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EDITORIAL

CYTOLOGICAL SPECIMENS IN THE MOLECULAR ERA OF METASTATIC MELANOMA: FROM DIAGNOSTIC ALTERNATIVE TO PRECISION TOOL

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With the increasing adoption of targeted therapies and immunotherapies, molecular profiling, particularly the assessment of BRAF, NRAS, and KIT mutations, has become integral to therapeutic decision-making in metastatic melanoma.

Current ESMO and ASCO guidelines do not provide explicit, stand-alone recommendations on the use of cytology for the diagnosis of suspected melanoma metastases, although it is widely acknowledged in clinical practice as a minimally invasive diagnostic tool. Both societies emphasize the need for adequate tissue sampling to confirm metastatic disease and to enable comprehensive biomarker testing. In particular, ASCO highlights the importance of obtaining histologic material – preferably through core needle biopsy – when molecular profiling is required to guide systemic therapy. Nevertheless, in selected clinical scenarios, we underline that essential molecular testing, especially for BRAF V600E, may be reliably performed on cytological material and, when positive, can be sufficient to promptly initiate targeted therapy (1, 2).

Indeed, Fine-Needle Aspiration Cytology (FNAC) is tolerated well in these environments and often gives diagnostic biomaterial. Therefore, cytological samples should satisfy the morphological and molecular factors to be clinically applicable (3). Cytological samples, despite being characterized by low cellularity, variable fixation, and the potential for nucleic acid degradation, have been shown to perform as well as histologically determined samples in detecting major molecular alterations when proper pre-analytical and analytical steps are taken (4, 5). The recent technology of NGS and techniques for higher-resolution DNA/RNA extraction have paved the way for cytological samples as a gold standard for molecular diagnosis, thus underscoring that cytopathology has played a key role for precision oncology (6-8). Examples of cytological samples applicable to molecular analysis include direct smear, cell block, needle rinses, and liquid-based methods. Air-dried and alcohol-fixed direct smears provide high-quality nucleic acids and have the advantage of selecting tissues rich in tumor via microdissection (3). Cell blocks (CB), derived from residual material, resemble formalin-fixed paraffin-embedded (FFPE) tissue, thus enabling their compatibility with immunocytochemistry (ICC) and many other ancillary assays (3). Recent improvements in cell block (CB) preparation (*e.g.*, introduction of CytoMatrix) can provide better preservation of cells and yields of nucleic acids (6), allowing to preserve excellent cytomorphological integrity while promoting molecular suitability. To maximize the yield from small tumoral cells samples, the proper disposition of cytologic biomaterial for both smears and ICC and molecular tests is cru-

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cial (3). Tumor cellularity impacts sensitivity to mutation detection greatly. In particular, a greater than 20% tumor fraction is often essential for reproducible polymerase chain reaction (PCR) or NGS-based assays (7). Fixation appears to be more important; indeed, alcohol-fixed or air-dried smears retain nucleic acids more effectively than formalin-fixed cytological biomaterial, but formalin-fixed cytological biomaterial is required for ICC assay (8). The inclusion of the needle rinses is a pragmatic means of obtaining additional tumor material for molecular tests without compromising the quality of cytological confirmation (9). It has been previously established in some studies that cytological and histological samples are highly congruent for BRAF mutation testing. Both Sanger sequencing and real-time PCR have > 95% concordance when detecting BRAF V600E in paired samples (5, 10). For example, mutation-specific immunocytochemistry for the detection of BRAF V600E with the VE1 clone antibody is being used for rapid, low-cost and inexpensive assays used in cell block section (10). These tests seem particularly useful when swift therapeutic decision-making is required or when molecular laboratory access is limited (10). Likewise, NGS to cytological samples has been successfully performed (7, 8), with superimposable results applied to those derived from FFPE tissue. In the case of molecular melanoma, therefore, these results indicate that in managed by validated workflow cytology is also sufficient and usually best (3). Still, some problems persist, despite this progress. The primary limiting factor is low tumor cellularity or tumor cells are distributed heterogeneously, which results in false negative diagnosis (3). The inhibition of DNA extraction and ICC analysis and subsequent adjustments, such as bleaching or image processing, may occur by melanin pigment (3). However, cytopathology has its advantages in relation to histopathology. Indeed, FNACs permit repeat minimally invasive sampling of metastatic sites. Cytological sampling reduces complications due to adverse effects and is generally well tolerated with the appropriate diagnostic and predictive quality (3). Integration of digital cytology with AI-based analysis can also tie cytomorphologic patterns to molecular profiles to predict mutational status and treatment response directly from the process of scanning slides (3). Cytology is thus rapidly developing into a powerful and real-time tool of precision oncology in a practical way (3). For optimal optimization of these for cytological samples for molecular applications, close collaborations within our interdisciplinary work are necessary. Close discussions between cytopathologists, oncologists, and molecular laboratories allow for the most efficient triage and selecting test in a laboratory setting (3, 10). It also means that our laboratories will find that their own laboratories can provide timely and appropriate sample selection (3, 10). Specifically, reflex testing protocols could have been established to auto-submission for BRAF or NGS analysis of cytology-confirmed melanoma metastases, substantially reducing turnaround times. To make such predictive evaluations available (*e.g.*, PDL1 detection) (10, 11), laboratories should define standard adequacy thresholds, enact protocols for non-destructive nucleic acid extraction, and establish storage practices that maintain biomaterial integrity. The reporting should be open to the public, accounting for the type of sample used, an estimated tumor fraction, and analytic sensitivity, highlighting the credibility and interpretive limitations of the analysis for clinicians (3, 10).

The major limitation of cytology in metastatic melanoma lies in predictive immunohistochemistry, particularly for PD-L1 detection, due to the very low clinically significant cut-off. While PD-1/PD-L1 testing is now standard, emerging immune-checkpoint biomarkers such as LAG-3 and TIGIT are under investigation with promising early results, but their evaluation often requires preserved tissue architecture, making cytology alone generally insufficient. Current ESMO and ASCO guidelines recommend adequate tissue sampling – typically via core needle biopsy – for histologic confirmation and molecular testing, although essential biomarkers like BRAF V600 can, in selected cases, be reliably assessed on cytological material to guide targeted therapy (1). In addition, BRAF testing on cytological specimens is a rapid and minimally invasive strategy for therapeutic stratification; however, most routine assays are primarily designed to detect canonical p.V600E mutations. Consequently, non-canonical class II/III variants or rare alterations may be missed, despite their potential clinical relevance and eligibility for targeted-therapy trials. In selected clinical contexts, negative cytology-based results should be confirmed with a second level testing, such as NGS, or alternatively because of lack of a significant number of neoplastic cells the core biopsy is needed (12).

In conclusion, cytology represents a valuable and pragmatic alternative to conventional biopsy with histological examination, offering minimal invasiveness, rapid turnaround time, and the possibility of repeated sampling. Nonetheless, its limitations

include reduced architectural assessment and, critically, limited tumor cellularity, which may constrain broad molecular profiling (*e.g.*, NGS) and the application of predictive immunohistochemistry. This is particularly relevant for PD-L1 assessment, whose evaluation may be challenging on scant material and is of specific clinical interest in metastatic melanoma. Beyond diagnosis, cytology remains pivotal in the assessment of suspicious lymph node metastases for staging purposes, enabling timely therapeutic stratification and potential access to neoadjuvant immunotherapy strategies associated with significant pathological responses and improved outcomes in selected patients.

FUTURE PERSPECTIVES

The future of cytology in molecular diagnostics is promising. Ongoing advances in low-input sequencing technologies, multiplex PCR, and microfluidic DNA extraction methods are making cytology-based molecular testing increasingly feasible and reliable. Expanded gene panels using compact platforms, as well as RNA fusion detection, can now be successfully performed even on limited material, thereby broadening the genomic scope assessable on cytological specimens.

Beyond DNA analysis, transcriptomic and proteomic approaches are progressively being applied to cytological preparations, offering the potential to integrate cytomorphology with functional molecular data. Artificial intelligence tools may further strengthen this integration by predicting molecular alterations directly from digital images and optimizing sample selection for downstream assays (1-8).

In conclusion, cytological specimens have evolved from a purely diagnostic tool to an integral component of the molecular diagnostic armamentarium in metastatic melanoma. They reflect the principle of obtaining maximal clinically relevant information through minimally invasive procedures. Within a well-coordinated multidisciplinary framework, current evidence supports cytology as a robust and reproducible substrate for molecular testing. Continued progress will depend on harmonized workflows, standardized quality assurance protocols, and expert-driven consensus guidelines for cytological material in melanoma. The formal validation of cytology as a molecular diagnostic platform will enhance precision oncology and facilitate timely, patient-centered testing in routine clinical practice.

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Publication ethics

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