BIOGRAPHICAL SKETCH

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NAME: **Singhal, Ashutosh**

eRA COMMONS USER NAME (credential, e.g., agency login): ashutoshsinghal

POSITION TITLE: Medical Research Data Scientist

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Kurukshetra University, Kurukshetra, India	BS	07/2000	Microbiology, Botany, Chemistry
Gurukula Kangri University, Haridwar, India	MS	07/2002	Microbiology
Central Drug Research Institute, Lucknow, India	PhD	12/2010	Molecular Biology of Infectious Disease & drug target discovery
UNT Health Science Center, Fort Worth, TX	Postdoc	08/2014	Neurovirology & Molecular gene therapy
Meharry Medical College, Nashville, TN	Postdoc	01/2020	Neurobiology & Cancer Immunotherapy
NIH Clinical Center, NIH	Certificate	2018	Clinical Research
Nashville Software School, Nashville, TN	Certificate	05/2019	Data Science

A. Personal Statement

My long-term interests and motivation are to contribute into transforming people's lives by discovering and developing new diagnosis or prognosis methods and treatments to achieve a healthy community irrespective of one's social status. Having worked on drug discovery in particularly difficult areas such as neglected tropical infectious diseases, rare or orphan disease, cancer and CNS and I experienced that the chance of a lead molecule discovered in the lab making into the clinical trial is less than 10%; and the probability of success in clinical trial is even worse. Bearing in mind complex nature of human biology, our basic bench research should be more integrated with the patient's response as we gather information from clinical trial results; and the same way clinical trials should leverage the advancements in biomarker and companion diagnostics in the lab to improve success rate. Therefore, analysis and cross-validation of data from/between lab and clinic is critical in designing such studies. Further, every cue, we uncover during the disease progression together with interventions at clinic has the potential of developing as biomarker, diagnosis or a therapeutic target and therefore, we need to analyze them wisely from the point-of-view of lab scientist or it will be a missed opportunity. However, it is often challenging to utilize strengths of clinicians or basic scientist in data science; similarly, data scientists often lack an understanding of complex biology and drug discovery process--both of which are indispensable to answer a myriad of important biological and clinical questions.

I have amalgamated my years of basic and translational biomedical research experience with clinical research and data science to help and facilitate clinical and translational R&D that may translate into better patient care and health outcomes. My current role as the Director of Medical Research, Development and Strategy is also to design and implement most advance infrastructure and tools of health information technology so that we are able to integrate data from multiple and disparate sources such as electronic health records (EHR), in-silico biology, genomic technologies, medical devices, biosensors, and social and environment. Harnessing these data has tremendous potential at-- the *macro*, population-level health as well as at the *micro*, evidence-based precision medicine level. Therefore, my overreaching goal is to contribute to gaining insights from complex data from lab, clinic and clinical trials to identify signals that we can develop as biomarkers or diagnostic tools, discover new therapies or vaccine OR design strategies/tools to prevent disease, manage chronic diseases, improve patient care and outcomes thus improving community health and health equity.

B. Positions and Honors

Positions and Employment

2020 –	Director, Medical Research, Development and Strategy, School of Applied Computational Sciences (formerly: The Data Science Institute); MMC, Nashville, TN
2020 – 2020	Medical Research Data Scientist, The Data Science Institute, MMC, Nashville, TN
2014 - 2020	Postdoctoral Research Associate, Meharry Medical College (MMC), Nashville, TN
2010 - 2014	Postdoctoral Research Associate, UNT Health Science Center, Fort Worth, TX

2005 - 2009	Senior Research Fellow, Central Drug Research Institute, Lucknow
2003 - 2005	Junior Research Fellow, Central Drug Research Institute, Lucknow

Other Experience and Professional Memberships

2014 - 2015	Associate Member, American Society of Biochemistry and Molecular Biology
2014 - 2015	Member, American Heart Association
2014 -	Associate Member, American Association for Cancer Research
2010 - 2014	Regular Member, Society for Neuroimmune Pharmacology
2007 -	Life Member, Indian Society for Parasitology

Honors

2017	Best poster presentation, 1st Prize, Vanderbilt Ingram Cancer Center
2016	Judge-Abstract reviewer, Annual Biomedical Conference for Minority Students-Tampa, FL
2016	Judge, 60th Annual Student Research Day, MMC, Nashville, TN
2013	Invited Panelist, Bio-Career Development Symposium, Fort Worth
2013	Early Career Investigator Travel Award, Society on Neuroimmune Pharmacology
2013	Arthur Falek Young Investigator Award, Society on Neuroimmune Pharmacology
2012	Judge, The 7th Annual Texas Conference on Health Disparities, Fort Worth
2012	Early Career Investigator Conference Award, Society on Neuroimmune Pharmacology
2011	Best Poster Award, 2nd Prize, UNT Health Science Center
2011	Judge, North Texas High Undergrad and School Research Symposium, Fort Worth
2010	Vice-President, Post-doctoral Research Association, UNTHSC, Fort Worth
2007	Prof. M.B. Mirza Award, Indian Society for Parasitology
2005	Senior Research Fellowship, Council of Industrial Research
2002	Junior Research Fellowship, Indian Council of Medical Research
2002	Junior Research Fellowship, Council of Industrial Research

C. Contribution to Science

Projects/apps published available online

1. Cancer and Determinants of Health in the Southern USA: Found that over 75% of counties in southern US have disproportionally higher rate of cancer deaths compared to the national average. Created an interactive county level map of cancer burden to visualize and gain insight. Developed machine learning models and identified key socio-economical and health behavioral factors associated with greater burden of cancer in counties.
Link of Repository: https://github.com/Ashutosh-Singhal/Cancer Social health determinants/

2. Does Health Insurance Status/Type Affect Early Diagnosis of Cancer? Analyzed data of all cancer types from 2007 to 2016 and found that patients with no insurance or government-sponsored insurance had higher percentage of late-stage diagnosis than patients with private insurance. Private insurance provided better chance of early detection of cancer; however, with launch of Affordable Care Act (ObamaCare), early diagnosis of cancer increased by ~10% with government-sponsored insurance.
Link of app: http://ashutosh-singhal.shinyapps.io/cancerstage_insurancetype

3. Opioid Crisis: This project sought to visualize the problem as it relates to opioid prescriptions. Analyzed the prescribing habits of a sample of 25,000 American doctors in 2014. We searched for insights and answers to how prescription opioids are linked to opioid deaths.
Link of app: https://ashutosh-singhal.shinyapps.io/OpioidCrisis_Shiny_App/

Recent publications:

a. Davogustto G. et al. High Rate of Arrhythmia Diagnoses Following Return of Pathogenic/Likely Pathogenic Variants in an Unselected Population. Circulation. 2020;142:A14663. (co-author)
b. Glazer AM. et al. Association of Incidental Rare Variants with Arrhythmia Phenotypes in 24,410 Individuals. ASHG conference 2020. (co-author)

Biomedical Research

1. Niemann-Pick type C (NPC) disease: A rare genetic disorder in children
NPC disease is caused by mutation in NPC gene defecting the trafficking of cholesterol and its massive accumulation in lysosomes of cells. We investigated the mode of action of HPBCD (2-hydroxypropyl-beta-

cyclodextrin) known to alleviate the symptoms in animal model and is currently in phase 2b/3 clinical trial. In our screening we found another cyclodextrin (CD) derivative, HPGCD (2-hydroxypropyl-gamma-cyclodextrin) showing the similar efficacy. It is interesting as unlike HPBCD, HPGCD did not show cholesterol binding affinity. Using a proteomic approach. We found that HPBCD or HPGCD treatment modulate the expression of most NPC-specific proteins including lysosome-associated membrane protein 1 (LAMP-1). Our gene knock out and over expression strategy confirmed LAMP-1 role in cholesterol trafficking. Confocal and electron microscopy showed that LAMP-1 augment lysosomal-ER connections, which are needed for cholesterol efflux or metabolized in the cells. Together, this study may help in developing novel therapeutic approaches for about 50 lysosomal storage diseases including NPC.

c. **Singhal A**, Krystofiak ES, Jerome WG, Song B. 2-Hydroxypropyl-gamma-cyclodextrin overcomes NPC1 deficiency by enhancing lysosome-ER association and autophagy. **Sci Rep**. 2020 May 26;10(1):8663. PubMed PMID: 32457374.
d. **Singhal A**, Szente L, Hildreth JEK, Song B. Hydroxypropyl-beta and -gamma cyclodextrins rescue cholesterol accumulation in Niemann-Pick C1 mutant cell via lysosome-associated membrane protein 1. **Cell Death Dis**. 2018 Oct 3;9(10):1019. PubMed PMID: 30282967.
e. Szente L, **Singhal A**, Domokos A, Song B. Cyclodextrins: Assessing the Impact of Cavity Size, Occupancy, and Substitutions on Cytotoxicity and Cholesterol Homeostasis. **Molecules**. 2018 May 20;23(5)PubMed PMID: 29783784.

2. Targeting Macrophages for cancer therapy:
Macrophages play critical role in tumor-associated inflammatory response, tumor establishment and invasion. Extensive engulfment of cellular debris during initial stage of tumor development may invoke proteotoxic shock in these cells, which in turn elevates Alu RNA, a non-coding RNAs. Alu RNA induces activation of NLRP3-inflammasome in macrophages and resulting inflammatory responses bring collateral death to the breast tumor cells. However, our study indicates that MMP-1 secreted from aggressive breast tumor cells downregulates AluRNA in tumor-associated macrophages. We found that MMP-1 activates proteases activating-receptor (PAR)-1 on activated macrophages, which lead to increase of cytosolic Ca2+ and calpain activation and degradation of TFIIIC110 subunit essential for Pol III-mediated Alu RNA synthesis. The decreased levels of Alu RNA biosynthesis fail to induce inflammatory response and cell death in macrophages. Therefore, we proposed that aggressive breast cancer cells manipulate tumor-associated macrophages by decreasing AluRNA and thereby tame the macrophages and keep them alive, critical for cancer cells to get growth factors and invasion.
Combinatorial adoptive cell immunotherapy for solid tumors:
Tumor-induced immune tolerance poses a major challenge for therapeutic interventions aimed to manage cancer. We found that treatment with a reversible proteasome inhibitor “Bortezomib” overcome T-cell suppression in murine breast and kidney adenocarcinomas, and lung fibrosarcoma expressing immunogenic antigens in mice. In bortezomib-treated 4T1HA tumor-bearing mice, CD4+T-cells showed increased IL-2 production, CD11c+ dendritic cells showed increased IL-12 and IL-15 production, and HA-specific activated CD8+T-cells showed enhanced expression of IFNγ, granzyme-B and transcription factor eomesodermin. Furthermore, bortezomib-treated CD8+T-cells showed increased phosphorylation of STAT5, mitogen-activated protein kinase p38, and Akt, which was abrogated by phosphatidylinositide 3-kinase (PI3K) inhibitor. These data support the therapeutic potential of bortezomib in conjunction with other immunotherapies to augment the strength of convergent signals from CD8+T-cell signaling molecules including TCR, cytokine receptors and downstream PI3K/Akt/STAT5 pathways to sustain CD8+T-cell effector function in the tumor microenvironment.

a. Pellom ST Jr, **Singhal A**, Shanker A. Prospects of combining adoptive cell immunotherapy with bortezomib**.** **Immunotherapy**. 2017 Mar;9(4):305-308. PubMed PMID: 28303766.
b. Pellom ST Jr, Dudimah DF, Thounaojam MC, Uzhachenko RV, **Singhal A**, Richmond A, Shanker A. Bortezomib augments lymphocyte stimulatory cytokine signaling in the tumor microenvironment to sustain CD8+T cell antitumor function. **Oncotarget**. 2017 Jan 31;8(5):8604-8621. PubMed PMID: 28052005.
c. **Singhal A**, Misra S, Chaudhuri G. Autocrine regulation of stress-induced cytotoxic Alu RNA expression in TNBC cells. American Association of Cancer Research Conference; 2015; Philadelphia, PA, USA. **Cancer Res**. 2015. 75 (15 suppl); 236.
d. **Singhal A**, Misra S, Chaudhuri G. Cancer cell-induced suppression of cytotoxic Alu RNA biosynthesis in the co-cultured macrophages. ASBMB Experimental Biology Conference; 2015; Boston, MA, USA. **The FASEB Journal**. 2015. 29 (1) Suppl: 578.10 (Selected for Talk).

3. Antioxidant Nanoparticles:
Oxidative stress is a common mechanism of neurodegenerative diseases and acute brain injury such as stroke. Disruption of blood supply to brain generate high levels of hydrogen peroxide (H2O2) and causes brain injury. We used H2O2-degrading enzyme, CATALASE enzyme, to formulate catalase-PLGA nanoparticles (NanoCAT). We found that NanoCAT alleviated cell death in human neurons and astrocytes and facilitated the repair. We formulated these nanoparticles surface with recombinant HIV-1 capsid protein to increase the delivery across

the blood-brain-barrier in CNS. NanoCAT is a promising therapeutic option to be developed as an antioxidant therapy for stroke as well as neurodegenerative disorders.

Neuro-AIDS project:

We demonstrated that during the HIV-1 infection in the brain, astrocytes secrete a protein known as tissue inhibitor of metalloproteinases-1 (TIMP-1), which protects neurons from HIV-1-associated neurotoxicity. We found that chronic inflammation decreases TIMP-1 expression levels and may leads to neurotoxicity. We investigated potential TIMP-1 receptor on neurons and underlying signaling pathways. Taking a translational approach, we constructed a TIMP-1 gene therapy vector specific to astrocytes and loaded to targeted-nanoparticles for gene delivery to CNS. We developed a novel arginine-polymer in this process for optimal gene delivery, which was not feasible with conventional delivery options. This work had significant translational potential in HIV-1-associated neurodisorders.

Alzheimer's Diseases (AD):

We found that high levels of cytokine CXCL8 in Alzheimer patient's brain tissue. We investigated role of CXCL8 in AD pathogenesis—we demonstrated neurons can produce CXCL8 when they are exposed to Aβ and/or pro-inflammatory cytokines, and CXCL8 inhibited Aβ-induced neuron death by increasing neuronal brain-derived neurotrophic factor (BDNF). We found that CXCL8 protects neurons possibly by paracrine or autocrine loop and regulates neuronal functions, therefore, may play a protective role in the AD pathogenesis.

a. **Singhal A**, Morris VB, Labhasetwar V, Ghorpade A. Nanoparticle-mediated catalase delivery protects human neurons from oxidative stress. **Cell Death Dis**. 2013 Nov 7;4:e903. PubMed PMID: 24201802.
b. **Singhal A**, Ghorpade A. A Nanotechnology approach to protect human neurons from oxidative stress. 19th SNIP Scientific Conference; 2013 April 03; San Juan, Puerto Rico, USA. **J Neuroimmune Pharm**. 2013. 8 (2), 382. (Selected for Talk).
c. **Ashutosh**, Chao C, Borgmann K, Brew K, Ghorpade A. Tissue inhibitor of metalloproteinases-1 protects human neurons from staurosporine and HIV-1-induced apoptosis: mechanisms and relevance to HIV-1-associated dementia. **Cell Death Dis**. 2012 Jun 28;3:e332. PubMed PMID: 22739984.
d. **Ashutosh**, Kou W, Cotter R, Borgmann K, Wu L, Persidsky R, Sakhuja N, Ghorpade A. CXCL8 protects human neurons from amyloid-β-induced neurotoxicity: relevance to Alzheimer's disease. **Biochem Biophys Res Commun**. 2011 Sep 9;412(4):565-71. PubMed PMID: 21840299.

4. Development of drug screening assay:

We developed a high-throughput screening (HTS) assay system to discover novel drugs against Leishmaniasis. This assay helped us to screen chemical libraries generated through computational modeling, docking and combinatorial chemistry. We genetically engineered Leishmania donovani parasite producing bioluminescence even when it is inside of human macrophage. We used parasite life stage-specific genetic elements to engineer the expression vector. Using these cell lines, we established a high-throughput screening assay and made it available as Institutional Service for routine screening. Since then this assay has been helping scientists in finding new chemical compounds effective against Leishmania disease. Several hundred compounds have been screened together with Drug for Neglected Disease Institute (DNDi) and CDRI and identified many lead molecules.

a. **Ashutosh**, Goyal N. Bioluminescent Leishmania donovani: Use in drug discovery. 17th National Congress of Parasitology", October 24-26; 2006; Dibrugarh, Assam, India (**Nominated for Young Scientist Award*).
b. Agarwal A, Ramesh, **Ashutosh**, Goyal N, Chauhan PM, Gupta S. Dihydropyrido[2,3-d]pyrimidines as a new class of antileishmanial agents. **Bioorg Med Chem**. 2005 Dec 15;13(24):6678-84. PubMed PMID: 16126395.
c. **Ashutosh**, Gupta S, Ramesh, Sundar S, Goyal N. Use of Leishmania donovani field isolates expressing the luciferase reporter gene in in vitro drug screening. **Antimicrob Agents Chemother**. 2005 Sep;49(9):3776-83. PubMed PMID: 16131481.
d. Chandra N, Ramesh, **Ashutosh**, Goyal N, Suryawanshi SN, Gupta S. Antileishmanial agents part-IV: synthesis and antileishmanial activity of novel terpenyl pyrimidines. **Eur J Med Chem**. 2005 Jun;40(6):552-6. PubMed PMID: 16003841.

5. Identifying the drug resistance mechanisms:

To identify the real mechanism of clinical drug resistance in Leishmaniasis we went to endemic area of India and collected patient samples. We found that parasites isolated from patients who were unresponsive to pentavalent antimonial drug were resistant to active form of the drug--trivalent antimony (SbIII), which confirmed a parasite's resistant phenotype. We compared the gene expression between drug resistant and sensitive strains using DNA microarray and identified potential genes involved in drug resistance. One of the gene was identified as a novel MAP-Kinase1 and sensitized the resistant parasite upon over expression. Therefore, we identified a novel gene responsible for Leishmania drug resistance phenotype in clinic.

a. **Ashutosh**, Sundar S, Goyal N. Molecular mechanisms of antimony resistance in Leishmania. **J Med Microbiol**. 2007 Feb;56(Pt 2):143-53. PubMed PMID: 17244793.

b. Mittal MK, Rai S, **Ashutosh**, Ravinder, Gupta S, Sundar S, Goyal N. Characterization of natural antimony resistance in Leishmania donovani isolates. **Am J Trop Med Hyg**. 2007 Apr;76(4):681-8. PubMed PMID: 17426170.
c. **Ashutosh**, Garg M, Sundar S, Duncan R, Nakhasi HL, Goyal N. Downregulation of mitogen-activated protein kinase 1 of Leishmania donovani field isolates is associated with antimony resistance. **Antimicrob Agents Chemother**. 2012 Jan;56(1):518-25. PubMed PMID: 22064540.

List of Published Work in My NCBI Bibliography:

https://www.ncbi.nlm.nih.gov/myncbi/ashutosh.singhal.1/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

I have served as lead key research personnel to following extramural grants:

Ongoing Research Support

PCORI, PCORnet®
The National Patient-Centered Clinical Research Network
Conduct health research, creating a large, highly representative network for conducting clinical outcomes research.
Role: Co-Investigator and Data Governance

COVID-19 Vaccine Hesitancy and Health Equity
Predictive modeling of social determinants of health and socially vulnerable index to investigate COVID-19 vaccine hesitancy and assessing health policy
Funded by Blue Cross Blue Shields of Tennessee (BCBST)
Role: Co-Investigator

Completed Research Support

07/12/13-06/30/17	**Defining the effects of bortezomib on NK cell activation in cancer**. 1SC1CA182843-01A1, NIH/NCI/NIGMS; Dr. Anil Shanker (PI); Role: Research Associate
08/05/14-07/31/16	**Manipulation of macrophage Alu RNA metabolism by breast cancer cells.** 1R21CA181920-01, NIH/NCI; Dr. Gautam Chaudhuri (PI); Role: Postdoc
02/01/05-04/04/19	**Neuronal Survival, HIV-1 and Astrocyte-TIMP-1.** 1R01 NS048837, NIH/NINDS; Dr. Anuja Ghorpade (PI); Role: Postdoc
06/01/11-05/31/16	**Stroke Therapy (Development of Antioxidant Nanoparticles).** 1R01 NS070896, NIH/NINDS; Dr. Vinod D. Labhasetwar (PI); Role: Postdoc