DIAGNOSIS

Improved diagnosis, therapy and outcomes for patients with CUP

F. Anthony Greco

Molecular cancer-classifier assays enable the diagnosis of a single cancer type for most patients with cancer of unknown primary (CUP), thus opening the door to the administration of site-specific therapies. Herein, I discuss how such therapies can improve the survival of patients with CUP, and the resulting paradigm shift towards tissue-of-origin diagnostics and treatments that is now becoming the standard of care for this patient population.

Refers to Moran, S. *et al.* Epigenetic profiling to classify cancer of unknown primary: a multicenter, retrospective analysis. *Lancet Oncol.* **17**, 1386–1395 (2016)

Historically, the treatment of patients with cancer of unknown primary (CUP) has been futile: these patients presented with metastatic cancers without a clinically detectable primary tumour, and thus could not be assigned a definitive tissue-of-origin cancer diagnosis — except upon autopsy. Analysis of serially collected autopsy samples from 884 patients with CUP[1] revealed that around 75% of the patients had only small (clinically occult) invasive primary tumours, at more than 25 disparate anatomical sites — most being carcinomas. Three decade ago, few metastatic cancers were known to be highly responsive to chemotherapy (predominantly lymphomas, and germ-cell, breast, or ovarian cancers) and those with metastases originating from these malignancies could not usually be identified in the wider group of patients with CUP.

In the 1980s, several patient subsets (accounting for 15% of patients with CUP) with more-favourable prognoses were recognized on the basis of clinical characteristics, such as sex, sites of metastasis, and/or histopathology[2]. Examples include patients with squamous-cell carcinoma in lymph nodes of the neck, women with axillary adenocarcinoma, and women with peritoneal serous adenocarcinoma — presumably reflecting primary tumour sites in the head and/or neck, breast, and ovary, respectively. Patients in these subgroups were intuitively treated with the same regimens used in the treatment of patients with metastatic lesions at the same sites and a detectable primary tumour; the outcomes were similar and superior to those observed in the overall population of patients with CUP. Indeed, the inability to detect the origin of the cancer was the only clinical difference between patients in these CUP subgroups and their counterparts with known primary tumours.

> “…the diagnosis of a cancer type in patients with CUP should guide the choice of site-specific therapy…”

Throughout the 1990s and early 2000s, empirical approaches to chemotherapy were evaluated in the 85% of patients with CUP not classified as being in a favourable subgroup[2], following the rationale that the origin of their cancers was unknown, and assuming that CUP comprised a biologically similar group of cancers. However, only a small minority of these patients obtained a clinical benefit from empirically determined regimens (with a median overall survival duration of 9 months)[2].

In the past decade, advances in immunohistochemistry (IHC) assay protocols[2], and, subsequently, the advent of molecular cancer-classifier assays (MCCA) have improved the diagnosis of cancer type and/or tissue of origin, leading to changes in care with appreciable clinical benefits for many patients with CUP[2,3]. Site-specific therapies have been recommended for a few selected patients with CUP based on immunohistochemical diagnoses alone[4], including those with a diagnosis of lymphoma, melanoma, or sarcoma, or a thyroid or germ-cell carcinoma. Improved outcomes of such patients treated with site-specific therapies have never been proven in phase III randomized studies; however, retrospective studies and anecdotal reports support the accuracy of these diagnoses, as well as the associated improvements in outcomes[2].

The diagnostic benefit of performing an MCCA in patients with CUP (that is, the ability to predict the cancer type) is now well established[2–5]. Only a minority (about 30%) of patients with CUP receive an accurate single-cancer type diagnosis based on IHC analyses[5,6]. MCCAs complement IHC and, when used in concert with assessments of clinical features, histopathology and IHC, enable a tissue-of-origin diagnosis in >90% of patients[2,3,5]. Such diagnoses are key for the recommendation of personalized therapies, and particularly important for patients harbouring cancers that are known to be responsive to specific therapies. Earlier this year, Moran *et al.*[6] reported the results of a multicentre retrospective study performed in 216 patients with CUP to evaluate the diagnostic value of an MCCA (EPICUP) based on an epigenetic DNA microarray platform that can enable identification of 38 different cancer types. A single tissue of origin (encompassing a total of 23 cancer types) was predicted for 188 of the 216 patients (87%)[6]; the use of EPICUP was accurate in predicting the cancer type in those patients who, months later, had a latent primary tumour identified (33 of 38 patients; 87%), and the MCCA findings were 100% in agreement with a single IHC-based diagnosis made for another 37 patients[6]. The main highlight of this retrospective study is that survival outcomes were positively correlated with the MCCA diagnosis for patients who had received site-specific therapy[6]. Clinical data supporting these conclusions were available for 114 patients: for the 31 patients who received chemotherapy that would be

considered standard-of-care and useful based on the MCCA prediction, the median overall survival duration was 13.6 months, compared with 6 months for the 61 patients who received empirically selected chemotherapy that was not considered the standard-of-care in light of the MCCA-based diagnoses ($P = 0.0051$)[6]. These data add to the growing evidence that MCCA-based selection of site-specific therapy has a positive effect on the outcomes of many patients.

The findings of Moran and co-workers[6] complement those from two previous prospective, and several retrospective studies. Hainsworth *et al.*[7] reported a multicentre prospective study of a 92-gene real-time/reverse transcriptase PCR (RT-PCR) assay (CancerTYPE ID) for the diagnosis of tissue of origin in 252 patients with CUP; a single tissue of origin (of one of 26 cancer types) was predicted for 247 of the patients (98%). The median overall survival duration was 12.5 months for the 194 patients who received site-specific therapies selected according to the MCCA-based diagnosis, compared with 9.1 months for the 396 patients in a historical control group who received empirical chemotherapy[7]. In addition, the median overall survival duration was 13.4 months for those patients with cancer types expected to be more-responsive (for example, germ cell, colorectal, breast, ovarian or renal cancer) to MCCA-selected site-specific therapy ($n = 115$) compared with 7.6 months for patients with cancers expected to be less responsive (examples include biliary tract, pancreatic, gastroesophageal and hepatocellular cancer) to therapies ($n = 79$ patients; $P = 0.04$)[7].

Yoon *et al.*[8] conducted a prospective study in 45 patients, all of whom received carboplatin, paclitaxel, and everolimus. Of these patients, 38 underwent MCCA testing to determine the tissue of origin of their tumour; the median overall survival duration of the 19 patients with cancers expected to respond favourably to the empirical treatment regimen delivered was 17.8 months, compared with 8.3 months for the other 19 patients who were not expected to respond favourably ($P = 0.005$). In three retrospective studies in patients with CUP who received an MCCA-based[9,10] ($n = 74$) or IHC-based[11] ($n = 74$) colorectal tissue-of-origin diagnosis, patients received site-specific therapies. For these patients, the median overall survival duration ranged from 21–37 months, a finding consistent with the values observed for patients with advanced-stage cancer originating from a known primary colorectal carcinoma. Another 22 patients who received an MCCA-based diagnosis of renal-cell carcinoma with an intermediate-to-poor risk category and, in most cases, who received sunitinib treatment, had a median overall survival duration of 13.4 months[2], consistent with the survival duration observed in patients with known metastatic renal-cell carcinoma. Finally, in another report[12], an MCCA-based single cancer type diagnosis was made in 25 of 30 patients with poorly-differentiated CUP (83%) that had not been specifically lineage-clarified following an extensive IHC analysis. Many of these patients had cancers considered to be highly responsive to treatment, such as lymphoma, germ-cell tumours, or melanoma. Seven patients received MCCA-diagnosis-based prospective therapies, and five of them remain without recurrence for periods ranging from >25 months to >72 months.

The importance of determining the cancer of origin in patients with CUP is more critical now than it was in the past. Patients with a variety of metastatic cancers can obtain survival benefits from the appropriate use of therapies and, in many instances, from multiple sequential therapies. Thus, the diagnosis of a cancer type in patients with CUP should guide the choice of site-specific therapy and also, for some cancer types (such as breast, colorectal, gastroesophageal junction, gastric, or lung cancers, or melanoma) prompt testing for known actionable genetic markers and/or therapeutic targets. The expanding number of advanced-stage malignancies for which immune-checkpoint inhibitors are recommended highlights the importance of considering these agents when these tumour types are diagnosed by IHC and/or MCCA in patients initially presenting with CUP. The importance of specific diagnoses for patients with CUP will further increase as therapies continue to improve for several difficult-to-treat cancers.

The studies using MCCA-based diagnoses discussed herein have limitations, but improved outcomes were reported in all of them for those patients diagnosed with cancer types for which effective therapies that improve survival were available. These aggregate data validate the diagnostic accuracy of MCCA — the improved outcomes are likely real, rather than a function of the shortcomings of the study designs. The consistency in the collective data is sufficient to support a change in the standard management of patients with CUP, now that the tissue of origin can usually be reliably determined. CUP biopsy samples should be tested using an IHC panel and, if necessary, an MCCA. Once a single cancer type is diagnosed, site-specific therapies should be considered for that patient, particularly if the diagnosed cancer type is known to be responsive to therapy; empirically selected chemotherapy should only be administered for the remaining few patients with unresolved CUP. In the absence of large-scale, adequately powered, randomized phase III trials, compelling data now support the use of this new management paradigm.

F. Anthony Greco is at the Sarah Cannon Cancer Center, Tennessee Oncology, 250 25th Avenue North Suite 100, Nashville, Tennessee 37203, USA.
fgreco@tnonc.com

doi:10.1038/nrclinonc.2016.194
Published online 29 Nov 2016

1. Pentheroudakis, G. Golfinopoulos, V. & Pavlidis, N. Switching benchmarks in cancer of unknown primary: from autopsy to microarray. *Eur. J. Cancer* **43**, 2026–2036 (2007).
2. Greco, F. A. & Hainsworth, J. D. in *Cancer: Principles and Practice of Oncology* 10th edn (eds DeVita, V. T., Lawrence, T. S. & Rosenberg, S. A.) 1720–1737 (Lippincott Williams and Wilkins, 2014).
3. Hainsworth, J. D. & Greco, F. A. Gene expression profiling in patients with carcinoma of unknown primary site: from translational research to standard of care. *Virchows Arch.* **464**, 393–402 (2014).
4. Ettinger, D. S. *et al.* Occult primary, version 3.2014. *J. Natl Compr. Canc. Netw.* **12**, 969–974 (2014).
5. Greco, F. A., Lennington, W. J. Spigel, D. R. & Hainsworth, J. D. Molecular profiling diagnosis in unknown primary cancer: accuracy and ability to complement standard pathology. *J. Natl Cancer Inst.* **105**, 782–790 (2013).
6. Moran, S. *et al.* Epigenetic profiling to classify cancer of unknown primary: a multicenter, retrospective analysis. *Lancet Oncol.* **17**, 1386–1395 (2016).
7. Hainsworth, J. D. *et al.* Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon Research Institute. *J. Clin. Oncol.* **31**, 217–223 (2013).
8. Yoon, H. H. *et al.* Gene expression profiling identifies responsive patients with cancer of unknown primary treated with carboplatin, paclitaxel, and everolimus: NCCTG N0187 (alliance). *Ann. Oncol.* **27**, 339–344 (2016).
9. Hainsworth, J. D., Schnabel, C. A. Erlander, M. G. Haines, D. W. & Greco, F. A. A retrospective study of treatment outcomes in patients with carcinoma of unknown primary site and a colorectal molecular profile. *Clin. Colorectal Cancer.* **11**, 112–118 (2012).
10. Greco, F. A., Lennington, W. J., Spigel, D. R. Varadhachary, G. R. & Hainsworth, J. D. Carcinoma of unknown primary site: outcomes in patients with a colorectal molecular profile treated with site specific chemotherapy. *J. Cancer Ther.* **3**, 37–43 (2012).
11. Varadhachary, G. R. *et al.* Carcinoma of unknown primary with gastrointestinal profile: immunohistochemistry and survival data for this favorable subset. *Int. J. Clin. Oncol.* **19**, 479–484 (2014).
12. Greco, F. A., Lennington, W. J. Spigel, D. R. & Hainsworth, J. D. Poorly differentiated neoplasms of unknown primary site: diagnostic usefulness of a molecular cancer classifier assay. *Mol. Diagn. Ther.* **19**, 91–97 (2015).

Competing interests statement
F.A.G. is on the Speakers' Bureau and serves as a consultant for Biotheranostics, Inc.