Cell Reports Medicine

CellPress
OPEN ACCESS

Spotlight

Harnessing the therapeutic vulnerability of MMR heterogeneity in colorectal cancer

Gayathri Anandappa[1] and Michael J. Overman[1,*]
[1]Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
*Correspondence: moverman@mdanderson.org
https://doi.org/10.1016/j.xcrm.2022.100908

In a recent issue of *Cancer Cell*, Amodio and colleagues report an interesting method of modulating immunosurveillance in colorectal tumors with DNA mismatch repair (MMR) heterogeneity.[1] By pharmacologically enriching the MMR deficient (MMRd) component using 6-thioguanine, they demonstrate improved tumor control in murine models.

The mismatch repair (MMR) protein machinery detects and replaces single nucleotide mismatches and small indels, thereby ensuring hi-fidelity DNA replication.[2] Loss of MMR protein function results in the accumulation of high mutational burden, primarily seen at areas of repeating sequences or microsatellites, resulting in high microsatellite instability (MSI-H). Approximately 5%–15% of colorectal cancers (CRC) have MMR deficiency (MMRd) or MSI-H and demonstrate robust clinical benefit to immune checkpoint inhibitors (ICIs) in both the early and metastatic settings.[3] The vast majority of metastatic CRC (95%), however, are microsatellite stable (MSS) or MMR proficient (MMRp) and are recalcitrant to ICI therapy. Strategies to overcome this primary resistance have been limited because of the complex heterogeneity of these tumors and lack of a full understanding of underlying biology, especially the tumor-immune microenvironment interactions. In the study under this spotlight, Amodio and colleagues explore a novel strategy to modulate MMR heterogeneity to promote immune surveillance.[1]

Clinical MMR heterogeneity is most commonly seen in the context of discordant test results between MMR and MSI testing. Explanations for these discrepancies primarily relate to pre-analytic or analytic factors such as tumor purity, suboptimal fixation, or antibody specificity. While patchy loss of MMR protein expression is often present, larger intermixed areas of MMRp and MMRd denotating intra-patient heterogeneous MMR protein expression have been reported with one study suggesting three distinct patterns—intraglandular, clonal, compartmental.[4–7] Chapusot and colleagues reported an incidence of 8% in 100 right-sided colon cancers, while in a larger case series of 1,855 samples by Loupakis and colleagues, there was a reported incidence of only 0.7%.[4,7] In a limited number of reports, intra-patient heterogeneous MMR protein expression was demonstrated to have biological relevance with absent or intact regions of MMR protein expression correlating with corresponding regions of MSI-H and MSS, respectively.[4,6,7] However, many unknowns exist. Clinical studies have not delineated the percentage of MSS/MMRp compared with MSI-H/MMRd in these heterogeneous cases, which limit the ability to determine the clinical relevance of the 20% MSI-H threshold utilized by Amodio et al. In addition, as the majority of reported cases are MSI-H tumors with MMR heterogeneity, the existence of biologically meaningful MMR heterogeneity in MSS cancers is less established. To date, there is only one published case report of a patient treated based on MMR heterogeneity. This patient received nivolumab and ipilimumab with an impressive disease control rate of 41 months[7] Interestingly, analysis of tumor tissue in this patient showed the MMRd component had high tumor mutational burden (TMB = 11 mutations/megabase) with high levels of tumor infiltrating lymphocytes (TILs) while the MMRp component had a low TMB (5.2 mutations/megabase) with no TILs. At disease progression the tumor biopsy revealed MMRd status.

Using populations of *Mlh*$^{+/+}$ and *Mlh*1$^{-/-}$ isogeneic cells in varying proportions (100%–0%, 20%–80%, 50%–50%, 0%–100%) in syngeneic mice, the authors aim to enrich the MMRd component in MMR heterogeneous tumors in this study.[1] They report that presence of only 20% of MMRd cells delayed tumor growth; increasing the proportion to 80% ceased tumor development. Using injections in opposite flanks, they demonstrate that immune responses elicited by MMRd cells abrogates both MMRd and MMRp cells locally but had no effect on distant MMRp sites. Resistant tumors were composed mainly of *Mlh*$^{+/+}$ with complete loss of *Mlh1*$^{-/-}$ in some cases. Flow cytometry demonstrated tumors with only 20% of MMRd cells recruited more CD8^{+} T cells than the controls. Depleting CD8^{+} T cells resulted in outgrowth of tumors composing mainly of MMRd cells while mice lacking γδ T cells had predominantly MMRp tumors suggesting alternate mechanisms of immune surveillance based on MMR status.

The group performed a genetic screen using a custom pooled CRISPR library targeting genes involved in DNA damage response and repair to establish a mouse model to assess response to 6- thioguanine (6TG), a compound used in the treatment in hematological malignancies and found to be inactive in MMRd cells. Treatment with 6TG resulted in the enrichment of MMRd cells as early as 96 h after drug exposure. Although the mouse models in this study neither recapitulate the molecular heterogeneity present in patients, nor capture the complex tumor immune

microenvironment, they provide early insights into the possibility of harnessing pharmacological modulation of molecularly heterogeneous MMR tumors.

Clinical trial efforts to modulate the immunogenicity of MMRp tumors by inducing heterogeneity are currently underway. Both the MAYA and the ARETHUSA study are therapeutically exploiting the hypermutant state induced by the alkylating agent temozolamide by inactivating mutations in MMR genes, thereby sensitizing a subset of MMRp CRC tumors with O-6 Methylguanine-DNA Methyltransferase (MGMT) methylation to ICIs.[8] Likewise, the observation that targeted therapy against the epidermal growth factor receptor (*EGFR*)/*BRAF*^{V600E} increased DNA damage and induced adaptive mutability in preclinical models has led to clinical trials combining anti-EGFR/BRAF targeted therapy with ICI.[9] This report by Amodio et al. provides a potential avenue to therapeutically harness MMR heterogeneity, albeit likely a rare phenomenon.

For this strategy to be successfully translated into the clinic, understanding the true frequency of clinical MMR heterogeneity is crucial. As routine multiregional testing of tumors is not practical in busy clinical pathology laboratories, liquid biopsies may offer a novel avenue to further explore clinically relevant MMR heterogeneity. The reasons for heterogeneity are unknown and may reflect subclonal variation in MMR gene inactivation or heterogeneity of *MLH1* promoter hypermethylation influenced by the tumor microenvironment.[10] Amodio and colleagues have made a strong case toward the need for improved biological understanding of MMR heterogeneous cancers.

DECLARATION OF INTERESTS

M.J.O. has management/advisory or consulting relationships with Simcere Pharmaceutical, Gritstone, Phanes Therapeutics, Takeda Pharmaceuticals, Pfizer, Merck, Glaxosmithkline, Nouscom, Tempus, Roche, and Bayer.

REFERENCES

1. Amodio, V., Lamba, S., Chilá, R., Cattaneo, C.M., Mussolin, B., Corti, G., Rospo, G., Berrino, E., Tripodo, C., Pisati, F., et al. (2022). Genetic and pharmacological modulation of DNA mismatch repair heterogeneous tumors promotes immune surveillance. Cancer Cell *41*, 196–209. https://doi.org/10.1016/j.ccell.2022.12.003.
2. Jiricny, J. (2006). The multifaceted mismatch-repair system. Nat. Rev. Mol. Cell Biol. *7*, 335–346. https://doi.org/10.1038/nrm1907.
3. Andre, T., Lonardi, S., Wong, K.Y.M., Lenz, H.J., Gelsomino, F., Aglietta, M., Morse, M.A., Van Cutsem, E., McDermott, R., Hill, A., et al. (2022). Nivolumab plus low-dose ipilimumab in previously treated patients with microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: 4-year follow-up from CheckMate 142. Ann. Oncol. *33*, 1052–1060. https://doi.org/10.1016/j.annonc.2022.06.008.
4. Chapusot, C., Martin, L., Bouvier, A.M., Bonithon-Kopp, C., Ecarnot-Laubriet, A., Rageot, D., Ponnelle, T., Laurent Puig, P., Faivre, J., and Piard, F. (2002). Microsatellite instability and intratumoural heterogeneity in 100 right-sided sporadic colon carcinomas. Br. J. Cancer *87*, 400–404. https://doi.org/10.1038/sj.bjc.6600474.
5. Hechtman, J.F., Rana, S., Middha, S., Stadler, Z.K., Latham, A., Benayed, R., Soslow, R., Ladanyi, M., Yaeger, R., Zehir, A., et al. (2020). Retained mismatch repair protein expression occurs in approximately 6% of microsatellite instability-high cancers and is associated with missense mutations in mismatch repair genes. Mod. Pathol. *33*, 871–879. https://doi.org/10.1038/s41379-019-0414-6.
6. Joost, P., Veurink, N., Holck, S., Klarskov, L., Bojesen, A., Harbo, M., Baldetorp, B., Rambech, E., and Nilbert, M. (2014). Heterogenous mismatch-repair status in colorectal cancer. Diagn. Pathol. *9*, 126. https://doi.org/10.1186/1746-1596-9-126.
7. Loupakis, F., Maddalena, G., Depetris, I., Murgioni, S., Bergamo, F., Dei Tos, A.P., Rugge, M., Munari, G., Nguyen, A., Szeto, C., et al. (2019). Treatment with checkpoint inhibitors in a metastatic colorectal cancer patient with molecular and immunohistochemical heterogeneity in MSI/dMMR status. J. Immunother. Cancer *7*, 297. https://doi.org/10.1186/s40425-019-0788-5.
8. Crisafulli, G., Sartore-Bianchi, A., Lazzari, L., Pietrantonio, F., Amatu, A., Macagno, M., Barault, L., Cassingena, A., Bartolini, A., Luraghi, P., et al. (2022). Temozolomide treatment Alters mismatch repair and Boosts mutational burden in tumor and Blood of colorectal cancer patients. Cancer Discov. *12*, 1656–1675. https://doi.org/10.1158/2159-8290.cd-21-1434.
9. Morris, V.K., Parseghian, C.M., Escano, M., Johnson, B., Raghav, K.P.S., Dasari, A., Huey, R., Overman, M.J., Willis, J., Lee, M.S., et al. (2022). Phase I/II trial of encorafenib, cetuximab, and nivolumab in patients with microsatellite stable, BRAFV600E metastatic colorectal cancer. J. Clin. Oncol. *40*, 12. https://doi.org/10.1200/jco.2022.40.4_suppl.012.
10. Kaplan, A.R., and Glazer, P.M. (2020). Impact of hypoxia on DNA repair and genome integrity. Mutagenesis *35*, 61–68. https://doi.org/10.1093/mutage/gez019.