from discovery to IND-enabling studies

- Managing the scientific teams: Early Discovery, Bioinformatics, Protein Engineering, Molecular Biology and Technology, Screening and Assay Development, and DNA Core
- Implemented drug discovery processes to identify targetable RNA sequences, maximize efficacy and minimize off-target effects, streamline SAR and molecule design, and de-risk immunogenicity to select safe and efficacious IND candidates
- Strategic and scientific leadership for new target identification and technology licensing
- Collaborating closely with *in vivo* pharmacology, vector development and CMC groups
- Contributing to pre-IND documentation and interactions with the FDA
- Representing the Drug Discovery efforts at Board of Directors meetings

Sr. Director, Head of RNA Biology & Drug Discovery 02/2020-12/2020

- Implemented procedures to streamline the discovery process and established the:
    - Early Discovery team to provide POC for emerging internal technologies and external technology transfers
    - Bioinformatics team to develop automated processes for Next Generation Sequencing analysis, identify target sequences in patient populations, and streamline off-target analysis and molecule design
    - Protein Engineering team to establish design guidelines, explore SAR, stability and expression, and create synthetic proteins and fusions implementing synthetic biology principles
    - Molecular Biology and Technology team to streamline modality design and gene regulation elements, develop methodologies to assess potency, and optimize early technologies
    - Screening and Assay Development team to establish high-throughput assays for efficacy, viability, cytotoxicity, immune responses, and off-target effects and develop physiologically relevant *in vitro*, *ex vivo* and patient-derived screening cell systems
- Expanded the company IP portfolio with new technology and targets
- Provided strategic and scientific leadership for new targets and disease areas
- Established documentation policies, Laboratory Information Management System (LIMS), protocols and SOPs
- Managed KOL relationships
- Contributed to the investor meetings for the successful $100M Series B Funding

MEDICAL DEVICE / SMALL MOLECULE PROJECT (STEALTH) San Francisco, CA

Sr. Director, Head of Preclinical R&D 02/2019-02/2020

- Led the preclinical department with 7 teams focused on: inhalation biology, *in vitro* systems toxicology, risk assessment, analytical chemistry, exploratory chemistry, screening, and project management/operations
- Oversaw GLP toxicology studies: *in vitro* genotoxicity/mutagenicity (Ames, MN, MLA) and *in vivo* toxicology (5-day MTD, 14-day and 90-day studies)
- Provided scientific, technical, and strategic leadership to support new product

development, and comply with FDA and international regulatory requirements
- Developed tools for integrated data management, and next generation, high-throughput analytical, respiratory biology and systems toxicology methodologies
- Established multi-disciplinary screening cascades to de-risk FDA submissions
- Established internal analytical chemistry, inhalation, systems biology and toxicology labs
- Partnered with global CROs to execute GLP preclinical studies and transfer newly developed methods to increase screening capacity and reduce turnaround times
- Interfaced and supported Regulatory, Clinical, Scientific and Medical Affairs, Quality, Engineering, Manufacturing, Formulation, Global Supply and Product Development teams

Director, Head of Preclinical R&D 04/2018-02/2019

- Built the preclinical department and established respiratory biology, toxicology, analytical chemistry, exploratory chemistry, risk assessment and project management teams
- Led the team to support cross-functional activities ranging from preclinical R&D, risk assessment, product development, clinical studies, prototyping and sustaining engineering, and regulatory compliance for multiple markets
- Expanded external testing capacity with multiple CROs, decreased turnaround times and reduced cost
- Established a custom LIMS system interfacing with CRO systems, to automate importing, analysis, QC and report generation dramatically reducing processing time

TRANSCRIPTX (MERGED WITH RECODE THERAPEUTICS) SF Bay Area, CA
EARLY STAGE MESSENGER RNA GENE THERAPY BIOTECH

Sr. Scientist, Team Leader 01/2016-04/2018

Head of *in vivo* Pharmacology 2017-2018

- Led the team to perform *in vivo* safety studies of nebulized therapeutics and proof-of-concept studies for therapeutic intervention in genetic lung diseases
- Established luminescence-based *in vivo* imaging and *ex vivo* biochemical methodologies for biodistribution and delivery to the upper airways and distal lung
- Assessed *in vivo* multiple distinct formulations for mRNA therapeutics (Lipid Nanoparticles, Polyplexes) and next-generation nebulization/aerosolization delivery systems for formulated mRNA
- Established the in-house vivarium and policies adhering to the IACUC guidelines

Head of Formulations & Analytical Development 2017

- Led the formulations team to produce and QC and Lipid Nanoparticle and Polyplex formulations for all preclinical programs
- Transferred methods and developed analytical SOPs for the QC of formulated material
- Established methodologies to consistently formulate Polyplexes with inexpensive portable devices that can be deployed at collaborator and CRO sites
- Established procedures for characterizing effects of aerosolization/nebulization on the

physicochemical properties of formulated particles and mRNA
- Developed novel assays to determine the capping efficiency of mRNA transcripts

Head of *in vitro* Screening and Safety Profiling 2016
- Led the team to establish methodologies to assess expression and safety of nebulized formulated mRNA *in vitro* and *ex vivo*
- Developed methodologies to detect protein expression from IVT products
- Led the development of *in vitro* safety, expression and immune cell activation assays
- Designed the initial mRNA-based gene therapy platforms for the preclinical programs

Hiring/Laboratory Setup
- Led the laboratory setup, method transfer and hiring of key personnel

REGULUS THERAPEUTICS San Diego, CA
MICRORNA THERAPEUTICS BIOTECH
Scientist, Project Team Leader 01/2012-11/2015

Project Team Lead, Target ID, Validation and Early Discovery 2014-2015
- Led the project team to identify, optimize and develop safe anti-miR-27 oligonucleotide lead candidates, showing improved liver function and reducing liver injury biomarkers in animal models of cholestatic disease with a genetic or autoimmune background
- Collaborated with academic labs to investigate potential treatments for orphan diseases
- Designed and executed in-house high-throughput cell-based screens to identify microRNAs that regulate metabolic pathways
- Developed molecular/transcriptomics assays to replace slow histopathology readouts

Project Lead, Basic Mechanisms of Action & Assay Development 2012-2013
- Led research on the mechanisms of action of microRNA inhibitors and published our findings
- Conceived and developed novel biochemical assays to evaluate pharmacological target engagement of microRNA inhibitors and assess off-target effects *in vitro* and *in vivo*
- Adapted direct target engagement assays developed for basic mechanism research to robust, high-throughput screening assays to advance portfolio projects
- Developed *in vitro* cell-based gene expression and flow cytometry assays to determine indirect target engagement for metabolic, oncology and immunology projects
- Identified target genes of microRNAs *in vivo*/*in vitro* to support biomarker selection

UNIVERSITY OF CALIFORNIA, SAN DIEGO San Diego, CA
Postdoctoral Fellow, RNA biology 10/2007-12/2011
Laboratory of Amy E. Pasquinelli
- Developed an innovative high-throughput biochemical and next generation sequencing method to comprehensively identify *bona fide* gene targets of microRNAs *in vivo* by

isolating Argonaute/ mRNA/microRNA complexes and analyzing their footprint on target mRNAs (CLIP-Seq)
- Led the discovery of novel functions of the microRNA pathway by identifying the first long non-coding RNA under Argonaute regulation and the autoregulation of the let-7 miRNA
- Investigated microRNA-mediated post-transcriptional regulation by engineering and employing transgenic *C. elegans* strains and genetic tools in human cells

Experience – Teaching

SAN DIEGO COMMUNITY COLLEGE DISTRICT – MESA COLLEGE San Diego, CA

Adjunct Professor of Microbiology, Genetics and Immunology 2014-2016
DEPT. OF BIOLOGY

- Lecture and Lab Professor for Microbiology - BIO205

Education

FEINBERG SCHOOL OF MEDICINE, NORTHWESTERN UNIVERSITY Chicago, IL

Ph.D. in Molecular Immunology & Cellular Biology 09/2001-08/2007
Advisor: Geoffrey S. Kansas

- Delineated the TCR-activated Ras/MAPK-mediated signal transduction pathways controlling the transcriptional regulation of glycosyltransferases in human T cells and mouse primary cells using viral transduction, pharmacological inhibition, transgenic mouse models and gene expression profiling
- Studied the transcriptional factors Stat3/4 and T-bet in the development of Th17 cells
- Investigated the tumor-suppressor properties of Ikaros, a master regulator of immune development

ARISTOTLE UNIVERSITY OF THESSALONIKI Greece

B.S., Molecular Biology & Immunology 1996-2001
Advisor: Lygeri Hadjipetrou-Kourounakis

- Studied the effects of adjuvants on cytokine and antibody profiles against the thymus-independent antigen dextran-FITC in mouse models for *Mycoplasma agalactiae* vaccines

Honors & Awards

Top 1% of Managers, internal company survey 2019
Regulus Star Award, for "contributing above and beyond the call of duty" 2013
Career Development Award, Lymphoma and Leukemia Society, 3 years 2010
Postdoctoral Fellowship Award, American Heart Association, 2 years 2010
Predoctoral Fellowship Award, American Heart Association, 1 year 2006
Travel Scholarship Award, Katten Muchin Rosenman Foundation 2005
Predoctoral Fellowship Award, American Heart Association, 2 years 2004
Graduate School Fellowship, Northwestern University, 2 years 2001
Excellence Award, Greek Ministry of Education, 6 consecutive years 1988 - 1994

Publications

1. Heine N, Ozvald AO, O'Regan D, Hiraki B, Landestoy PV, Lansang M, **Zisoulis DG**.
A Proton Transfer Reaction Mass Spectrometry methodology for real-time, online identification and quantification of aerosol constituents *(manuscript in preparation)*

2. Hand JN, Tran P, Liu J, Friedman JR, **Zisoulis DG**.
Therapeutic inhibition of miR-27 improves liver function and reduces injury biomarkers in mouse models of cholestatic disease *(to be submitted once cleared for publication)*

3. Bochnakian A, Zhen A, **Zisoulis DG**, Idica A, KewalRamani VN, Neel N, Daugaard I, Hamdorf M, Kitchen S, Lee K, Pedersen IM
Interferon-inducible miR-128 modulates HIV-1 replication by targeting TNPO3 mRNA
***Journal of Virology*, 2019**; Sep 30;93(20)

4. Fung L, Guzman H, Sevrioukov E, Idica A, Park E, Bochnakian A, Daugaard I, Jury D, Mortazavi A, **Zisoulis DG**, Pedersen IM.
miR-128 Restriction of LINE-1 Retrotransposition Is Dependent on Targeting hnRNPA1 mRNA
***International Journal of Molecular Sciences*, 2019**; Apr 21;20(8)

5. Guzman H, Sanders K, Idica A, Bochnakian A, Jury D, Daugaard I, **Zisoulis DG**, Pedersen IM.
miR-128 inhibits telomerase activity by targeting TERT mRNA
***Oncotarget*, 2018**;9(17):13244-13253

6. Daugaard I, Sanders K, Idica A, Vittayarukskul K, Hamdorf M, Krog J, Chow R, Jury D, Hansen LL, Hager H, Lamy P, Choi CL, Agalliu D, **Zisoulis DG**, Pedersen IM.
miR-151 induces partial EMT by targeting E-cadherin in NSCLC cells
***Oncogenesis*, 2017**; 6(7):e366

7. Pedersen MI, **Zisoulis DG**.
Transposable elements and miRNA: Regulation of genomic stability and plasticity
Mobile Genetic Elements*, 2016;* 6(3)

8. Hamdorf M, Idica A, **Zisoulis DG***, Gmelin L, Martin C, Sanders K, Pedersen IM.
miR-128 represses L1 retrotransposition by binding directly to L1 RNA
***Nature Structural and Molecular Biology*, 2015**; 22(10):824-31
*** co-first author**

9. Hogan DJ, Vincent TM, Fish S, Marcusson EG, Bhat B, Chau NB, **Zisoulis DG**.
Anti-miRs competitively inhibit microRNAs in Argonaute complexes
***PLoS ONE*, 2014**; 9(7): e100951

10. Hunter SE, Finnegan EF, **Zisoulis DG**, Lovci MT, Melnik-Martinez KV, Yeo GW, Pasquinelli AE.
Functional genomic analysis of the let-7 regulatory network in *Caenorhabditis elegans*
***PLoS Genetics*, 2013**; 9(3): e1003353

11. **Zisoulis DG**, Kai ZS, Chang, RK, Pasquinelli AE.
Auto-regulation of miRNA biogenesis by let-7 and Argonaute
***Nature*, 2012**; 486(7404):541-4

12. **Zisoulis DG**, Lovci MT, Wilbert ML, Hutt KR, Liang TY, Pasquinelli AE, Yeo GW.
Comprehensive discovery of endogenous Argonaute binding sites in *Caenorhabditis elegans*
***Nature Structural and Molecular Biology*, 2010**; 17(2):173-9

13. Mathur AN, Chang HC, **Zisoulis DG**, Stritesky GL, Yu Q, O'Malley JT, Kapur R, Levy DE, Kansas GS, Kaplan MH.
Stat3 and Stat4 direct development of IL-17-secreting Th cells
***Journal of Immunology*, 2007**; 178(8):4901-7

14. Mathur AN, Chang HC, **Zisoulis DG**, Kapur R, Belladonna ML, Kansas GS, Kaplan MH.
T-bet is a critical determinant in the instability of the IL-17-secreting T-helper phenotype
***Blood*, 2006**; 108(5):1595-601

15. Underhill GH, **Zisoulis DG***, Kolli PK, Ellies LG, Marth JD, Kansas GS.
A crucial role for T-bet in selectin ligand expression in T helper 1 (Th1) cells
Blood, **2005**; 106(12):3867-73 ***co-first author**

16. **Zisoulis DG**, Kansas GS.
H-Ras and phosphoinositide 3-kinase cooperate to induce alpha(1,3)-fucosyltransferase VII expression in Jurkat T cells
***Journal of Biological Chemistry*, 2004**; 279(38):39495-504

17. Barry SM, **Zisoulis DG**, Neal JH, Clipstone NA, Kansas GS.
Induction of FucT-VII by the Ras/MAP kinase cascade in Jurkat T cells
***Blood*, 2003**; 102(5):1771-8

Book Chapters

1. **Zisoulis DG**.
Determination of oligonucleotide association with miR:Argonaute complexes *in vivo*
Drug Target microRNA: Methods and Protocols, Editor Schmidt M.
Springer, 2016

2. **Zisoulis DG**.
Quantification of oligonucleotide association with Argonaute complexes *in vitro*
Drug Target microRNA: Methods and Protocols, Editor Schmidt M.
Springer, 2016

3. **Zisoulis DG**, Yeo GW, Pasquinelli AE.
Comprehensive identification of miRNA target sites in live animals
MicroRNAs in Development: Methods and Protocols, Editor: Talmas D.
Humana Press, 2011

Guest Talks

1. ***Cas & Non-Cas Proteins for RNA Modulation: RNA Editing & Beyond***
2nd RNA Editing Summit, December 1-3 2020, Virtual Event

2. ***RNA as target and drug***
Summer Workshop, National Research Focus (NCCR) on RNA and Disease & the Swiss National Science Foundation
August 24-28 2015, Saas-Fee, Swiss Alps

Conference Talks

1. **Zisoulis DG**, Kai ZS, Chang, RK, Pasquinelli AE.
Auto-regulation of miRNA biogenesis by let-7 and Argonaute.
17th Annual Meeting of the RNA Society at Ann Arbor, MI, June 2012

2. **Zisoulis DG**, Kai ZS, Chang, RK, Pasquinelli AE.
Auto-regulation of miRNA biogenesis by let-7 and Argonaute.
18th International C elegans Meeting at University of California, Los Angeles, CA, June 2011

3. **Zisoulis DG**, Lovci MT, Liang TY, Wilbert ML, Yeo G, Pasquinelli AE.
Comprehensive identification of endogenous Argonaute-bound miRNAs and mRNA target sequences at nucleotide level resolution *in C. elegans*.
17th International C elegans Meeting at University of California, Los Angeles, CA, June 2009

4. **Zisoulis DG**, Lovci MT, Liang TY, Yeo GW, Pasquinelli AE.
Massive identification of miRNA target sites in endogenous mRNAs.
The Biology of RNA Silencing (Keystone Symposia) at Victoria, BC, Canada, April 2009

5. **Zisoulis DG**, Lovci MT, Liang TY, Yeo GW, Pasquinelli AE.
Massive identification of miRNA target sites in endogenous mRNAs.
Plant and Animal Genome XVII Conference at San Diego, CA, January, 2009

6. **Zisoulis DG**, Kansas GS.
H-Ras mediates FucT-VII induction via Raf and PI3K
Experimental Biology/35th Int. Congress of Physiological Sciences at San Diego, CA, April 2005

7. **Zisoulis DG**, Kansas GS.
H-Ras induces FucT-VII expression via Raf and PI3K
Glycobiology Meeting 2004 at Honolulu, HI, November 2004

Management Experience

- Experience with building and leading diverse teams of PhDs and RAs
- "Building High-Performance Teams" Management Course
- "Lab to Leadership" Management Course

Patents

- Antibody-based "Reagent for the isolation and purification of miRISC complexes". Licensed through UCSD to Thermo Scientific Pierce (PA1-031)
- 2 Patent Applications under review, 5 Provisional Applications in preparation

References

Provided upon request and include former managers, colleagues and direct reports

Technical Skills

Gene expression

- NGS library preparation and sequencing
- Microarray profiling
- Nanostring
- qPCR (384 format)
- Statistical data analysis (Prism, JMP)

in vitro

- Cell culture (primary cells/cell lines)
- Cytotoxicity/cell viability/proliferation assays
- Transfection/electroporation
- Automated Liquid Handler programming
- Recombinant cell line generation
- Gene silencing

in vivo

- IP/IV/IN administration
- IVIS Imaging
- Tissue harvesting: spleen, liver, lymph nodes, bone marrow, blood

Immunostaining

- Flow cytometry (7-color)
- Immunocytochemistry
- Fluorescent microscopy
- ELISA-spot

Protein/RNA/DNA biochemistry

- HITS-CLIP/PAR-CLIP (CLIP-seq)
- RNA, Protein & Chromatin Immuno-precipitations (RIP, ChIP)
- Western, Northern blot analysis
- ELISA, electrochemi-luminescence based assays (MSD)
- Protein/antibody purification
- Serum Biochemistry
- Bead-based multiplexing of multiple analytes (Luminex xMap)
- Homogeneous lysate platform (Alphalisa)

Formulations

- Cationic Polymers
- Lipid NanoParticles
- Encapsulation efficiency
- RNA Integrity analysis
- Tangential Flow Filtration

Aerosols/Nebulizations

- Jet and Mesh Nebulizers
- Mass Median Aerodynamic Diameter (MMAD)
- Mercer Style Cascade Impactor Analysis

Molecular biology

- DNA cloning & mutagenesis
- Viral gene delivery systems (AAV, RV)
- Transient expression systems
- RNAi-induced gene silencing
- Sequencing library generation
- Library screening
- Custom expression vectors engineering for screening
- CRISPR and MosSCI based genome engineering
- RNAi interference: siRNA/shRNA/ASO

Information Technology

- Office and Image Editing
- Linux, OS X, Windows
- Python, R, HTML
- SharePoint administration