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Commentary

Cancer of unknown primary site (CUP), practice-changing diagnosis and therapy in the molecular era: a commentary

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Introduction

Cancer of unknown primary (CUP) is a clinicopathological syndrome of metastatic cancers without an identifiable anatomical primary site and has remained a diagnostic and treatment enigma for decades. Clinically occult primary tumor sites have been found by autopsies in about 75% of cases from more than twenty different sites [1], but some primaries are buried deep in tissues/organs and would require thousands of blind tissue sections to find. The hidden primary tumors within the CUP syndrome seem to represent a small percentage of many different cancers which share a common presentation. The mechanisms responsible for very small clinically undetectable primary tumors that do not grow larger but produce metastasis which grow and spread remains a puzzle, however it is likely explained by unique genomic, immunologic, transcriptomic and/or epigenetic alterations [2].

The CUP diagnosis is not rare. The 2025 incidence in the United States was 37,350 patients [3], greater than the annual incidence of multiple myeloma (36,110), chronic lymphocytic leukemia (23,690), extensive stage small-cell lung cancer (33,997) and many other *de novo* stage IV metastatic cancers. A major difficulty in the past was the inability to determine the primary cancer type in most patients which has traditionally been required in order to plan site-specific therapies. CUP has been considered by many as a unique single type of cancer with a common clinical behavior/biology. Therefore, without the means to diagnose specific cancers broad spectrum empiric chemotherapy (one size fits all) became the standard for the last four decades but yielded dismal outcomes (median survival 9–12 months) [2,4]. The development of gene expression profiling (GEP) and comprehensive molecular profiling (CMP)/next generation sequencing delivered new genomic insights, becoming the operational levers for molecular diagnosis, classification and characterization thus fulfilling a critical unmet need and has transformed the management of many CUP patients.

Advances in Molecular Diagnostics

The previous belief that CUP was a single cancer type is no longer tenable and is supported by considerable clinicopathological and molecular evidence [5]. There has been a fundamental change in diagnosis, classification and treatment of the many cancers within the CUP syndrome primarily related to advances in molecular profiling. Clinicopathological evaluation and molecular testing with both GEP for tissue of origin diagnosis and CMP for detection of genomic alterations represent dramatic inflection points for management of patients providing new improved avenues for both organ specific and agnostic therapies.

The molecular revolution in CUP diagnosis and characterization has unveiled the concealed

heterogeneity of CUP. GEP has been particularly important in revealing multiple specific cancer types with a diagnostic accuracy of 80%-90% [2] allowing for site-specific precision-based therapy rather than nonspecific empiric chemotherapy. A presumptive primary-defined primary tumor diagnosis has become one of the critical factors in establishing care for CUP patients [2,4]. There are platform variations in GEP assays and other limitations in their diagnostic capabilities including the inability to diagnose cancers not in their developmental databases/libraries of cancers they recognize. Therefore, most rare cancers are not in their databases and cannot be diagnosed by molecular profiling alone. There are occasional overlapping diagnoses of certain cancers with somewhat similar molecular profiles (pancreaticobiliary carcinomas, ovary/endometrium, breast/salivary gland carcinomas) which can occasionally lead to an erroneous diagnosis. However, even with these diagnostic limitations GEP remains relatively accurate and requires interpretation in the context of clinicopathological features.

CMP platforms are also variable and have limitations but some may be capable of accurately diagnosing primary tumors but have not yet been adequately validated in CUP. The ability of CMP to detect actionable biomarkers in many different known cancers has facilitated molecular targeted therapy and immunotherapy either alone or as components with chemotherapy and the prognosis of many patients with known advanced cancers has been remarkably improved. Furthermore, actionable biomarkers are often shared across the spectrum of neoplastic diseases providing the basis for agnostic targeted therapy which is now applicable for many advanced cancers. Molecular assays are continuing to improve having now ventured into liquid biopsies with ctDNA/cfDNA analysis which are useful [2]. Molecular studies have not yet revealed an alteration unique to CUP or a CUP specific therapeutic target but have revealed multiple actionable alterations consistent with the many different cancers within the CUP syndrome.

Implications for TNM Staging

There has been no staging system for most CUP patients since an anatomical primary tumor was not detectable. In the most recent American Joint Committee on Cancer (AJCC) TNM staging system manual [6] CUP was only briefly addressed and lacked defined staging criteria for most solid tumors. However, the primary tumor category T0 (no evidence of primary tumor) was listed as a possibility for establishing a specific presumptive occult primary tumor for all solid tumors but only if all data (clinicopathological, molecular, others) highly suggested a primary tumor site. A few rare CUP examples were illustrated including T0 occult breast carcinoma (women with axillary node carcinoma consistent with breast carcinoma), T0 squamous carcinoma in neck nodes (nasopharyngeal carcinoma-Epstein-Bar virus mediated and oropharyngeal carcinoma- human papillomavirus mediated), and T0 unknown primary cutaneous melanoma [2,4,6]. These patients along with a few other subgroups with clinicopathologic features highly suggestive of a occult primary represent about 15–20% of all CUP patients and have been historically defined as "favorable CUP" since their prognosis has been substantially better with site-specific therapy (similar to the same cancers with identifiable primaries) [2,4] and superior to the 80–85% of whom a primary tumor could not be assumed who were designated "unfavorable CUP".

Today several other CUP patients within the unfavorable group may have their presumptive primaries-defined by the combined use of clinicopathological evaluation and molecular testing making them eligible for TNM staging (T0 primary tumor type) as a specific metastatic cancer type [4]. Therefore, assigning TNM T0 specific cancers and treatment for that cancer has critical therapeutic implications as opposed to nonspecific empiric chemotherapy. These CUP patients are best defined as presumptive primary-defined CUP subsets (CUP/Lung, CUP/prostate, CUP/breast, CUP/colorectal, CUP/renal, etc.). These designations will benefit clinicians in directing care for their patients and eventually pending further therapeutic comparative studies the CUP label may no longer be necessary.

Therapeutic Decision Framework

Two recent prospective randomized controlled studies [7,8] as well as a systematic review and meta-analysis [9] have documented the critical clinical role of both GEP-guided site-specific precision-based therapies [7] and CMP-guided agnostic targeted therapies/immunotherapies [8]. Both approaches significantly improve the survival of patients compared to the decade's old standard of empiric chemotherapy and represent a paradigm shift in patient management. These recent positive results have created a therapeutic dichotomy for those previously defined as unfavorable CUP. A stepwise algorithm for the new CUP management is illustrated in **Table 1**.

A presumptive primary tumor diagnosis has become one of the critical factors in establishing care for CUP patients [2,4,7,9]. Initial empiric chemotherapy or immediate agnostic targeted therapy should be tempered in most patients in order to better define, if possible, their specific cancer types. CUP may be considered the poster child of metastatic cancers in the molecular era as most patients require

Table 1. Stepwise algorithm for CUP management.

1.	**Initial Diagnostic Evaluation: Clinical assessment, histopathology, immunohistochemistry and medical imaging; favorable versus unfavorable CUP**
2.	**Molecular testing:** GEP and CMP testing from tissue or liquid biopsy; diagnosis of presumptive primary-defined CUP subsets and recognition of actionable alterations and biomarkers.
3.	**Selection of treatment:** Favorable CUP-site-specific therapy; Integration of molecular and clinicopathological information in unfavorable CUP; options depend on the findings and include site-specific therapy, agnostic targeted therapy, empiric chemotherapy or clinical trials as listed below. **3a. Presumptive primary-defined CUP subset diagnosed:** site-specific precision- based therapy **3b. Actionable biomarker found without presumptive primary-defined CUP subset diagnosis**: agnostic targeted therapy **3c. No Actionable biomarker found and without presumptive primary-defined CUP subset diagnosis:** empiric chemotherapy or novel clinical trials

genomic testing at diagnosis before planning therapy. Patients with suspected CUP require a multipronged evaluation to exclude an identifiable anatomical primary site. The evaluation should include clinicopathological findings (history, physical examination, medical imaging, histology, immunohistochemistry and selected serum tumor markers) along with GEP and CMP. Standard histopathology and immunohistochemistry staining of a biopsy may highly suggest a primary site in about 33% of cases by the combination of positive and negative stains [2]. The addition of GEP and CMP platforms performed on biopsies or blood (liquid biopsies) used in concert with standard clinicopathological features improves the presumptive diagnosis of the primary site to about 80%–90% [2,4] of patients and makes them eligible for TNM staging (primary tumor=T0). CMP is also essential in all patients since a substantial number have actionable molecular targets. Molecular findings have become as important as standard pathologic findings in diagnosis and therapy selection.

Molecular targeted therapy and immunotherapy either used alone or combined with chemotherapy have become the new improved standards for several known solid tumors many which present as CUP including those from the lung, melanoma, breast, kidney, urothelial tract, gastroesophageal junction/gastric, liver, biliary tract and others. Agnostic targeted therapy and immunotherapy becomes a major consideration as a component of first-line therapy in CUP depending on whether or not a presumptive primary -defined subset is diagnosed. The choice of therapy is often difficult since several targeted therapies and immunotherapies are indicated along with chemotherapy or used alone in many known metastatic cancers and may be the preferred therapy if a presumptive primary-defined diagnosis is made of a responsive tumor. If a presumptive primary tumor diagnosis is not associated with a beneficial or minimally beneficial standard therapy or in those without a presumptive primary tumor diagnosis, agnostic therapy should be considered in those with actionable biomarkers.

The number of beneficial agnostic therapies is rapidly expanding [10] and now includes targeting biomarkers (BRAF V600E mutations, HER2 alterations, NTRK fusions, RET fusions, microsatellite instability-MSI-High-MSI-H/dMMR, tumor mutation burden-TMB high and a high percentage immunohistochemistry PDL-1 positive stain.

A small minority of patients fall through the cracks and have neither a presumptive primary-defined diagnosis or an actionable biomarker placing them in a category to receive either empiric chemotherapy or novel clinical trials including investigations of potentially new biomarkers.

Summary and Future Directions

Aggregate data clearly show the CUP syndrome is comprised of many different cancers with varying biologies. Although patients share a common clinical presentation, the concept of CUP as a single cancer type should be retired [5]. Molecular profiling coupled with standard clinicopathological evaluation has become paramount for the diagnosis of a presumptive primary-defined cancer and actionable biomarkers guiding site-specific precision-based or agnostic therapy for many patients rather than empiric chemotherapy. Although molecular findings are not helpful to plan therapy for every patient, the stakes have become very high for those with unmasked specific cancers, particularly given the documented remarkable effectiveness of molecular targeted therapy and immunotherapy for many advanced cancers. GEP and CMP are complementary and not mutually exclusive tests necessary for optimal management [2,4,7–9].

The most recent clinical practice guidelines of the National Comprehensive Cancer Network (NCCN) and the European Society of Medical Oncology (ESMO) for the diagnosis and therapy for CUP patients did not recommend GEP in their most recently published clinical practice guidelines. Their decision was based on the results of two randomized controlled studies started more than thirteen years ago [2,9] before the advent of molecular targeted therapies and immunotherapies which did not support GEP guided therapy. The recent randomized studies reporting significant superiority of molecular guided therapies versus the old standard of empiric chemotherapy [7,8] should change NCCN and ESMO recommendations in their anticipated updated guidelines and hopefully facilitate more general implementation of molecular testing.

Further studies are required of the emerging technologies associated with the ongoing evolution and integration of multi-omics with artificial intelligence [11] which will likely further improve the ability to more accurately diagnose the tissue of origin, recognize actionable molecular targets, and find new biomarkers predictive of responses to molecular guided therapies. The complex array of genomic diseases termed “cancers” will in the future likely be precisely and successfully treated based on an understanding of genomic/transcriptomic/epigenetic/immunologic mechanisms and defining primary tumor sites may no longer be clinically relevant.

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