Excellence in research work for which the Sun Pharma Research Award is claimed

The nominee Prof. Benu Brata Das, has made outstanding contributions in the area of DNA repair and genome stability, which advances our understanding of genomic alterations underpinning carcinogenesis and neurodegeneration. The main focus of the nominee is to understand the fundamental role of DNA topoisomerases 1 (Top1) both in the nucleus and mitochondria during DNA replication and transcription; characterizing cellular lesions induced by trapping of Top1-DNA covalent complexes (Top1cc), elucidating the cellular responses/pathways elicited in response to such lesions; identify new regulators and novel post-translational modifications of repair proteins for genome maintenance, with a long-term goal to develop molecules to target the pathology.

The nominee also discovered the novel role of Protein arginine methyltransferase 5 (PRMT5), an epigenetic factor, in repair of trapped Top1-DNA lesions. They show that TDP1 is dimethylated at R361 and R586 by PRMT5, and that arginine methylation of TDP1 is a critical modulator of the catalytic activity of TDP1. TDP1-methylation facilitates its interaction with XRCC1 for the repair of Top1cc-mediated DNA damage. Finally, the methylation of R361 and R586 stimulate TDP1 catalytic activity and repair function and promote cell survival in response to CPT. *The nominee is the pioneer to elucidate the novel role of PRMT5 in Top1cc repair* (Rehman *et al*., Nucleic Acids Research, 2018), which opens new avenue to co-target PRMT5 with Top1 in cancer.

In a recent study, Prof. Das reveal mechanistic crosstalk between two post-translational modifications (Arginine methylation and ubiquitylation of TDP1) that is critical for the repair of trapped Top1cc and maintaining genome stability. By generating CRISPR-mediated PRMT5 KO cells, they show that loss of TDP1 arginine methylation results in compromised TDP1 proteostasis, which leads to the accumulation of enzymatically less active TDP1 protein that failed to rescue cells from CPT-induced cytotoxicity. The study also demonstrates that methylation of TDP1 at R586 promotes ubiquitin/proteasome-dependent TDP1 proteostasis to maintain its steady-state level within cells. The current work offers evidence that both the arginine methylation sites of TDP1 facilitate the DNA repair activity. (Bhattacharyya et al., Cell reports, 2022). (Bhattacharyya et al., STAR protol, 2022). This study unravels this mechanism in details and implicates it in a combinatorial chemotherapy. PRMT5 inhibitor GSK3326595 has been approved as a monotherapy in phase II clinical trials of cancer. Therefore, our current work published in Cell Reports provides a new rationale for the combination of Top1-PRMT5 inhibitors in tumorigenesis. This work has received several international and national attentions in the field and has a proposition for clinical trials.

Poly(ADP-ribose) polymerases (PARP1) is a DNA repair protein and FDA approved clinical target in BRCA-defective breast and ovarian cancer. PARP1 catalyzes poly(ADP-ribose) (PAR) chains to various proteins including themselves and Top1. The nominee discovered a novel role of PARP1 in the regulation of Top1-nuclear mobility through Top1-PARylation. They establish that inhibition of Top1-PARylation efflux Top1 from the nucleolus to the nucleoplasm, which results marked increase in Top1 trapping. The CPT resistant Top1 (N722S) patient mutant shows defective nuclear dynamics due to faulty PARylation (Das SK *et al*., Nucleic Acids Research, 2016).

The nominee discovered that PARP1 is a key molecular component of TDP1 for the repair of Top1cc. TDP1 is recruited to DNA damage sites by PARP1 through TDP1-PARylation. Cancer cells exposed to the combination of PARP inhibitors with Top1 inhibitors substantially increased Top1 trapping which is due to increased unrepaired Top1cc-associated DNA double strand breaks (DSBs) and

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lethality. This work provides a molecular mechanism explaining the synergism between PARP and Top1 inhibitors by showing that PARP1 and TDP1 are epistatic that is highly relevant for the ongoing clinical trials combining PARP inhibitors and top1 inhibitors in cancer (Nucleic Acids Research, 2014).

Establishing *Leishmania donovani* TDP1 as a potential drug target

***Leishmania donovani*, a unicellular protozoan parasite, causes a wide range of human diseases including fatal visceral leishmaniasis. Tyrosyl DNA-phosphodiesterase 1 (TDP1) hydrolyzes the phosphodiester bond between DNA 3'-end and a tyrosyl moiety of trapped topoisomerase I-DNA covalent complexes (Top1cc). Leishmania harbors a TDP1 gene (LdTDP1), however, the biological role of TDP1 remains largely unknown. In the present study, Prof. Das group has generated TDP1 knockout *L. donovani* (LdTDP1-/-) promastigotes and has shown that LdTDP1-/- parasites are deficient in 3'-phosphodiesterase activities and were hypersensitive to Top1-poison like camptothecin (CPT), DNA alkylation agent like methyl methanesulfonate, and oxidative DNA lesions generated by hydrogen peroxide but were not sensitive to etoposide. They have also detected elevated levels of CPT-induced reactive oxygen species triggering cell cycle arrest and cell death in LdTDP1-/- promastigotes. LdTDP1-/- promastigotes accumulate a significant change in the membrane morphology with the accumulation of membrane pores, which is associated with oxidative stress and lipid peroxidation. To our surprise, we detected that LdTDP1-/- parasites were hypersensitive to antileishmanial drugs like amphotericin B and miltefosine, which could be rescued by complementation of wild-type TDP1 gene in the LdTDP1-/- parasites. Notably, multidrug-resistant L. donovani clinical isolates showed a marked reduction in TDP1 expression and were sensitive to Top1 poisons. Taken together, our study provides a new role of LdTDP1 in protecting *L. donovani* parasites from oxidative stress-induced DNA damage and resistance to amphotericin B and miltefosine (Roychowdhury *et al*., *The FASEB Journal*, 2022)**

TDP1 is a key repair enzyme that hydrolyses the trapped Top1 from DNA. A homozygous mutation of human TDP1(H493R) is responsible for the severe neurodegenerative syndrome, spinocerebellar ataxia with axonal neuropathy (SCAN1). Das group provides first evidence that mitochondria in cells harboring SCAN1-mutant TDP1 are selectively trapped on the mitochondrial DNA (mtDNA), generates mtDNA damage, shows increased fission rates. TDP1H493R trapping prevents mitochondrial transcription, energy production and mitobiogenesis. SCAN1-TDP1 trapping triggers autophagy that allows identification and removal of dysfunctional mitochondria in neurons through mitophagy. (Gosh et al., SCIENCE ADVANCES, 2019)

Detail of patents.

US Patent: Bicycle topoisomerase i inhibiting compounds, process for preparation and use thereof Application number# 17059289 Date: 2021/8/12,

Indian Patent filed on dated 29.05.2018 with application No. 201811020003. Title: Bicyclic compounds as topoisomerase I inhibitors

Publications:

1. Bhattacharjee S, Richardson J., ***Das, B.B**.** FRET-based assay to estimate modulation of TDP1 activity through arginine methylation.

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STAR protocol, 2023, 4, (2), 102218.(Cell press); IF: 5.1

2. Chowdhuri SP, Dhiman S, Das SK, Meena N, Das S, Kumar A, ***Das, B.B**.** Novel Pyrido[2',1':2,3]imidazo[4,5-c]quinoline Derivative Selectively Poisons Leishmania donovani Bisubunit Topoisomerase 1 to Inhibit the Antimony-Resistant Leishmania Infection *in Vivo*. ***J Med Chem***, 2023 Feb 23. (ACS; IF: 8.01)

3. Bhattacharjee S, Rehman I, Basu, S., Nandy S, Richardson J., ***Das, B.B****. **2022** The interplay between symmetric arginine dimethylation and ubiquitylation regulates TDP1 proteostasis for the repair of topoisomerase I-DNA adducts. ***Cell Reports***, 39, 110940 (Cell press: IF: 9.99)

4. *Rehman, I.; Basu, S.; Das, S.K.; Bhattacharjee, S.; Ghosh, A.*; Pommier, Y.; and ***Das, B.B****. **2018.** PRMT5-mediated arginine methylation of TDP1 for the repair of topoisomerase I covalent complexes. ***Nucleic. Acids Research***., **46**: 5601-5617. (IF: 19.1)

5. Roy Chowdhury S., Das SK., Banerjee B., Paul Chowdhuri S., Majumder H.K., and ***Das, B.B****. **2022**. TDP1 knockout *Leishmania donovani* accumulate Topoisomerase1-linked DNA damage and are hypersensitive to clinically used antileishmanial drugs. ***The FASEB Journal***, , 36(4): e22265. (IF: 5.3)

6. *Majumdar, P*, Bathula C, *Basu S.M., Das, S.K.*, Agarwal R, Hati S, Singh A, Sen, S*, ***Das, B.B.****. **2015.** Design, synthesis and evaluation of thiohydantoin derivatives as potent topoisomerase I (Top1) inhibitors with anticancer activity. ***Eur J Med Chem.*** ;102:540-5. (IF: 7.08)

7. Ghosh, A., Bhattacharjee, S., Paul Chowdhuri, S., Mallick, A, Rehman, I., Basu, S., and ***Das, B.B*.*** 2019. SCAN1-TDP1 trapping on mitochondrial DNA promotes mitochondrial dysfunction and mitophagy. ***SCIENCE ADVANCES***, ***2019***, ***5, eaax9778***. *(IF: 14.1)*

8. 5.Das SK, Ghosh A, Paul Chowdhuri S, Halder N, Rehman I, Sengupta S, Sahoo KC, Rath H*, ***Das BB*** #*. **2018** Neutral Porphyrin Derivative Exerts Anticancer Activity by Targeting Cellular Topoisomerase I (Top1) and Promotes Apoptotic Cell Death without Stabilizing Top1-DNA Cleavage Complexes. ***J. Med. Chem***., 61 (3), 804–817. (ACS; IF: 8.01)

9. Kundu, B., *Das, S. K., Chowdhuri, S. P.*, Pal, S., Sarkar, D., *Ghosh, A.*, Mukherjee, A., Bhattacharya, D., ***Das, B.B***.* Talukdar, A. **2019**. Discovery and Mechanistic Study of Tailor-Made Quinoline Derivatives as Topoisomerase 1 Poison with Potent Anticancer Activity. ***Journal of Medicinal Chemistry (ACS)***., **62**: 3428-3446. (ACS; IF: 8.01)

10. Kundu, B., Sarkar, D., *Chowdhuri, S. P.*, Pal, S., *Ghosh, A., Das, S. K.*, Mukherjee, A., Bhattacharya, D., ***Das, B.B***.* Talukdar, A.* **2020**. Development of a metabolically stable topoisomerase I poison as anticancer agent. ***Eur J Med Chem.;202:112551.***. (IF: 7.08)

11. Das, S.K., Rehman, I., Ghosh, A., Sengupta, S., Majumder, P., Jana, B and ***Das BB*#.*** Poly(ADP-ribose) polymers regulate DNA topoisomerase I (Top1) nuclear dynamics and camptothecin sensitivity in living cells. **2016**. ***Nucleic. Acids Res.*** . 44, 8363-75. (IF: 19.1)

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