Immunotherapy sensitivity of mismatch repair deficient cancer: Mutation load is not enough

Marco Gerlinger[1,2]

1. The Institute of Cancer Research, London, UK
2. The Royal Marsden Hospital, GI Cancer Unit, London, UK
marco.gerlinger@icr.ac.uk

Abstract:

Abundant neoantigens are considered responsible for the immunotherapy sensitivity of mismatch repair deficient (MMRd) cancers. In this issue of *Cancer Cell*, two papers show that *MLH1* mismatch repair gene loss promotes cGAS-STING activation, interferon secretion and T cell priming. This may be essential for the high immunotherapy sensitivity in MMRd cancer.

Main text:

DNA mismatch repair deficient (MMRd) tumors are among the most sensitive cancers to immunotherapy with checkpoint inhibitors (Le et al., 2017). The accepted paradigm is that the high mutation load of MMRd tumors leads to the presentation of a large number of mutated neoantigens on MHC molecules, making these cancer cells highly recognizable by T cells (Fig. 1). Yet, a substantial proportion of MMRd tumors does not respond to immunotherapy. This questions how some tumors can avoid recognition and destruction by immune cells despite high mutation loads. The hypermutator phenotype of MMRd tumors leads to extreme intratumor heterogeneity and fosters the evolution of immune evasion mechanisms, for example through genetic inactivation of genes with critical roles in antigen presentation (Ozcan et al., 2018; von Loga et al., 2020). This has been considered a main pathway to escape from immune recognition. Now two papers in this issue of Cancer Cell add a further intriguing possibility (Guan et al., 2021; Lu et al., 2021).

Adaptive immunity does not only depend on the presence of non-self antigens but requires additional triggers to prime an immune response (Woo et al., 2015). For example, during viral infections, the immune system is kick started by cellular sensors that detect pathogen associated molecular patterns such as DNA in the cytoplasm. Cytoplasmic DNA is sensed by the cGAS-STING pathway which triggers secretion of type I interferons. These activate antigen-presenting dendritic cells which then prime T cells of the adaptive immune system. The T cells can subsequently recognize and destroy cells displaying non-self antigens on the cell surface.

Now, Lu et al. investigate the role of the cGAS-STING pathway in engineered MMRd murine cancers generated by deletion of *MLH1* with CRISPR (Lu et al., 2021). Consistent with an increased immune recognition, MMRd hypermutated tumors grow more slowly in immunocompetent mice than the parental cancers. Intriguingly, this effect disappears when *STING* is inactivated. Growth control of MMRd tumors furthermore depends on the activation of dendritic cells by interferon, supporting the critical role of T cell priming by these antigen presenting cells.

Importantly, *STING* inactivation but also restoration of *MLH1* activity in MMRd tumor cells impairs the activity of the type I interferon pathway downstream of STING, linking *MHL1* deficiency in cancer cells directly to STING activation. Loss of STING in cancer cells furthermore impairs the effective priming of T cells *in vitro* and reduces T cell infiltrates in tumors *in vivo* (Fig. 1).

To investigate whether cGAS-STING activity is relevant for immunotherapy responses, the authors test how *MLH1* deficiency and loss of *STING* impact the response to immune checkpoint inhibitor treatment in their murine models. Intriguingly, MMRd tumors respond to checkpoint inhibitors but restoring *MLH1* expression abrogates the response, even though both model systems harbor high mutation loads. *STING* knockout in the MMRd tumor model similarly impairs the efficacy of checkpoint blockade. In addition, Lu et al. show in small patient cohorts that higher cGAS and STING expression correlates with better and longer responses to immune checkpoint inhibitors in MMRd cancers and melanomas.

This provides new evidence that effective T cell recognition of *MLH1* deficient MMRd tumors is not only the consequence of high neoantigen loads. It furthermore requires cGAS-STING activity in tumor cells and this occurs as a programmed consequence of *MLH1* loss. This raises the questions how *MLH1* loss triggers cGAS-STING activation. Lu et al. provide a first clue by showing a significant increase in cytosolic DNA in *MLH1* deficient cells.

In a second paper from the same groups, Guan et al. investigate the hypothesis that the Exo1 DNA exonuclease is poorly controlled when *MLH1* is lost which drives chromosomal instability and escape of nuclear DNA into the cytosol (Guan et al., 2021). Physiologically, Exo1 is not only involved in mismatch repair but also performs DNA end-resection during homologous recombination mediated double strand break repair. Guan et al. first show that *MLH1* deficiency causes DNA release into the cytoplasm and that this further increases when double strand breaks are induced by ionizing radiation (IR). Furthermore, *MHL1* deficiency confers delayed resolution of double strand breaks as well as cGAS-STING activation. Subsequent knockout of *EXO1* in *MLH1* deficient cells reduces the impact of *MLH1* loss: cytosolic DNA, the delayed double strand break resolution phenotype and cGAS-STING activation are diminished in untreated and IR treated cells. This supports the hypothesis that Exo1 activity is essential for cGAS-STING pathway activation through *MLH1* loss.

By analysing several mutant Exo1 proteins in an *in vitro* DNA excision assay, Guan et al. confirm that the physical interaction with MLH1/PMS2 dimers regulates the DNA excision activity of Exo1. A cellular reporter system shows that *MLH1* deficiency permits the Exo1 nuclease to resect at least 3,500 bp of single stranded DNA from an experimentally induced double strand break. In comparison, resection is usually terminated near the strand break in wild-type cells. They furthermore find that the excessive single stranded DNA generation in *MLH1* deficient cells exhausts RPA proteins. This has previously been linked to genomic instability. Taken together, Exo1 activity leads to DNA damage in the absence of *MLH1* and is associated with a 3-5 fold increase of chromosomal abnormalities in *MHL1* deficient cells. The authors hypothesize that chromosomal instability promotes the release of nuclear DNA into the cytoplasm where it triggers cGAS-STING signalling (Fig. 1).

The results in these two papers provide important insights into the regulation of immune recognition in MMRd tumors and provoke new ideas for the development of urgently needed predictive immunotherapy biomarkers and indeed better immunotherapies. *MLH1* loss is one of the commonest causes of MMRd but loss of other mismatch repair proteins, such as MSH2, are alternative mechanisms (Salem et al., 2020). *MLH1* loss leads to cGAS-STING activation, higher immune cell infiltrates and response to checkpoint inhibitors but these downstream effects cannot be assumed for other causes of MMRd. It is hence possible that the specific pattern of MMR gene

loss influences immunotherapy responses. This should be readily testable in existing clinical trials in MMRd tumors as the expression of the MMR proteins MLH1, MSH2, MLH6 and PMS2 has been routinely assessed before patient inclusion. Moreover, previous work (Xia et al., 2016) and the current publication by Lu et al. find that cGAS and STING expression are frequently absent in cancer cells from various tumor types. It is conceivable that loss of cGAS or STING expression enables immune evasion and their role as predictive biomarkers should be investigated in immunotherapy trials. Moreover, combination of immune checkpoint inhibitors with type I interferons or cGAS agonists that recently entered clinical development (Le Naour et al., 2020) may be rational strategies to improve immunotherapy responses in MMRd tumors with intrinsically low cGAS-STING activity.

Taken together, these new data show that cancer cell intrinsic cGAS-STING is a critical promoter of immune recognition and possibly a prerequisite for effective immunotherapy even when neoantigen loads are high.

Acknowledgements: The author receives research funding from the European Research Council, the UK National Institute of Health Research, Merck KG, Bristol Myers Squibb and Roche.

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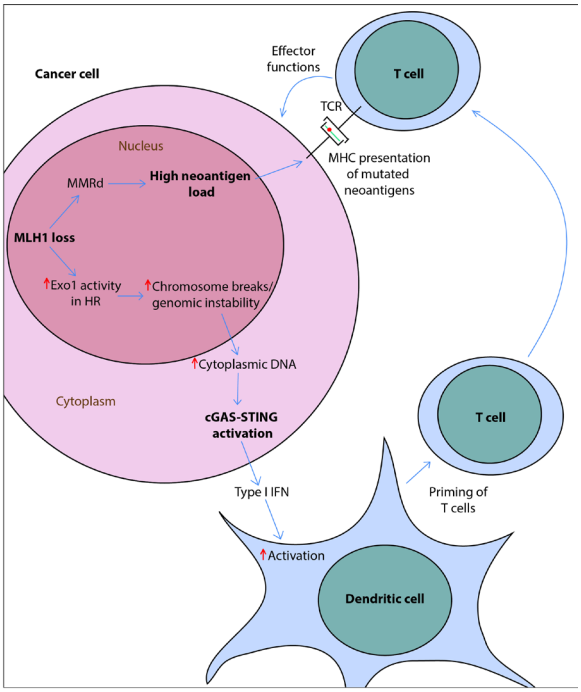

Figure 1. Immune recognition of *MLH1* deficient MMRd tumors is promoted by two mechanisms. Firstly a high neoantigen load which is the result of DNA mismatch repair deficiency. MLH1 also regulates the DNA exonuclease Exo1 during mismatch repair and homologous recombination mediated double strand repair. Mlh1 loss therefore secondly leads to Exo1 hyperactivity which causes excessive DNA damage and chromosome breaks. This promotes release of double stranded DNA into the cytoplasm where it activates the cGAS-STING pathway. sGAS-STING triggers type I interferon secretion which activates antigen cross-presenting dendritic cells, leading to the priming of T cells with tumor-derived antigens. Primed neoantigen specific T cells are then able to recognize and kill cancer cells presenting neoantigens on MHC molecules on the cell surface. IFN: interferon, TCR: T cell receptor.