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Development and validation of a novel sSTRIDE-MMR functional assay to study the efficiency of MMR inhibitors

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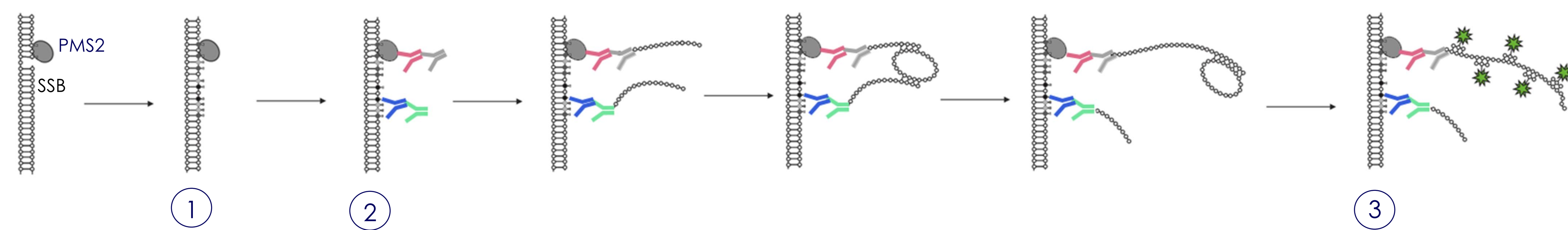
INTRODUCTION

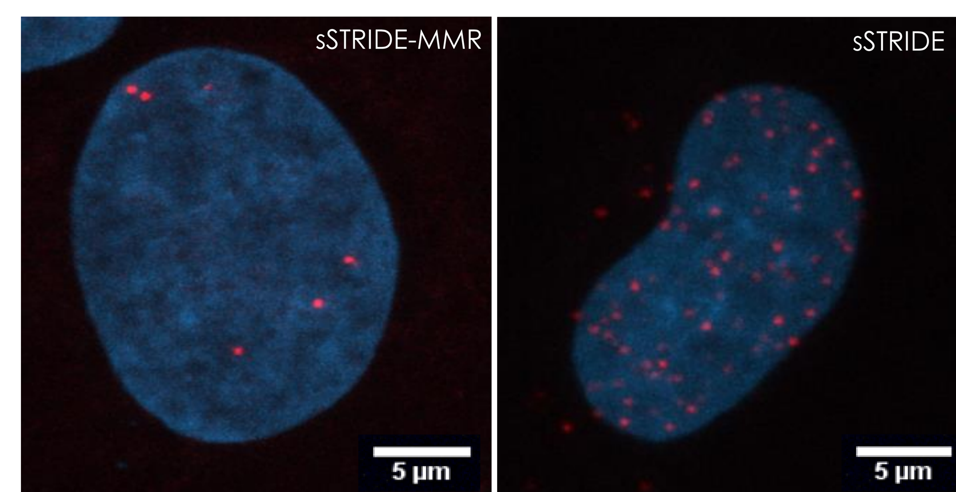
Mismatch repair (MMR) is one of the fundamental pathways that guards genome stability. Decreased efficiency or inactivation of this pathway may promote cancer but also lead to neoantigen formation within a tumor, which activates the immune response. Therapeutic strategies aiming at inhibiting MMR are thus under development, yet methods informing about the status of MMR are lacking. We report here the development and validation of a first cell-based MMR-specific functional assay. The signals occur only at sites of coincidence of a single-strand DNA break and PMS2 – an endonuclease that forms the MutLa heterodimer.

Figure 1. Schematic representation of the sSTRIDE-MMR assay.

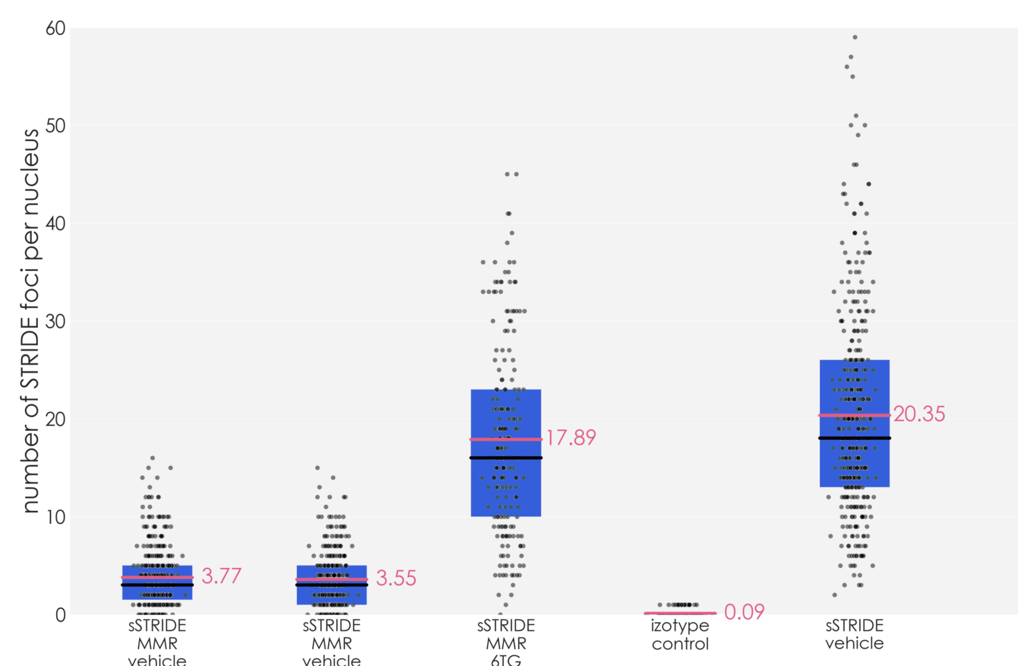


sSTRIDE-MMR assay allows direct detection of a SSB and PMS2 protein. The main steps in the procedure consist of 1) enzymatic incorporation of modified nucleotides, 2) antibody-based detection of modified nucleotides and the MMR-specific protein and 2) signal amplification using RCA reaction.

Figure 2. Detection of MMR-specific SSBs (single-strand DNA breaks) in U2OS cells.

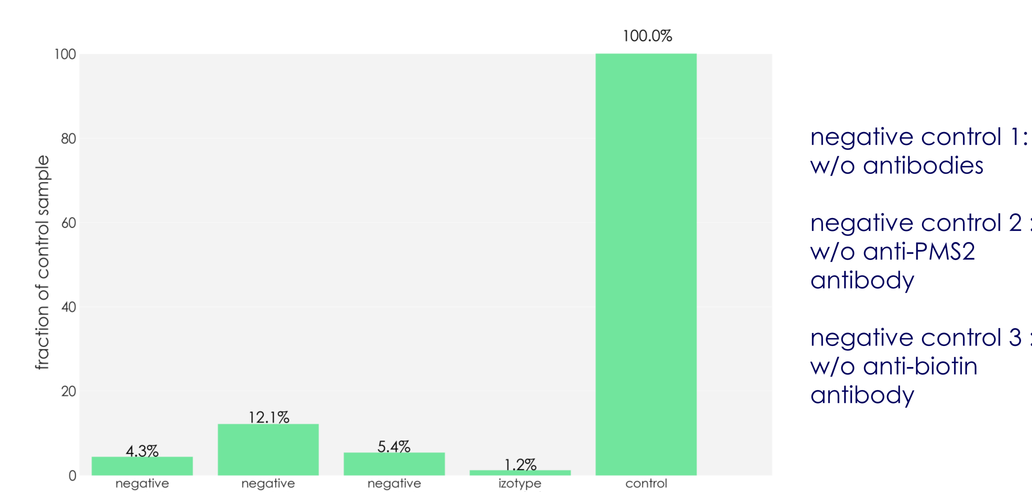


Nuclei with MMR-specific SSBs (left) and all types of SSBs.



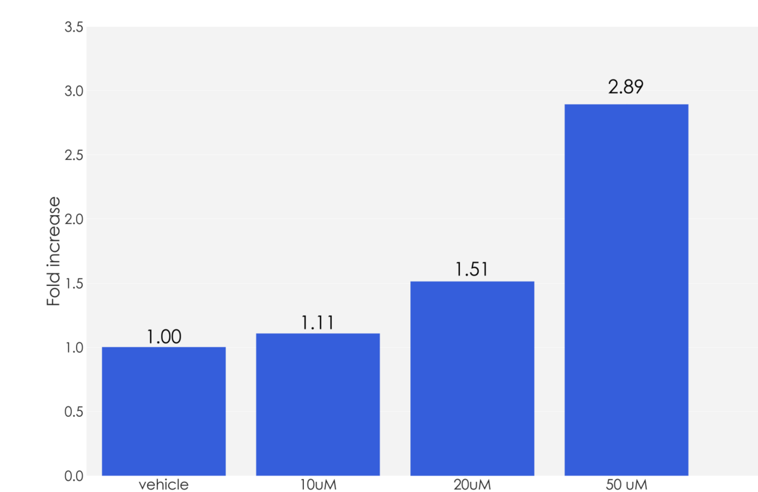
A comparison of MMR-specific SSBs in vehicle and 6TG treated sample, as well as traditional sSTRIDE and isotype control, based on a 3D quantitative image analysis. Each dot represents counts from a single cell. The mean value is depicted as a red horizontal line, and median as a black one.

Figure 3. Assay validation: negative controls in U2OS cells.



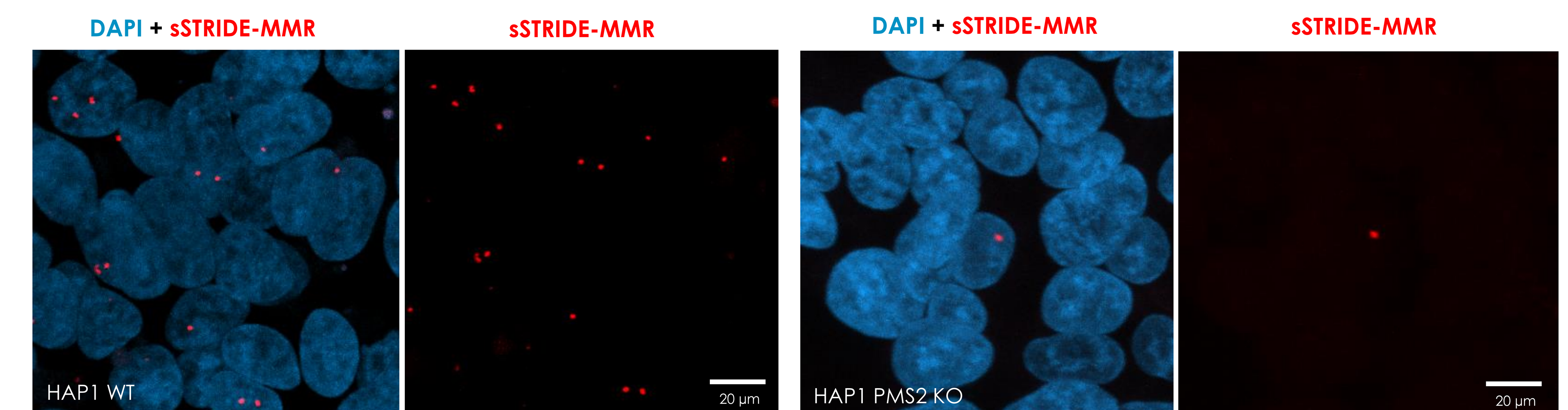
All negative controls show an order of magnitude lower unspecific signal when compared with control in sSTRIDE-MMR assay.

Figure 4. Assay validation with MMR-specific SSBs inducing agent (6TG).



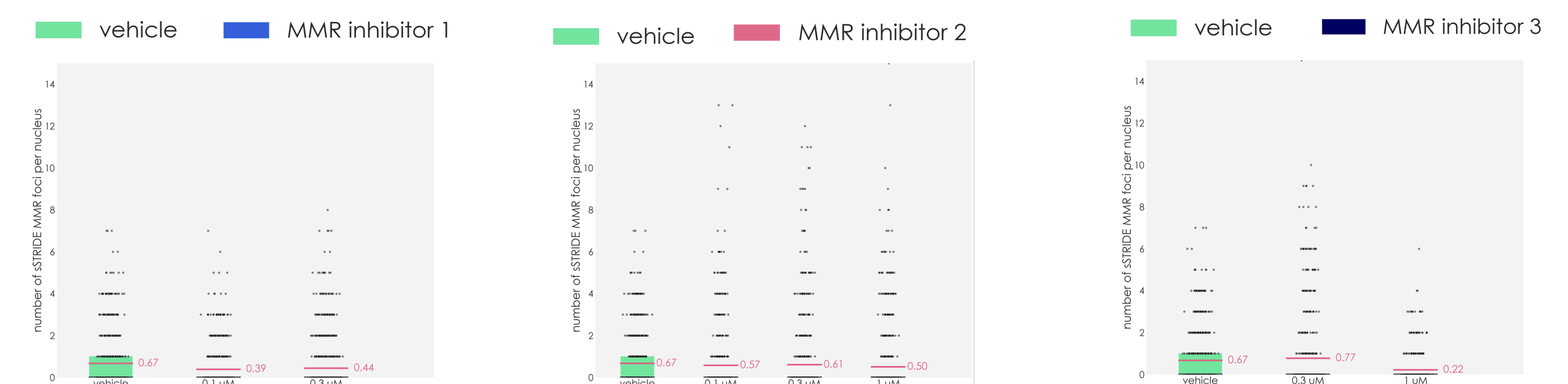
Treatment of the U2OS cell line with an increasing concentration of 6TG induces an increasing number of MMR-specific SSBs. The number of MMR-specific SSBs was established based on a 3D quantitative image analysis and then normalized to the number of counts in the vehicle.

Figure 5. Assay validation in PMS2 KO cells.

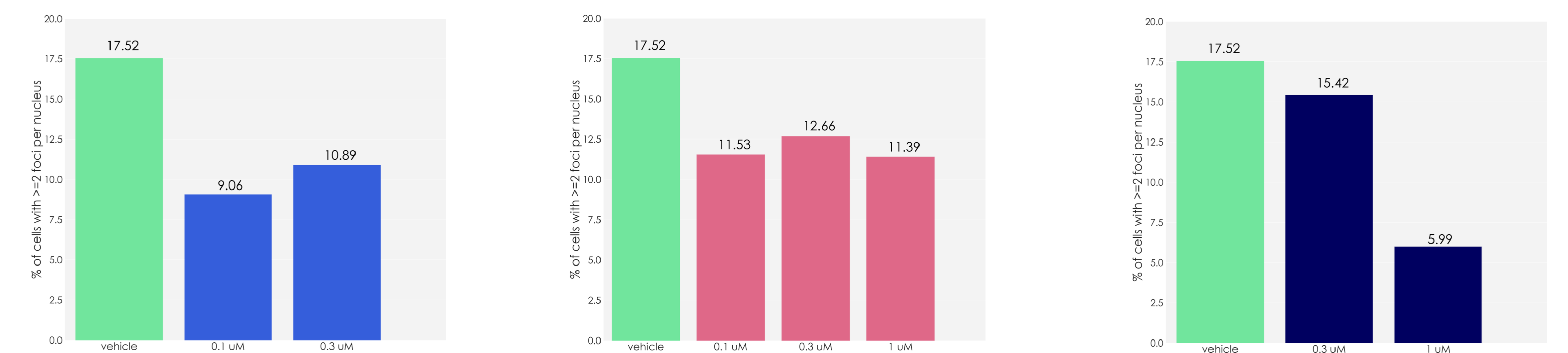


sSTRIDE-MMR in HAP1 WT (left) and HAP1 PMS2 KO (right) cells. Very few signals were detected in PMS2 KO cells.

Figure 6. Application of sSTRIDE-MMR assay to inform about the effect of MMR inhibitors.



Level of MMR-specific SSBs in HAP1 cells before (vehicle) and after treatment with MMR inhibitor 1 (left), MMR inhibitor 2 (center), and MMR inhibitor 3 (right). Quantitative results were obtained based on a 3D image analysis, where each dot represents counts from a single cell, and mean and median are marked with red and black horizontal lines, respectively.



Percentage of cells with two or higher MMR-specific SSBs per nucleus before (vehicle) and after treatment with MMR inhibitors. 2 SSBs per nucleus is the 90th percentile threshold established based on MMR-specific counts in vehicle sample. Quantitative results were obtained based on a 3D image analysis.

CONCLUSIONS

Assay validation and preliminary results obtained with MMR inhibitors confirm that the sSTRIDE-MMR assay can be successfully applied to inform about the status of MMR in cell models. Straightforward quantification, very low level of false positive readouts and the direct detection of MMR-related SSBs make the assay a useful tool that can assist the development of MMR inhibitors. Further studies are needed to assess whether the assay can also be used to inform about the status of MMR in patients.