# BIOGRAPHICAL SKETCH

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NAME: Zakrzewski, Johannes L.

eRA COMMONS USER NAME (agency login): ZAKRZEWJ

POSITION TITLE: Associate Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

| INSTITUTION AND LOCATION | DEGREE (if applicable) | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| Friedrich-Alexander University | MD | 04/2000 | |
| University Hospital Hamburg-Eppendorf | Resident | 05/2000 | |
| Bone Marrow Transplantation Center Idar-Oberstein | Resident | 08/2001 | Pediatric Bone Marrow Transplantation |
| University Hospital of Essen | Resident | 03/2004 | Pediatrics |
| Memorial Sloan Kettering Cancer Center | Postdoctoral Fellow | 06/2008 | Immunology |
| SUNY Downstate Medical Center | Resident | 06/2010 | Pediatrics |

## A. Personal Statement

The goal of this application is to build a preclinical and translational research program geared towards the development of a novel method for CAR T cell-based cancer immunotherapy. I have extensive clinical expertise in oncology and hematopoietic stem cell transplantation, as well as in cancer and transplantation immunology. As a scientist I have had a longstanding interest in the development of strategies to enhance T cell immunity, in particular in the setting of tumor immunotherapy. Over the past decade I have taken a strong leadership role in developing innovative interdisciplinary experimental approaches involving immunology, molecular biology, nanotechnology, drug development, cell engineering and biomedical engineering. In summary, I have a demonstrated record of accomplished and productive research projects in areas of high relevance for adoptive cell therapy and cancer immunotherapy, and my expertise and experience have prepared me to lead the proposed project.


1. Zakrzewski JL et al. Adoptive transfer of T-cell precursors enhances T-cell reconstitution after allogeneic hematopoietic stem cell transplantation. Nat Med 2006; 12: 1039-1047. PMID: 16936725.
2. Zakrzewski JL et al. Tumor immunotherapy across MHC barriers using allogeneic T cell precursors. Nature Biotechnol 2008; 26: 453-461. PMCID: PMC2731996.
3. Shono Y, Tuckett AZ, Ouk S, Liou HS, Altan-Bonnet G, Tsai JJ, Oyler JE, Smith OM, West ML, Singer NV, Doubrovina E, Pankov D, Unhad CV, Murphy GF, Lezcano C, Liu C, O'Reilly RJ, van den Brink MRM, Zakrzewski JL. A small molecule c-Rel inhibitor reduces alloactivation of T cells without compromising anti-tumor activity. Cancer Discovery 2014; 4: 578-591. PMCID: PMC4011979.
4. Shono Y, Tuckett AZ, Liou HC, Doubrovina E, Derenzini E, Ouk S, Tsai JJ, Smith OM, Levy ER, Kreines F, Ziegler CGK, Scallion MI, Doubrovin M, Heller G, Younes A, O'Reilly RJ, van den Brink MRM, Zakrzewski JL. Characterization of a c-Rel inhibitor that mediates anticancer properties in hematologic malignancies by blocking NF-kappaB-controlled oxidative stress responses. Cancer Research 2016, 76: 377-389. PMID: 26744524.


## B. Positions and Honors

## Positions and Employment

2007 - 2009 Adjunct Professor, NYU Polytechnic School of Engineering

2010 - 2012 Clinical Fellow in Pediatric Hematology/Oncology, Memorial Sloan Kettering Cancer Center
2012 - 2017 Assistant Attending Pediatrician, Memorial Sloan Kettering Cancer Center
2014 - 2017 Instructor in Pediatrics, Weill Cornell Medical Center
2018 Visiting Investigator, Memorial Sloan Kettering Cancer Center
2018 - Assistant Clinical Professor in Pediatrics, Weill Cornell Medical Center
2018 - Associate Scientist, Hackensack University Medical Center
2018 - Member, Georgetown Lombardi Comprehensive Cancer Center

## Other Experience and Professional Memberships

2010 - Member, COG
2012 - Member, ASBMT
2013 - Member, ASH
2014 - Scientific Advisory Board, ImmuneTarget Inc.
2017 - Scientific Advisory Board, BioIVT

## Honors

2005 Fellowship Award, German Research Foundation
2006 Fellowship Award, Lymphoma Research Foundation
2008 David G. Nathan Award in Hematology/Oncology, Society for Pediatric Research

## C. Contribution to Science

1. Stem cell or T cell-based therapies have been used for decades to confer immunity and anti-tumor activity to T cell-deficient patients. However, these cell therapies are associated with a variety of limitations, in particular a limited availability of suitable cells: donor and recipient have to be either the same person, or donor and recipient have to be at least partially HLA matched. As a research fellow from 2004 to 2008 I pioneered a cell culture-based immunotherapy method for the treatment of T cell deficiency in cancer patients and HSCT recipients. This approach is based on adoptive transfer of in vitro generated T cell precursors using a co-culture system with a stromal cell line expressing the Notch 1 ligand DL1 to generate large numbers of T lineage committed precursor cells ex vivo. These T cell precursors can engraft in the thymus upon adoptive transfer into irradiated recipients and give rise to a single wave of T cell development resulting in enhanced T cell reconstitution and graft-versus-tumor activity in the absence of graft-versus-host disease (GVHD). Moreover, I demonstrated that precursor T cells that were genetically targeted to the CD19 antigen could mature in fully mismatched recipients and yield T cells with potent anti-lymphoma activity without causing GVHD in tumor-bearing mice. The cells have been shown to mature into functional T cells that are host MHC restricted and host tolerant, likely due to intrathymic selection and education, negating the need for HLA matching. This procedure is not only laborsaving and cost-effective (since virtually unlimited quantities of precursor cells can be generated and stored for “off-the-shelf” use), but it facilitates the application of gene transfer technology to generate antigen- specific or otherwise enhanced designer cells.

   a) Zakrzewski JL et al. Adoptive transfer of T-cell precursors enhances T-cell reconstitution after allogeneic hematopoietic stem cell transplantation. Nat Med 2006; 12: 1039-1047. PMID: 16936725.

   b) Zakrzewski JL et al. Tumor immunotherapy across MHC barriers using allogeneic T cell precursors. Nature Biotechnol 2008; 26: 453-461. PMCID: PMC2731996.

   c) Zakrzewski JL, van den Brink MRM, Sadelain M. Methods for off-the-shelf tumor immunotherapy using allogeneic T-cell precursors. United States patent application 20110052554.

2. Imaging-guided percutaneous injection of lymphoid organs has the potential to open up new exciting avenues for cell, drug, and gene therapy. I am currently leading several projects on the development of preclinical protocols for the treatment of T cell deficiency associated with HSCT, aging, and radiation-induced injury using ultrasound-guided injection of lymphoid organs. In addition to manipulation of the

thymic microenvironment this research program explores bioengineering and nanotechnology methods to develop strategies promoting thymus-independent T cell generation. These methods utilize molecularly tailored biomaterials, micro and nanofabrication techniques to create an artificial implantable hematopoietic niche providing the microarchitecture and signaling requirements for T cell development.

a) Tuckett AZ, Thornton RH, Shono Y, Smith OM, Levy E, Kreines F, van den Brink RM, Zakrzewski JL. Image-guided intrathymic injection of multipotent stem cells supports life-long T cell immunity and facilitates targeted immunotherapy. Blood 2014; 123: 2797-2805. PMCID: PMC4007607.

b) Zakrzewski JL, van den Brink MRM, Hubbell JA. Overcoming immunological barriers in regenerative medicine. Nat Biotechnol 2014; 32: 786-794. PMCID: PMC4409427.

c) Tuckett AZ, Zakrzewski JL, Li D, van den Brink MRM, Thornton RH. Free-hand ultrasound guidance permits safe and efficient minimally invasive intrathymic injections in both young and aged mice. Ultrasound in Medicine and Biology 2015; 41: 1105-1111. PMCID: PMC4346466.

3. Separation of GVHD from anti-tumor activity is one of the most important challenges in the field of allogeneic HSCT, and even though a number of experimental approaches have been developed, none of them has been established in mainstream clinical practice. A potential molecular target for a strategy to separate GVHD from graft-versus-tumor (GVT) activity is the NF-kB family member c-Rel, a transcription factor that upon antigen receptor triggering regulates lymphocyte survival and proliferation and is known to play dominant roles in inflammation, autoimmune disease, as well as oncogenesis. Using high-throughput screening our team identified a small molecule that acts as a highly specific direct inhibitor of c-Rel activity. We discovered that inhibition of c-Rel diminishes alloactivation while preserving antigen-specific T-cell receptor activation, revealing redundancy of c-Rel in T-cell mediated anti-tumor activity of both mouse and human T-cells. This study provides a highly innovative immunomodulatory approach with broad therapeutic implications including tolerance induction after HSCT and solid organ transplantation, as well as anti-tumor therapies.

   a) Shono Y, Tuckett AZ, Ouk S, Liou HS, Altan-Bonnet G, Tsai JJ, Oyler JE, Smith OM, West ML, Singer NV, Doubrovina E, Pankov D, Unhad CV, Murphy GF, Lezcano C, Liu C, O'Reilly RJ, van den Brink MRM, Zakrzewski JL. A small molecule c-Rel inhibitor reduces alloactivation of T cells without compromising anti-tumor activity. Cancer Discovery 2014; 4: 578-591. PMCID: PMC4011979.

4. The aim of this research program is to study the potential of NF-kB and oxidative stress pathways as targets for cancer therapy. Our recent work revealed that modulation of the redox homeostasis in cancer cells, including inhibition of NF-kB and Nrf2 dependent antioxidant responses, is safe and can be exploited for targeted cancer therapy. We anticipate that our work will produce drug candidates for clinical trials in the near future.

   a) Shono Y, Tuckett AZ, Liou HC, Doubrovina E, Derenzini E, Ouk S, Tsai JJ, Smith OM, Levy ER, Kreines F, Ziegler CGK, Scallion MI, Doubrovin M, Heller G, Younes A, O'Reilly RJ, van den Brink MRM, Zakrzewski JL. Characterization of a c-Rel inhibitor that mediates anticancer properties in hematologic malignancies by blocking NF-kappaB-controlled oxidative stress responses. Cancer Research 2016, 76: 377-389. PMID: 26744524.
   b) Liu HY, Tuckett AZ, Fennell M, Garippa R, Zakrzewski JL. Repurposing of the CDK inhibitor PHA-767491 as a NRF2 inhibitor drug candidate for cancer therapy via redox modulation. Invest New Drugs. 2018 Jan [Epub ahead of print]. PIMD: 29297149
   c) Zakrzewski J, Liou H-C, Ouk S. Treatment of hematological malignancy with small molecule NF-kB inhibitors. United States Provisional Application Serial No. 62439335.

## D. Research Support

## Ongoing Research Support

Sponsored Research Agreement with ImmuneTarget Inc.
Zakrzewski, Johannes L. (PI)

10/01/18-07/31/2021
Targeted therapy of multiple myeloma via ROS and NF-kB-mediated mechanisms.

## Completed Research Support

Hyundai Scholar Hope Grant
Zakrzewski, Johannes (PI)
01/2017 – 12/2017
Targeted therapy of pediatric hematologic malignancies via NF-kB-mediated mechanisms.

6465-15, Leukemia and Lymphoma Society Translational Research Program
Zakrzewski, Johannes L. (PI)
10/01/14-09/30/17
Strategies to overcome transplantation barriers and treat hematologic malignancies enabled by small molecule-mediated inhibition of the transcription factor c-Rel.

K08 CA160659-05
Zakrzewski, Johannes (PI)
08/10/11-07/31/16
Strategies to enhance thymus-independent T cell development in cancer patients

Inaugural Tellabs Foundation Research Grant, Leukemia Research Foundation
Zakrzewski, Johannes L. (PI)
07/01/13-06/30/14
A novel therapy for graft versus host disease without compromising tumor immunity

Nanotechnology Center Grant, Memorial Sloan Kettering Cancer Center
Zakrzewski, Johannes L. (PI)
01/07/12-01/06/14
Engineering of T cell regenerating tissue enabled by native ECM nanofibers and synthetic biodegradable polymer nanofibers