STRIDE™ as a technology platform for accurate measurement of DNA breaks and breaks-associated repair proteins

kamil@intodna.com
magda@intodna.com
www.intodna.com

Anna Uherek, Karolina Stępień, Olga Wójcikowska, Franek Sierpowski, Zsombor Prusci, Monika Jarosz, Maja Białecka, Jakub Lechowski, Karolina Korpanty, Magdalena Kordon-Kiszala, Kamil Solarczyk

Accurate assessment of DNA breaks level is crucial in maximizing the therapeutic potential of synthetic lethality approaches related to the DNA Damage Response (DDR). Currently utilized IHC or IF methods for DNA damage detection require active DDR and often lack specificity and sensitivity. Unlike standard methods, STRIDE™ allows direct detection and quantitative measurement of total pool of double- (dSTRIDE™) or single-strand (sSTRIDE™) DNA breaks, independent of DNA repair processes. However, these assays can be customized to give an insight into the involvement of specific DNA repair proteins at DNA breaks sites. Here, we present several STRIDE™ functional assay variants targeting different DNA repair pathways and proteins and how they can be used to support the development of DDR targeting therapeutics.

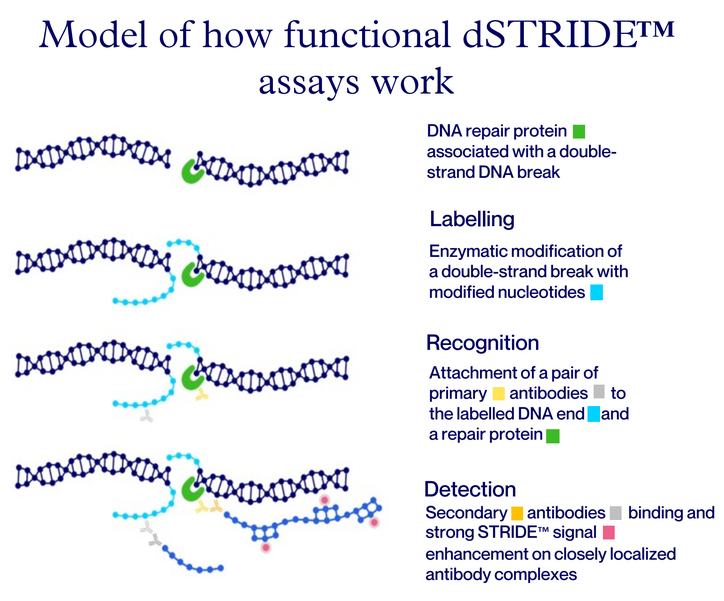

Figure 1 Schematic representation of main steps of functional sSTRIDE™ assays.

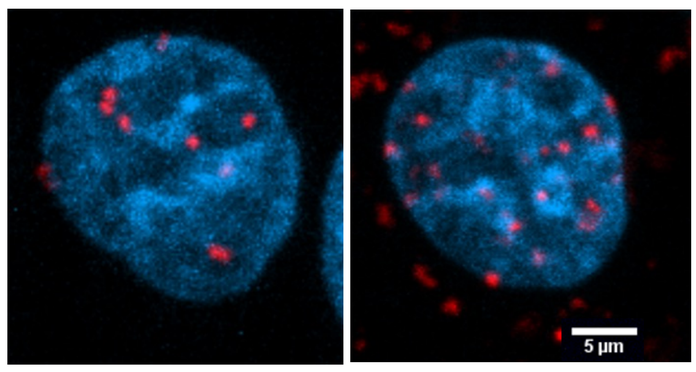

Figure 2 sSTRIDE-WRN: detection of WRN-specific DSBs and all types of DSBs in HCT116 cells. Nuclei with dSTRIDE™-WRN (left) and dSTRIDE™ signals (right).

WRN recruitment to DSBs

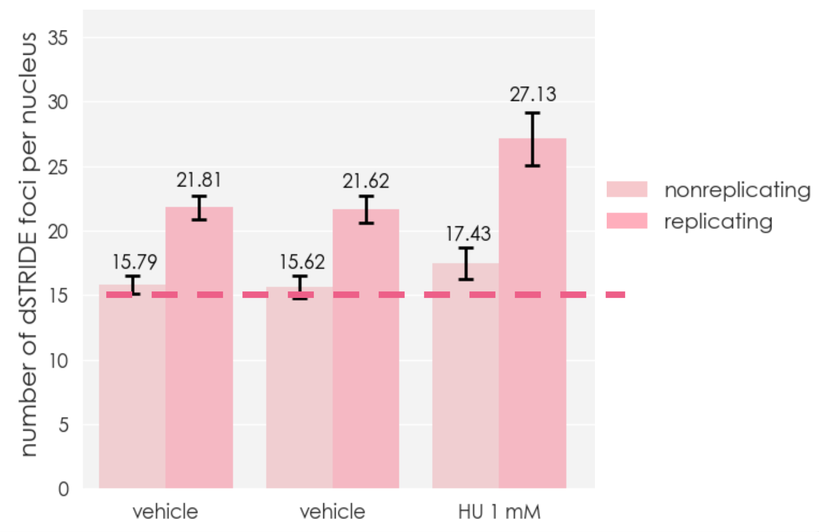

Figure 3 dSTRIDE™-WRN: treatment with hydroxyurea enhances WRN association with DSBs in replicating cells.

Increase in the number of WRN-associated DSBs upon HU treatment when compared to the vehicle in the population of replicating HCT116 cells. Technical duplicates of untreated samples show very good reproducibility and exhibit similar level of dSTRIDE™-WRN foci count. Replicating cells were distinguished based on PCNA co-staining. Bar plot showing mean values of dSTRIDE™-WRN foci per nucleus.

HR pathway activity

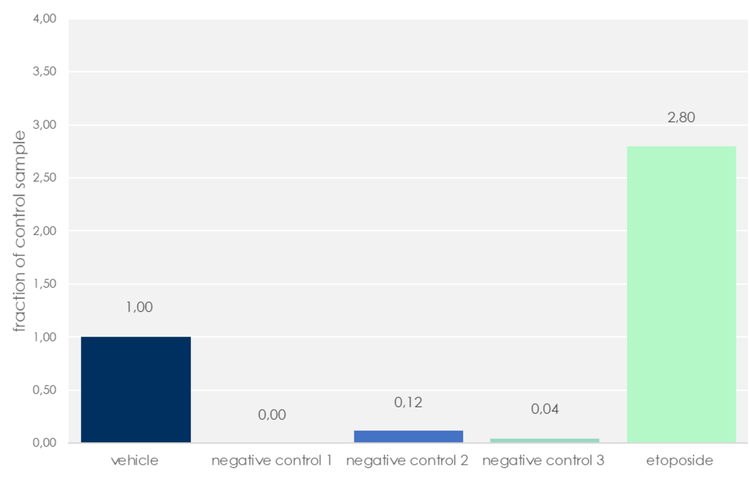

Figure 4 dSTRIDE™-RPA70: set of controls for assay validation in DLD1 cells.

Technical negative controls exhibit very low dS-RPA70 foci count in comparison to control (vehicle) sample, confirming assay specificity. Enhanced response for etoposide treatment proves that RPA70 is recruited to repair double strand DNA breaks. Bar plot showing mean values of dSTRIDE™-RPA70 foci per nucleus.

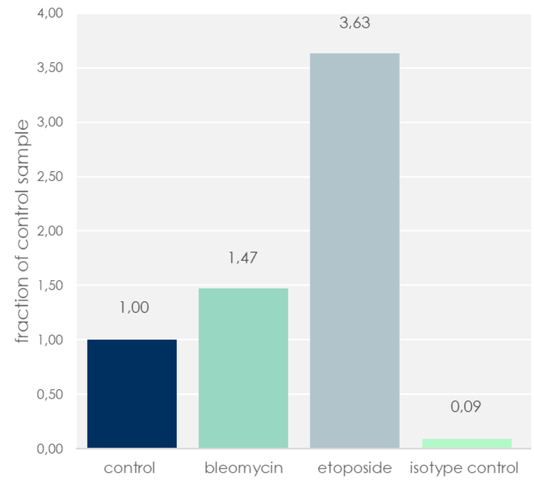

Figure 5 dSTRIDE™-RAD51: level of RAD51 associated with DSBs after DNA damage induction.

Treatment with bleomycin and etoposide leads to increased numbers of detected dSTRIDE™-RAD51 foci in U2OS cells. Isotype control exhibits low level of nonspecific signal. Bar plot showing mean values of dSTRIDE™-Rad51 foci per nucleus

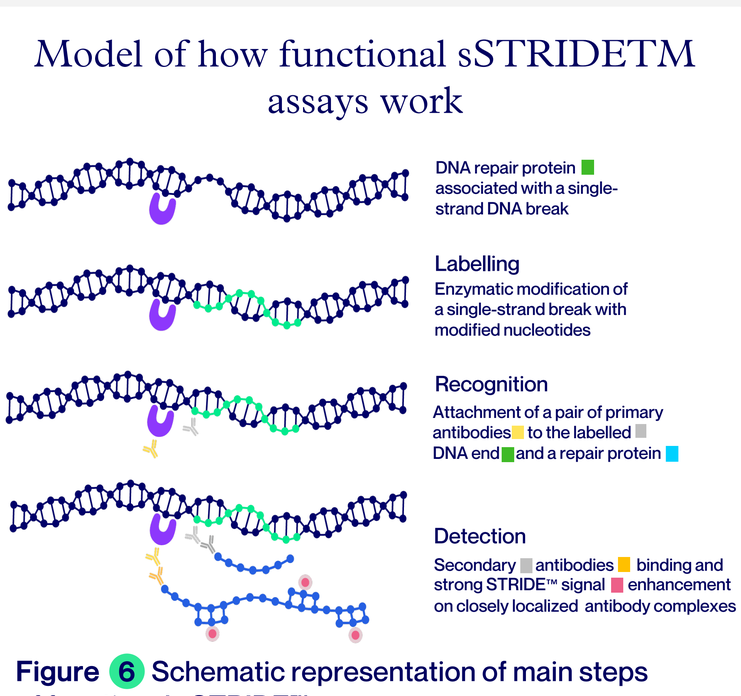

Figure 6 Schematic representation of main steps of functional sSTRIDE™ assays.

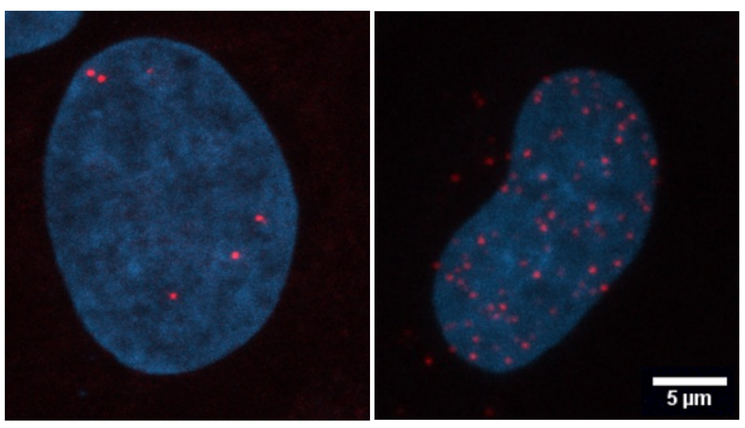

Figure 7 sSTRIDE-MMR: detection of PMS2-specific SSBs (sSTRIDE™-MMR) and all types of SSBs in U2OS cells. Nuclei with sSTRIDE™-MMR(left) and sSTRIDE™ signal (right).

MMR pathway activity

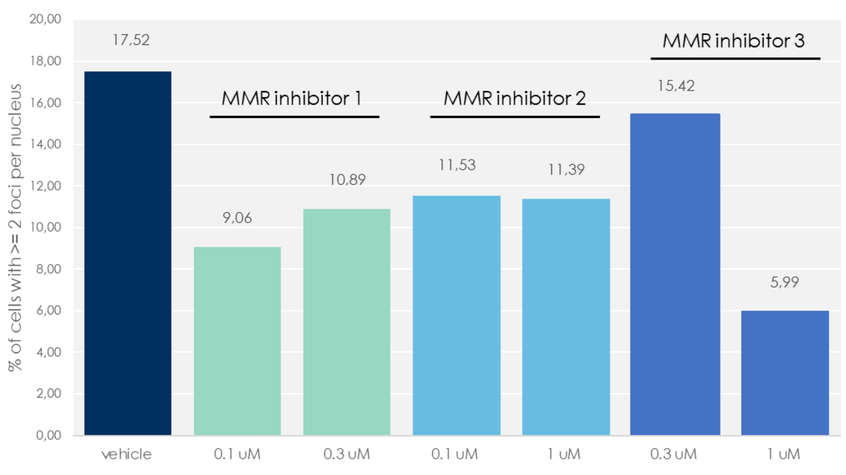

Figure 8 sSTRIDE-MMR: assessment of MMR inhibitors efficiency

Level of PMS2-associated SSBs in HAP1 cells: untreated (vehicle) and treated with various MMR inhibitors. Bar plot showing percentage of MMR-positive cells, i.e. cells with two or more MMR-specific SSBs per nucleus. Quantitative results were obtained based on a 3D image analysis.

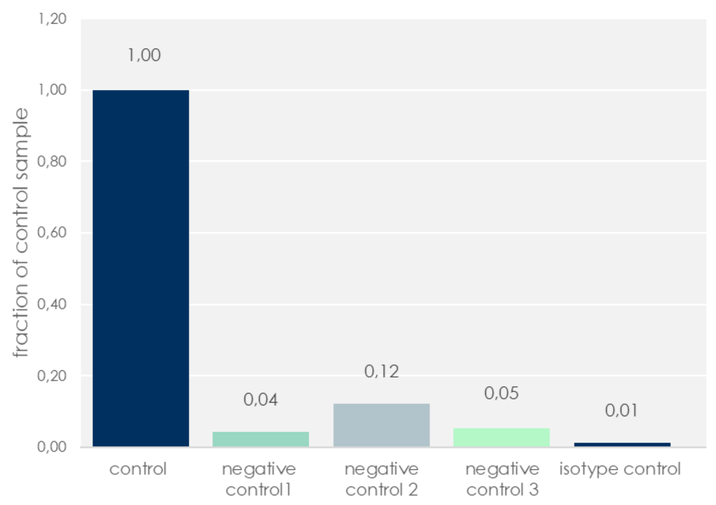

Figure 9 sSTRIDE™-MMR: set of technical negative controls for assay validation in U2OS cells.

All technical negative controls show a neglectable fraction of signal in comparison to control sSTRIDE™-MMR sample. Bar plot showing mean values of sSTRIDE™-MMR foci per nucleus

BER pathway activity

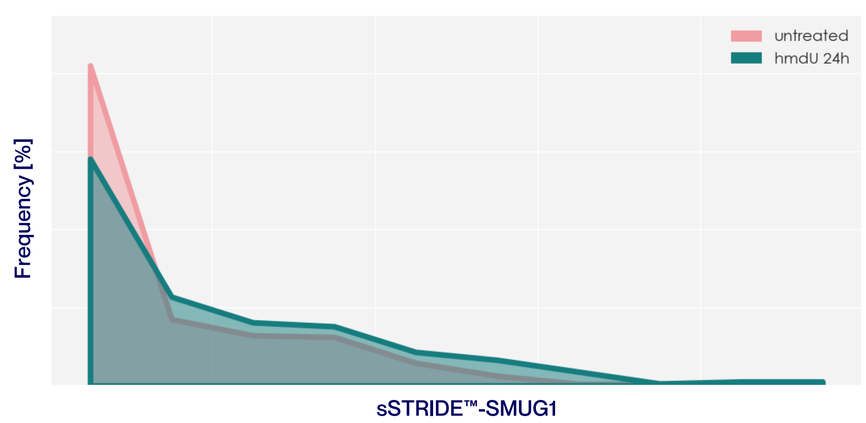

Figure 10 sSTRIDE-SMUG1: increase in the number of SMUG1- associated SSBs after 24h treatment with hmdU

Elevated levels of sSTRIDE™-SMUG1 foci after treatment with hmdU confirms that SMUG1 is involved in removal of the cytotoxic nucleoside from the genomic DNA. Histogram showing the distribution of sSTRIDE™-SMUG1 foci in untreated and hmdU treated HAP1 cells.

Inhibition of various DNA repair pathways in DDR-deficient tumors is a well established anti-cancer treatment strategy. In recent years, many new drug candidates targeting repair proteins emerged, introducing a need for a precise tool to confirm their target specificity. STRIDE™, with direct detection of DNA-bound repair proteins, enhanced signal to noise ratio and the possibility of multiplexing with standard IF staining, can be a valuable tool to assess the performance of various DNA repair pathways and gain knowledge about the mechanism of action of drug candidates.