Results of real-world MSI/dMMR testing in tumors outside the Lynch syndrome spectrum: is there a role for NGS?

A.Lebedeva[1,2], I.Demidova[3], N.Savelov[3], A.Barinov[3], A.Tsukanov[4], A.Druy[5], M.Makarova[6], A.Semenova[7], M.Biakhova[7], M.Ivanov[1], A.Taraskina[1,2], V.Mileyko[1,2]

1.OncoAtlas LLC (Moscow, Russia); 2. Sechenov University (Moscow, Russia); 3. Moscow City Oncological Hospital № 62 (Moscow, Russia); 4. Ryzhikh National Medical Research Center for Coloproctology (Moscow, Russia); 5. Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology (Moscow, Russia); 6. Evogen (Moscow, Russia); 7. S.S. Yudin City Clinical Hospital, Oncology Center №1 (Moscow, Russia).

Background

- Accurate mismatch repair deficiency (dMMR) and microsatellite instability (MSI) detection is crucial for the diagnostics of Lynch syndrome and immunotherapy treatment.
- Testing strategies in tumors with low frequency of dMMR/MSI remains a challenge. Here, we present results of the real-world routine dMMR/MSI testing with gold standard methods (GSM) and NGS-based MSI testing using a small amplicon panel.

Methods

- Patients who underwent routine MMR/MSI testing with IHC/PCR/NGS and MMR gene sequencing and displayed any positive signal from 2022 to 2024 were included in the analysis.
- Patients with diverse tumor types (both common for Lynch syndrome [LSS] and outside of the Lynch syndrome spectrum [non-LSS]) were analyzed.
- Patients' FFPE samples were tested via 5-loci PCR, IHC with 4 antibodies and amplicon NGS panel (Solo-test Driver, OncoAtlas), which analyzed 59 STRs. NGS classified tumors as MSI, MSS or MSE (equivocal results).
- For MMR gene sequencing, Sanger or WES on normal tissue was performed.

Results

- We identified 24 (11.9% of all samples analyzed) samples with positive signal of MSI/dMRR or MMR gene mutation were from patients affected by non-LSS tumors.
- The most commonly observed non-LSS tumors (n=24) were neuroblastoma (n=7) and breast cancer (n=6).
- Among patients with non-LSS tumors, 92% were found to carry MMR gene mutations (somatic/germline). Of those, 8 had confirmed Lynch syndrome, and 5 patients carried mutations in genes (e.g. MLH3) or variants of unknown significance (VUS).
- NGS was successful for 21 (87.5%) of the FFPE samples. Of those, 16 (76.2%) samples were MSS, 2 (9.5%) were MSI and 3 (14.3%) were MSE.
- Agreement between NGS and gold standard methods (IHC, PCR) was lower in tumor types outside LS spectrum than in tumors typical for LS (**Table 1**).
- When comparing MSI vs MSS in common tumors MSI score was 3.5 (mean, 1.1-13.8) vs 0.4 (0.32-0.5), in rare tumors MSI scores were 0.9 (0.64-0.69) vs 0.4 (0.32-0.49). Among patients with rare tumors with IHC results, 5 (24%) had focal staining or loss of single proteins, all of these cases were MSS by NGS.
- Of all non-LSS cases analyzed, only one case was MSI-positive by PCR, where a shift of 1-3 nucleotides was observed, and IHC was consistent with dMMR (loss of MSH2/MSH6). However, NGS results were equivocal (MSE) (**Table 2**).
- Any protein loss by IHC was observed in 8 cases. Of those, 1 case was MSI-positive by NGS. Cases with isolated single protein loss were MSS (n=4) or MSE (n=1).
- Two patients (neuroblastoma, glioblastoma) had validated Lynch syndrome or CMMRD (glioma). These patients had MSS or MSE results (NGS).

	Tumor types outside the Lynch Syndrome spectrum	Tumor common for Lynch Syndrome
PPA	20.0% (4-62%)	83.7% (70-91%)
NPA	92.3% (67-99%)	90.9% (72-98%)
OPA	72.2% (49-88%)	86.2% (76-93%)

Table 1 (left). Agreement between NGS and gold standard methods (PCR or IHC; tumor was considered positive if at least one of the methods indicated MSI/dMMR) in tumor types common and uncommon for Lynch Syndrome

Figure 1. The distribution of NGS-bases MSI scores in common (typical for LS) and rare (non-LSS) tumors. There were two non-LSS MSI-positive cases. MSI scores were higher for common tumors, although the sample size was small.

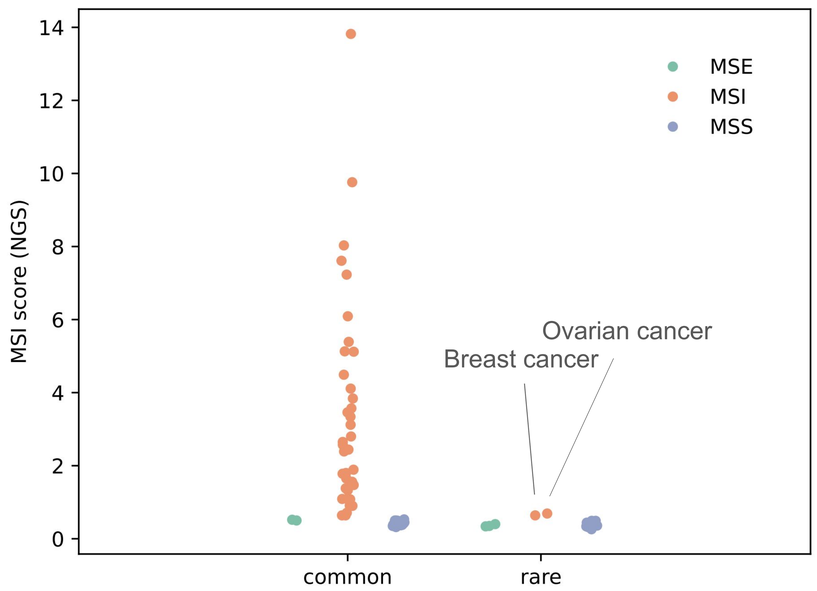

Table 2. Results of gold standard methods (IHC, PCR) and NGS, as well as Lynch syndrome assessment for cases that had MSI or MSE NGS result. Each row represents one case.
*CMMRD – constitutive mismatch repair deficiency syndrome

Tumor type observed	Lynch syndrome	PCR result	IHC result	NGS result
Breast cancer	No, possibly somatic MLH1	MSS	MLH1/PMS2	MSI
Ovarian cancer	No	MSS	pMMR	MSI
Kidney cancer	No, possibly somatic PMS2	MSI	MSH2/MSH6	MSE
Neuroblastoma	Yes, MLH1	MSS	MLH1/PMS2	MSE
Glioma	CMMRD*	MSS	MSH6 (isolated)	MSE

Conclusions

- PCR results may be influenced by common polymorphisms and the lower rate of MSI accumulation in non-LSS tumors.
- In non-LSS tumors, NGS appears to be more informative for MSI analysis as compared to gold standard methods.
- Cases with focal staining/single protein loss are likely MSS.
- Optimized MSI score cutoffs for non-LSS tumors may be more sensitive for the correct stratification of patients.

Poster #3058eP

For contact:
lebedeva@oncoatlas.ru

Disclosures:
All authors declare no conflict of interest.