Why is the panel recommending IHC over running an NGS panel which would include other biomarkers? This is important especially when there is a small amount of specimen/tissue being used. NGS undoubtedly provides more genomic information. However, the specific question the panel is addressing is which testing modality provides the most accurate results for detecting DNA mismatch repair or microsatellite instability. NGS panels perform quite well in detecting DNA MMR and high levels of microsatellite instability in adenocarcinomas from the GI tract. However, there is significant fall-off in the reliability of these tests for cancer types outside the GI tract. From the literature, it looks like NGS assays may need to be optimized for individual cancer types, which is likely not feasible for most clinical laboratories.

Should the MMR IHC and MSI PCR be done simultaneously or sequentially? The panel did not address this specific issue. Certainly, in clinical practice it is often easier to perform these tests simultaneously to get results faster. Keep in mind that the panel found that the accuracy of the standard MSI-PCR assay drops off for cancer types outside of the GI tract. Similar to NGS assays, there is evidence that MSI-PCR could be optimized for individual cancer types.

There are recommendations that are strong but have a limited or low level of evidence. How did the panel arrive at the strength of recommendations? CAP expert panels now employ a well-organized and standardized tool, GRADE (Grading of Recommendations Assessment, Development and Evaluation) Evidence to Decision Framework. In this system, level of evidence is an important factor in the determination of strength of recommendation, but it is not the only factor. Other contributing factors include balance of benefits and harms and considered judgments by the expert panel around values and preferences in relation to outcomes, resources, health equity, accessibility, and feasibility.

Moving forward, it would greatly benefit all CAP guidelines if the strength of the published evidence is improved. The acknowledged gold standard is the prospective, randomized clinical trial. We acknowledge that this gold standard may not be possible or feasible for assessment of biomarkers. A real problem is that many published pathology biomarkers studies use archived tissues that are not systematically collected. The archived (or retrospective) part is not the problem per se. Rather, it how these retrospective samples were collected. In many published studies these archived samples are "convenience" samples that are not systematically collected. The patients who are the sources of the tissues were treated differently over a broad period of time. All these factors introduce bias into the study, which contributes to lowering the certainty of the evidence. Archived tissues can be used, but ideally they would be derived from a prospective trial or from well-defined clinical scenarios in...
which all the patients were treated uniformly. Then, the findings would be validated using different samples from a similarly designed study.

**PD-L1 test should be added to the panel**
PD-L1 testing was out of scope for the panel’s consideration. An acknowledged complicating feature of assessment of patients for checkpoint inhibitor therapy is that PD-L1 appears to be preferred in some cancer types while assessment of MMR/MSI is preferred in others. Right now, there is not good evidence to help guide us on whether all advanced cancers should be tested for PD-L1, MMR, and MSI. It is known, however, that many cancer types do not have overlapping PD-L1 positivity, MMR defects, MSI-high, and high tumor mutation burden (TMB). As an example, in head and neck squamous cell carcinoma, MMR defects are less common, so PD-L1 IHC is a logical first step in assessment. There is currently no data on the utility, if any, of a stepwise addition of further testing in patients with melanoma and lung cancer who have PD-L1 negative tumors.

**Validation on all these “other cancer types” will be a burden for many laboratories. Does the panel suggest how to adequately validate for the different cancer types as suggested in the recommendations?**
As a first step, the panel hopes that publication of the evidence-based guideline will help to promote the idea that these assays (MMR IHC, MSI-PCR, MSI-NGS, and TMB-NGS) are for the most part not interchangeable. One common feature of many past publications is that the methodology for assessing mismatch repair was quite murky. Unfortunately, this meant that for nearly all these studies, it was not possible for the panel to determine which specific tests were associated with the best patient outcomes and survival. The panel recognizes that it would be extremely difficult to optimize multiple NGS assays for individual cancer types. This is one of the factors that contributed to the panel suggesting MMR IHC as a good first step for cancer types outside the GI tract. MMR IHC has its pitfalls as well, however. Interpretation of MMR IHC can be difficult, and many pathology residents and fellows are not specifically trained in its assessment. The good news here is that all of these hurdles can be overcome now that we recognize their existence.

**Do these guideline recommendations only suggest the use of FDA-approved assays? How about those laboratories that do not use these specific assays?**
The expert panel did not examine the issue of FDA-approved assays because it is out of the project scope. The panel recognizes that many clinical laboratories currently use highly effective LDTs to assess MMR IHC, MSI-PCR, and MSI-NGS. It is important that the laboratory follows their local accreditation bodies’ validation requirements.