Details of Research Work

My lab at inStem is developing innovative biomaterials and chemical biology approaches to solve unmet clinical needs. My lab has adopted innovative conceptual approaches, bridging chemistry and biology to develop clinically translatable technologies. While our contributions to discovery-based research are evidenced in our publications, our laboratory has been motivated by the development of technologies to address unmet clinical needs. My lab strength lies in taking established chemistry and bringing it to address a challenging and open question in the biological/clinical space.

Some of the outcomes of my lab's endeavors in the area of fundamental and translational research are as follows: My lab recently developed electrospun nanofibrous sheets-based technology as a novel blood bag to scavenge damage-associated molecular patterns to enhance the quality and shelf-life of stored blood (***Nature Communications*** 2022, *13,* 7394). Furthermore, my lab has developed a prophylactic topical gel (***Science Advances*** 2018, *4*, eaau1780), and protective clothing as a medical textile (***unpublished***), with the aim to protect farmers from pesticide-induced toxicity and lethality, which is of particular relevance in the agricultural community. Additionally, we have seamlessly adopted the "biomimicry-based drug discovery" method and developed new chemical entities (NCEs) as potential drugs for the treatment of Inflammatory Bowel Diseases (IBD) (***Nature Communications*** 2019, *10*, 89). Thus far, approaches for the treatment of IBDs have been palliative and directed at inflammation management or immune suppression. My approach, which holds the promise of repair of gut barrier dysfunction, has been regarded as an innovative advance in the field. We have also developed self-assembled hydrogels-based biomaterials for clinical applications such as preventing the rejection of transplanted organs (***Science Translational Medicine*** 2014, *6*, 249ra110). While developing technologies for solving clinical needs, in parallel, I am interested in carrying out fundamental research to understand molecular behavior. For example, along with collaborators, we have systematically studied the basic principles of chemical fuel-driven living and transient supramolecular polymerization (***Nature Communications*** 2019, *10*, 450). Other than these research goals, I am also interested in the career development of next-generation bioengineers to work at the forefront of regenerative medicine and clinical translational research.

My research vision is "Innovation with a societal impact"

1. **Developing 'first-in-class' new chemical entities for the treatment of inflammatory bowel diseases:**

IBD consisting of Crohn's and Ulcerative Colitis are resultant of dysregulation of the immune system leading to intestinal inflammation and microbial dysbiosis. Gut barrier integrity is maintained by the tight junction proteins such as claudins (Cldn), Zona occludin-1 (ZO1), and occludin (Ocln) that are critical for epithelial cell barrier functions. It is known that levels of tight junction proteins are significantly down regulated under IBD conditions leading to increased gut permeability to microbial ligands and noxious metabolites resulting in systemic inflammatory responses. However, thus far, all treatment methods are using anti-inflammatory agents and immunosuppressants. Therefore, the current drugs can only manage the disease but do not cure. We hypothesized that restoring the gut barrier function by repairing the damaged epithelial layer by inducing the tight junction proteins (TJPs) will have enhanced efficacy in the treatment of IBDs.

Microbiota and their metabolites are in close proximity to the gut epithelium that constitutes a single cell-layer separating host components from the external environment. Consumption of diets containing berries and pomegranates have demonstrated significant beneficial effects on human health. It has been suggested that further downstream metabolites of ellagic acid (EA) known as 'urolithins' generated by gut microbiota render potential health benefits, in vivo. Among urolithins, Urolithin A (UroA) displayed potent anti-inflammatory, anti-oxidative and anti-ageing properties compared to other metabolites. Thus, not only the consumption of diets enriched in polyphenols is required but also the presence of microbes that convert them into beneficial metabolites is critical for manifestation of their health effects. At this time, the targets or pathways through which such microbial metabolites regulate physiological processes are largely unknown. Therefore, we have systematically investigated the role of UroA in providing beneficial effects and identified a novel mechanism in which UroA exhibits its efficacy. Briefly, we examined the activities of UroA identified that in addition to the anti-inflammatory activities, this compound strongly enhanced the gut barrier function. We demonstrated that UroA enhances barrier function by inducing tight junction proteins through activating aryl hydrocarbon receptor (AhR)-nuclear factor erythroid 2–related factor 2 (Nrf2)-dependent pathways (Figure 1).

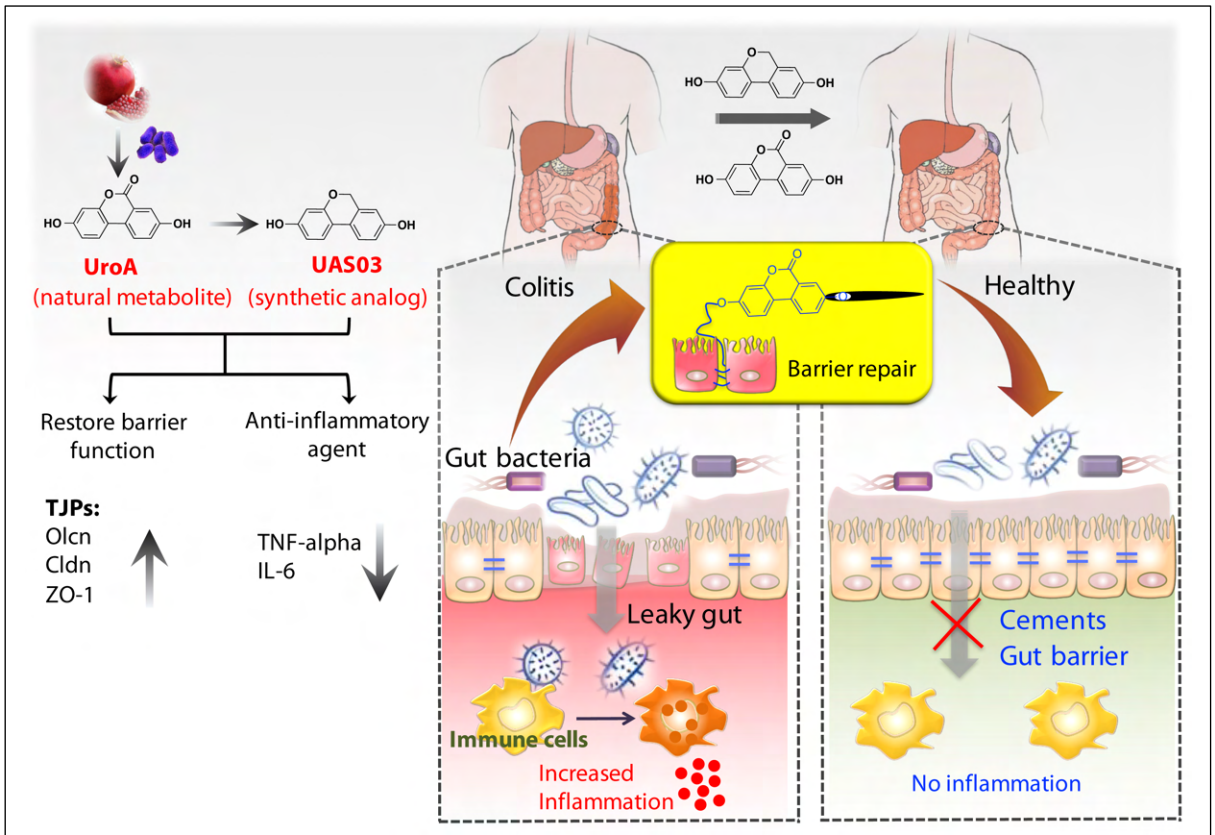


Figure 1: A schematic picture of protection of gut barrier function using urolithins. Gut microbes produce metabolite Urolithin A (UroA) from pomegranate rich-diet, and a synthetic analog has been developed. Both molecules enhance barrier function through overexpression of tight junction proteins, and they act as anti-inflammatory agents.

UroA has a lactone that connects two mono-hydroxyl phenyl rings leading to a planar structure. Gastric pH or digestive enzymes can hydrolyze the lactone ring, which opens the ring resulting in the loss of the planar structure and potentially its activities. To generate more stable and potent compounds, we synthesized non-hydrolyzable cyclic ether derivative, UAS03. The stability studies showed that UAS03 indeed is stable at gastric pH and also in the presence of gastric enzymes e.g., esterases and proteases.

Anti-inflammatory activities of UroA and UAS03 were examined in vivo in a LPS-induced peritonitis mouse model. UroA or UAS03 treatment significantly reduced the LPS-induced increase in serum IL-6 and TNF-α levels. Our results suggest that UAS03 is a potent structural analogue of UroA with increased anti-inflammatory activities. Our studies highlight the critical requirement for AhR-Nrf2 in protecting from barrier dysfunction. It is possible that UroA/UAS03 are exerting colitis protective activities by two pronged mechanisms of action. These compounds directly act on immune cells (e.g., macrophages) to prevent LPS/bacterial induced inflammation as well as exhibit anti-oxidative activities through AhR-Nrf2 pathways. Most importantly, these metabolites have direct impact on gut epithelium and gut barrier function by upregulating tight junction proteins. Enhanced barrier function reduces the bacterial leakage in the gut leading to significant reduction in systemic inflammation (***Nature Communications*** 2019, *10*, 89). Overall, UroA/UAS03 will not only be efficacious in IBD-related diseases but may also have significant translational implications in other disorders involving barrier dysfunction and inflammation such as alcohol liver diseases, neurological disorders, and colon cancers.

Based on this work, Vemula co-founded a spin-off company, Artus Therapeutics, Inc. Furthermore, Vemula has done three rounds of medicinal chemistry to identify four lead compounds that exhibited optimal safety and toxicity profile in IND-enabling study. Based on this invention, Vemula's team will be filing Investigative New Drug (IND) application to the US-FDA to start the Phase I clinical trials in coming years. If their lead compound show promising results in clinical trials, it will impact >250 millions of IBD patients, worldwide.

For developing this technology, thus far, we have not received any awards/fellowships.

Additionally, multiple other inventions from my lab have potential to make societal impact. A couple of them described here.

2. **Developing prophylactic dermal gel to deactivate pesticides to prevent pesticide-induced toxicity and death:**

Farmers are the backbone of India, and providing "first-in-class" technologies to protect their health from pesticide-induced lethality is an impactful contribution to society.

To understand the awareness of agricultural workers about pesticide-induced toxicity and the lethal effects on the ground, Vemula's team interacted with >200 farmers and their families and visited their agriculture fields from 60 villages in Telangana State. Their interactions revealed that they were aware of pesticide-induced toxicity, as they experience pain/toxic effects right after spraying pesticides in the field. However, the suffering is not alleviated because of the lack of protective technologies. Inspired by these interactions and awareness of the extent of the burden, Vemula's team has developed the prophylactic skin gel with the singular aim to prevent pesticide exposure during spraying. Pesticide exposure damages neuro-muscular function; therefore, farmers would not be able

to do fieldwork productively. The prophylactic technologies developed by Vemula's lab might be able to protect millions of farmers from pesticide-induced toxicity/lethality and enable farmers to conduct their fieldwork efficiently.

A platform technology with enormous potential to address a major unmet clinical need, developed in Vemula's lab is described here. This has particular significance for the agricultural farming community in India. The use of organophosphate-based pesticide sprays is widespread and the mainstay of the farming community, which nonetheless constitutes a health hazard for the farmers following inhalation or contact through nasal and dermal routes, respectively. Exposure to pesticides while spraying leads to a plethora of toxic effects, including neuronal toxicity, loss of neuromuscular function, paralysis and death. For example, a worrying statistic in 2017 alone, in one district in India, pesticide spraying led to over 60 deaths, 25 cases of lost vision, and >1000 hospitalizations. (http://www.livemint.com/Politics/YtxwY9FhCdap9ra8aBWx4J/Vidarbha-pesticide-deaths-Probe-panel-may-file-report-this.html and http://www.thehindu.com/news/national/pesticides-cause-farmer-deaths-illnesses-in-yavatmal/article19803158.ece)
This incident is just one example that demonstrates the severity of the unmet need. Existing personal protective equipment such as plastic gowns are not user-friendly to use in open fields under the sunlight, and there is an urgent requirement for safe and effective alternative approaches to protect farmers from this toxicity. Furthermore, there is no existing technology to prevent pesticide exposure in farmers.

Therefore, to solve this unmet need, Vemula's lab at inStem has developed a nucleophilic polymer-based topical hydrogel, which can be applied to the skin like a cream. This cream chemically deactivates pesticides through nucleophile-mediated hydrolysis to prevent pesticide-induced toxicity and mortality, *in vivo* (Figure 2). Additionally, his team has adapted this further to generate an active cloth, which could be made into a bodysuit and a facial mask to prevent pesticide-exposure through dermal and inhalation routes, respectively. Vemula's lab has demonstrated in animals that the presence of a thin layer of nucleophilic *poly*-Oxime hydrogel or wearing active protective cloth drastically prevented pesticide-induced neuro-muscular dysfunction, loss of endurance, and locomotor coordination. Therefore, it may protect farmers from losing their productive fieldwork. A 100% survival is observed in rats following topical pesticide administration in the presence of *poly*-Oxime hydrogel. Such a prophylactic gel and protective cloth may, therefore, are likely to be of enormous impact in preventing pesticide-induced toxicity and mortality (***Science Advances*** 2018, ***4***, eaau1780).

Preclinical toxicity and safety studies revealed that the topical gel is entirely safe and could be tested on humans. Based on this technology, inStem's spin-off company has been formed in Bangalore (Sepio Health Pvt. Ltd.) Through this company, further development is being done to commercialize the technology. The team is engaging with the Central Drug Standard Control Organization (CDSCO) to initiate the human trials at the earliest.

Because of its societal impact, this invention has been recognized by *Gandhian Young Technologist Award-2019* (by SRISTI-BIRAC, Biotechnology Industrial Research Assistance Council), and *DBT-Product, Process, Technology Development and Commercialization Award 2020* (Department of Biotechnology, Govt of India).

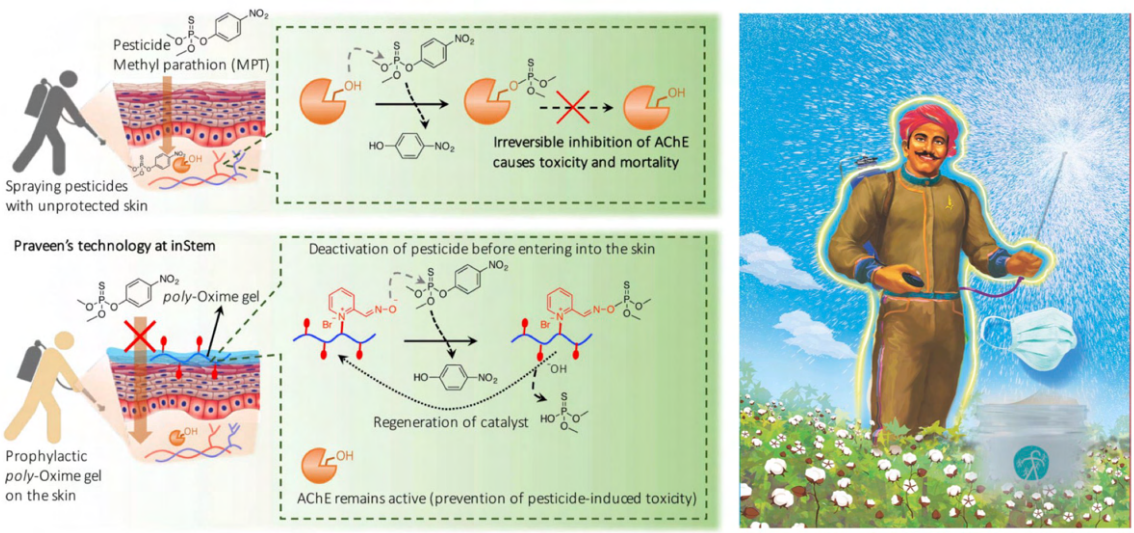


Figure 2: ***Left*** Schematic of pesticide penetration through the skin, which leads to the inhibition of Acetylcholinesterase (AChE) enzyme. AChE inhibition causes neuronal dysfunction, toxicity, and death. A topical gel that was developed in Vemula's lab can be applied to the skin, which deactivates pesticides, therefore prevents their entry into the body. Hence, this gel can protect from pesticide-induced toxicity and mortality. ***Right:*** Schematic of protective topical hydrogel and an active clothing, which have the ability to deactivate pesticide to protect farmers from pesticide-induced toxicity.

3. Developing novel quaternary ammonium salts based Germicidal Fabric to prevent infections:

When India was entering a lockdown to contain the spread of the novel coronavirus, SARS-CoV-2, the Department of Biotechnology and its autonomous institutes have encouraged scientists to develop technologies to contribute to the global response to the impending COVID-19 crisis. At inStem, Vemula lab has immediately started developing potential germicidal molecules to prevent the virus spread/infection. The pandemic forced people to wear masks to reduce the virus spread. However, the current masks, including cloth, surgical and N95 masks, are efficient in filtering viruses but do not deactivate viruses. Recently studies have shown that viruses including SARS-CoV-2 can remain active on various surfaces, including on these masks, for up to seven days, which poses the potential risk of infection while touching the masks. Therefore, Vemula lab has developed novel chemicals based on the quaternary ammonium salts (QAS) that can efficiently interact and rupture the membrane of the enveloped viruses & bacteria to deactivate them. They have developed an efficient process to immobilize these novel antiviral QAS on the fabric, hence the genesis of germicidal fabric technology. Germicidal technology has shown a 99.99% kill rate against a wide range of enveloped viruses, such as lentivirus, Sendai virus, and human respiratory viruses, including COVID-19 causing coronavirus (SARS-CoV-2) and influenza virus (H1N1 and H5N5 flu) and against various harmful bacteria, including gram-negative and gram-positive bacteria. Additionally, Vemula has closely engaged with Color Threads, licensee of germicidal fabric technology, to facilitate the industrial-scale production of antiviral molecules and re-usable antiviral masks. In a short time, these masks have reached millions of people.

By recognizing the impact of this technology, *the American Chemical Society* has selected this technology as one of the few impactful technologies developed worldwide in response to the COVID-19 pandemic. They have published a detailed article in ACS Central Science (***ACS Cent. Sci.*** 2020, *6*, 1469–1472).

Based on this technology, a medical textile company, Color Threads Pvt Ltd was formed. The technology has been licensed to the company from inStem. In November 2020, based on this technology, affordable and reusable antiviral face masks (G99+ antiviral), antiviral apparel (hoodies, track- suits and athleisure) have been launched in the market. Additionally, a partnership was formed with Aditya Birla Fashion and Retail Limited (Van Heusen), and launched wide range of antiviral apparel. During the peak of pandemic, these antiviral products have been used by millions of people to prevent spread potential infections.

In recognition of the societal impact of germicidal fabric technology, the Department of Biotechnology, Government of India has given prestigious National Biotechnology Innovation Award-2023 to Praveen Vemula.

4. A novel blood bag technology to enhance the quality and shelf-life of stored blood:

Blood transfusion is often a lifesaving practice for patients in the intensive care unit (ICU), where ~50-70% of ICU patients are transfused with blood units during their stay**.** Typical indications for blood transfusion include sickle cell crisis, anaemia, and severe blood loss. While RBC transfusion has therapeutic benefits, transfusion of storage-aged RBCs may cause deleterious effects on the recipients. Upon storing RBCs, a wide range of damage-associate molecular patterns (DAMPs), such as cell-free DNA, nucleosomes, free-hemoglobin, and poly-unsaturated-fatty-acids are generated. DAMPs can further damage RBCs; thus, the quality of stored RBCs declines during storage and limits their shelf-life. Thus far, previous studies have focused on improving storage conditions through alternative cryopreservation protocols, anaerobic storage, and using additives/rejuvenation solutions. Although these approaches have shown encouraging results, till now, no efforts have been made to scavenge DAMPs to prevent damage to RBCs. Therefore, efficient technologies to scavenge the spectrum of DAMPs and further reduce their formation in stored RBCs remain an unmet need. Hence, our lab has been focused on developing nanomaterial-based technologies to scavenge DAMPs to enhance the quality of stored RBCs. Since these DAMPs consist of positive or negative charged species, we developed taurine and acridine containing electrospun-nanofibrous-sheets (*Tau-Acr*NFS), featuring anionic, cationic charges and a DNA intercalating group on their surfaces. We showed that *Tau-Acr*NFS are efficient in scavenging DAMPs from stored human and mouse RBCs *ex vivo* (***Nature Communications*** 2022, *13,* 7394). We find that intermittent scavenging of DAMPs by *Tau-Acr*NFS during the storage reduces the loss of RBC membrane integrity and discocytes-*to*-spheroechinocytes transformation in stored old RBCs. We performed RBC-transfusion studies in mice to reveal that intermittent removal of DAMPs enhances the quality of stored-old-RBCs equivalent to freshly collected RBCs, and increases their shelf-life by ~22%. Such prophylactic technology may lead to the development of novel blood bags or medical device, and may therefore impact healthcare by reducing transfusion-related adverse effects (Figure 3).

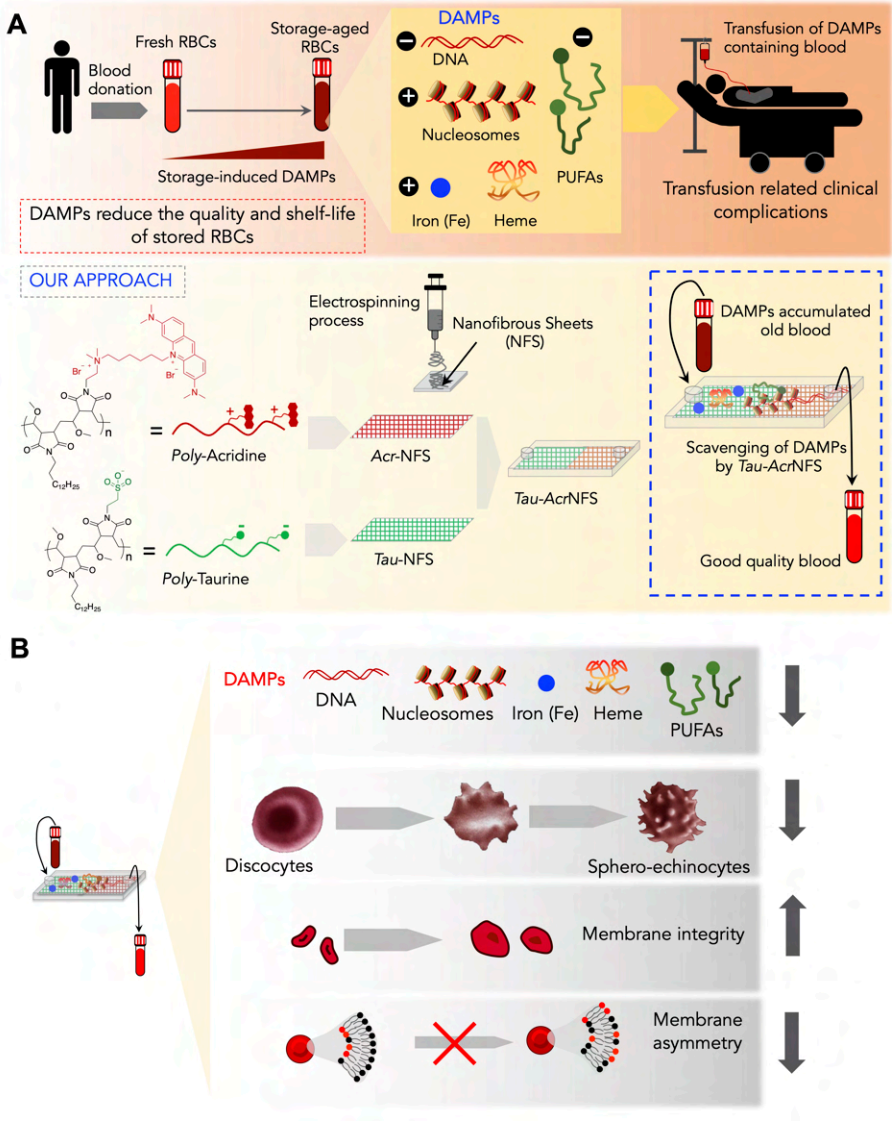
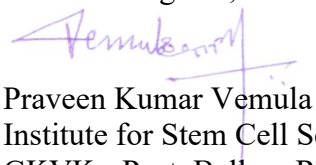


Figure 3: A) Stored RBCs produce damage-associated molecular patterns (DAMPs). Transfusion of DAMPs containing RBCs could lead to transfusion-related complications, including systemic inflammation and organ injury. *In our approach*, charge-bearing electrospun nanofibrous sheets (*Tau*-NFS and *Acr*-NFS) were prepared to scavenge charged DAMPs from stored RBCs. **B)** Scavenging of DAMPs remarkably enhanced the quality and shelf-life of stored RBCs by reducing DAMPs, transformation of discocytes to sphero-echinocytes and membrane asymmetry, while increasing the membrane integrity.

These are some of the examples of my laboratory contributions in the field of drug discovery, drug delivery and translational research.

I hereby confirm that based above give details of research is true. Hence, submitting the nomination for this work is the Sun Pharma Science Foundation Research Award 2023.

With best regards,



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