BIOGRAPHICAL SKETCH

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NAME: Varadarajan, Navin

eRA COMMONS USER NAME (agency login): NVARADARAJAN

POSITION TITLE: Assistant Professor

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
University of Madras	BS	08/1998	Chemistry
Indian Institute of Science	MS	08/2001	Chemistry
University of Texas, Austin	PHD	12/2006	Chemistry & Biochemistry

A. PERSONAL STATEMENT

The overall goal of my research group is to facilitate the clinical translation of human immunotherapies/vaccines, right from the discovery/engineering of novel protein therapeutics to the evaluation of single-cell functionality in adoptive cell therapy, by employing custom high-throughput nanowell assays. I am fully dedicated to a career in tumor immunology and envision a career path engineering methods and therapeutics to solve clinical problems. Towards this goal, I collaborate extensively with clinicians at University of Texas M.D. Anderson Cancer Center, University of Texas Health Science Center, Baylor College of Medicine and the University of Texas Medical Branch at Galveston. Our focus is largely on human immune cells and to a smaller extent on advanced non-human primate models. My training is in the fields of chemistry, molecular biology, immunology and chemical engineering. With regards to the current project, our lab has developed methods for molecular engineering of proteins and for studying their efficacy on different subsets of immune cells. We also have expertise in engineering enzymes for modifying their substrate specificity and catalytic activity, and in studying their biochemistry. In collaboration with clinicians at MD Anderson we are now applying these methodologies towards understanding single-cell functionality to improve immunotherapy.

B. POSITIONS AND HONORS

Positions and Employment

2007 - 2008 Post-doctoral fellow, University of Texas, Austin, TX
2008 - 2010 Post-doctoral fellow, Massachusetts Institute of Technology, Cambridge, MA
2010 - Assistant Professor, University of Houston, Houston, TX

Other Experience and Professional Memberships

2006- American Chemical Society
2010- American Institute of Chemical Engineers
2012- American Association of Cancer Research
2012- American Society for Biochemistry and Molecular Biology
2013- American Association of Immunologists

Honors

1998 Integrated PhD Fellowship, Indian Institute of Science
2011 New Faculty Award, University of Houston
2013 Stewart-Rahr Young Investigator Award, Melanoma Research Alliance
2013 Cullen College of Engineering Outstanding Teaching Award, University of Houston
2014 Cullen College of Engineering Outstanding Researcher Award, University of Houston

C. Contribution to Science

1. My graduate PhD work was dedicated to engineering the substrate specificity of enzymes. Working in the laboratories of Drs. Georgiou and Iverson, I developed a single cell methodology to enable the screening of population of bacterial cells for identifying rare variants displaying the engineered protein of interest. We implemented a flow cytometric assay to directly report on the catalytic activity of the *Escherichia coli* outer membrane protease, OmpT. We employed a dual substrate strategy, screening for the desired catalytic activity but also simultaneously counter-selecting against the parental substrate reactivity. We envisioned that this would be important for switching the substrate specificity as opposed to relaxing the substrate specificity, and presented a new paradigm in the enzyme engineering field. By constructing large libraries of OmpT variants, and by utilizing this flow cytometric assay, we were able to isolate OmpT variants with altered substrate specificity. These results were particularly impressive since we demonstrated the ability to alter the substrate specificity without compromising overall catalytic efficiency. Encouraged by our initial findings, we then systematically altered the substrate specificity of OmpT to recognize a wide variety of non-cognate substrates including post-translationally modified amino acids like nitro-Tyrosine and sulfo-Tyrosine. Furthermore, we reported the engineering of an OmpT variant that recognize the Glu-Arg sequence, a dipeptide sequence that is not recognized by any naturally occurring protease, thus enabling the design of synthetic specificities. This comprehensive engineering of protease substrate specificity onto a single parent scaffold without compromising catalytic efficiency represents a significant milestone in protease engineering.

    - Varadarajan N, Gam J, Olsen MJ, Georgiou G, Iverson BL (2005) 'Engineering of protease variants exhibiting high catalytic activity and exquisite substrate selectivity' *Proc Natl Acad Sci U S A.* 102:6855-60.
    - Varadarajan N, Rodriguez S, Hwang BY, Georgiou G, Iverson BL (2008) 'Engineering a Family of Highly Active and Selective Endopeptidases with Programmed Substrate Specificities' *Nature Chemical Biology* 4(5):290-4
    - Varadarajan N, Georgiou G, Iverson BL (2008) 'Engineered Proteases that Cleave Specifically after Sulfalted Tyrosine for the Detection of Post-Translationally Modified Peptides' *Angew Chem Intl Ed* 47(41):7861-3
    - Varadarajan N, Cantor J, Georgiou G, Iverson BL (2009) 'Construction and flow-cytometric screening of targeted enzyme libraries' *Nat Protoc* 4(6):893-901.
    - Varadarajan N, Pogson M, Georgiou G, Iverson BL (2009) 'Proteases that can distinguish among different posttranslational forms of tyrosine engineered using multicolor flow-cytometry' *J Am Chem Soc* 131(50):18186-90

2. My postdoctoral research at MIT with Dr. Christopher Love and Dr. Bruce Walker at MGH was focused on the development and implementation of high-throughput single cell assays for monitoring the functionality of immune cells in the context of HIV-1 infection. This assay allowed us to directly monitor the ability of a patient's immune cells to kill HIV-infected cells, at the single-cell level. By utilizing this assay, we demonstrated that the effector functions of $CD8^+$ T cells, secretion of IFNγ and cytotoxicity are independently regulated. This work highlighted the fact that the procedure for evaluating vaccine efficacy, at least in HIV-infection, is suboptimal. Next, we performed characterization of immune cells isolated from gut of HIV-infected patients. Applying this single-cell method to assess HIV-specific T-cell responses demonstrated that it is possible to establish clonal $CD8^+$ T-cell lines that represent the most abundant specificities present in circulation using 100- to 1,000-fold fewer cells than traditional approaches require and without extensive genotypic analysis a priori.

    - Varadarajan N, Julg B, Yamanaka YJ, Chen H, Ogunniyi AO, McAndrew E, Porter LC, Piechocka-Trocha A, Hill BJ, Douek DC, Pereyra F, Walker BD, Love JC (2011) 'A high-throughput single-cell analysis of human $CD8^+$ T cell functions reveals discordance for cytokine secretion and cytolysis' *J Clin Invest* Nov 1;121(11):4322-31.
    - Varadarajan N, Kwon DS, Law KM, Ogunniyi AO, Anahtar MN, Richter JM, Walker BD, Love JC (2012) *Proc Natl Acad Sci U S A.* 109(10):3885-90.

3. My lab here at UH is focused on combining molecular engineering and engineering single-cell methodologies for translational cancer immunotherapy, and for diagnosis and treatment of autoimmune diseases.

To enable the detection of autoreactive B cells in rheumatoid arthritis, we recently reported the utility of a high-throughput single-cell assay, microengraving, for the screening, characterization and isolation of anti-citrullinated protein antibodies (ACPA) from peripheral blood mononuclear cells (PBMC) of patients. Stimulated B cells were profiled at the single-cell level in a large array of sub-nanoliter nanowells ($\sim 10^5$), assessing both the phenotype of the cells and their ability to secrete cyclic-citrullinated peptide (CCP)-specific antibodies. Single B cells secreting ACPA were retrieved by automated micromanipulation, and amplification of the immunoglobulin (Ig) heavy and light chains was performed prior to recombinant expression to confirm specificity.

- Sendra V, Lie A, Romain G, Agarwal SK, Varadarajan N (2013) Detection and isolation of auto-reactive human antibodies from primary B cells. **Methods** Dec 1; 64(2):153-9. doi: 10.1016/j.ymeth.2013.06.018

In collaboration with the Georgiou group at UT Austin, we assisted the development of a single cell nanowell based methodology for the paired sequencing of antibody heavy and light chains from B cells.

- Dekosky BJ, Ippolito GC, Deschner RP, Lavinder JJ, Wine Y, Rawlings BM, Varadarajan N, Giesecke C, Dörner T, Andrews SF, Wilson PC, Hunicke-Smith SP, Willson CG, Ellington AD, Georgiou G (2013) High-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire. **Nat Biotech** Jan 20; 31(2):166-. doi: 10.1038/nbt.2492.

Chimeric antigen receptors (CAR) are synthetic molecules that typically combine the specificity and affinity of single-chain antibodies with selected intracellular signaling domains of the T-cell receptor complex. When expressed on genetically modified T cells, CARs redirect specificity independently of human leukocyte antigen to recognize tumor-associated antigens (TAA). Data from clinical trials infusing genetically modified T cells targeting B-cell leukemias have demonstrated dramatic responses, even in heavily pretreated patients. Despite these success however, responses are not universal and the mechanistic basis for the efficacy of the inoculum are poorly defined. We have recently reported Timelpase Imaging Microscopy In Nanowell Grids (TIMING) as a high-throughput methodology to enable the dynamic monitoring between CAR^+ T cells and NALM-6 tumor cells. We used the technology to compare the efficacy of $CD4^+$ CAR^+ T cells (CAR4 cells) and $CD8^+$ CAR^+ T cells (CAR8 cells) and demonstrated that the CAR4 cells kill slower than CAR8 cells likely due to decreased Granzyme B content. Furthermore, for both sets of T cells the fate was dependent on functional activation through multiple tumor cells. Our study demonstrated for the first time that like $CD8^+$ T cells, $CD4^+$ T cells can participate in multi killing events via simultaneous conjugation to multiple targets.

- Liadi I, Roszik J, Romain G, Cooper LJN, Varadarajan N (2012) 'Quantitative High-Throughput Single-Cell Cytotoxicity Assay for Chimeric Antigen Receptor T cells' **J Vis Exp** Feb 2;(72). doi:pii: 50058. 10.3791/50058.
- Liadi I, Singh H, Villamizar NR, Romain G, Merouane A, Kebriaei K, Huls H, Qiu P, Roysam B, Cooper LJ, Varadarajan N (2015) Individual motile $CD4^+$ T cells can participate in efficient multi-killing through conjugation to multiple tumor cells **Cancer Immunol Res** 2015 3(5); 1–10
- Liadi I, Singh H, Romain G, Roysam B, Cooper LJ, Varadarajan N (2015) Defining the potency of CAR+ T cells: Fast and furious or Slow and steady **Oncoimmunology** doi: 10.1080/2162402X.2015.1051298

The efficacy of most therapeutic monoclonal antibodies (mAbs) targeting tumor antigens results primarily from their ability to elicit potent cytotoxicity through effector-mediated functions. We have recently engineered the Fc region of the mAb targeting the leukemic cell antigen, CD33, and utilized TIMING to analyze NK cell mediated antibody dependent cell mediated cytotoxicity. We demonstrated that the Fc engineered antibodies allowed more NK cells to participate in killing and serial killing. Enhanced target killing also increased the frequency of NK cells undergoing apoptosis but this effect was donor dependent.

- Romain G, Senyukov VV, Villamizar NR, Merouane A, Kelton W, Liadi I, Mahendra A, Georgiou G, Roysam B, Lee DA, Varadarajan N (2014) Antibody Fc-engineering improves frequency and promotes kinetic boosting of serial killing mediated by natural killer cells **Blood** 2014 Nov 20;124(22):3241-9. doi: 10.1182/blood-2014-04-569061

D. RESEARCH SUPPORT

Ongoing Research Support

RP120241 (PI: Cooper) 12/01/2011-05/01/2015 0.25 summer
Cancer Prevention and Research Co-Investigator
Institute of Texas
T-Cell Therapy after Hematopoietic Stem Cell Transplantation
To generate tumor-specific T cells for infusion in clinical trials

1R01CA174385 (PI: Varadarajan) 09/01/2012-08/31/2016 3.0 summer
NIH/NCI/NIBIB/OD
Quantitative single-cell biomarkers of T-cells to optimize tumor immunotherapy
The objective of this proposal is to identify in vitro biomarkers of CD19-specific chimeric antigen receptor T cells

RP130570 (PI: Varadarajan) 12/01/2012-11/30/2014 3.0 academic
Cancer Prevention and Research
Institute of Texas
Single-cell biomarkers of clinical-grade T cells and NK cells to optimize tumor immunotherapy

272833 (PI : Varadarajan) 05/01/2013-04/30/2016 0.0
Melanoma Research Alliance
Quantitative single-cell biomarkers of melanoma immunotherapy

Completed Research Support

5U54AI057156-08 (PI: Walker) 03/01/2011-02/28/2013 1.0 summer
NIH/NIAID
Western Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research (WRCE)
Subproject: RP003 Project leader
Quantitative mapping of CD8+ T-cell responses during chikungunya vaccination
The objective of the proposal is to enable the quantitative mapping of the breadth and the functionality of CD8+ T-cell responses elicited in response to CHIK vaccination in *M. fascicularis* using a novel high-throughput assay
Overlap: None

E-1774 (PI: Varadarajan) 06/01/2011-05/31/2014 0.0
Welch Foundation
Engineering chymotrypsin to selectively cleave after phosphotyrosine
The objective of the proposal is to engineer the substrate specificity of chymotrypsin to recognize the post-translationally modified amino acid, phosphotyrosine by employing a novel flow-cytometric surface display assay.
Overlap: None