Identifying the Primary Site Using Gene Expression Profiling in Patients with Carcinoma of an Unknown Primary (CUP): A Feasibility Study from the GEFCAPI

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Carcinomas of an unknown primary site (CUP) rank among the 10 most frequent cancers worldwide and its prognosis is notoriously poor with median survival rates attaining 8 months [1]. CUP are heterogeneous tumors whose origin is unidentifiable at the time of the diagnosis but they share the unique clinical feature of metastatic disease. New molecular diagnosis tools with DNA array could be a promising technology in the search of lost CUP origins [2, 3]. Using this large database, Horlings developed a diagnostic gene expression based classifier (CupPrint[®], Agendia BV, Amsterdam, The Netherlands) in order to diagnose the histogenic background of CUP in daily practice [4]. The CupPrint assay was, at the time of the study, commercialized by Agendia.

We report here the results of a prospective study of feasibility conducted by the GEFCAPI (Groupe d'Etude Français des Carcinomes de site Primitif Inconnu), in collaboration with the Agendia team [4]. The aim of this study was to evaluate the feasibility of integrating an oligonucleotide microarray, that contains 495 genes selected as highly differentially expressed between 49 tumor types (CupPrint). The sample's gene expression profile is compared to a previously built tumor gene expression data set encompassing 49 primary tumor types to identify the tissue of origin in patients with CUP. Formalin-fixed, paraffin-embedded (FFPE) specimens from diagnostic biopsies of 22 patients with CUP were prospectively collected. The assay was successfully performed on

specimens from 18 of the 22 patients (82%). In the remaining 4 cases the test could not be performed because of low RNA preservation in the specimens. Results concerning the 18 patients are reported in the table 1, with 5 suggested tumor types according to the gene expression profile and final suggested diagnosis, integrating analysis of medical history, immuno-histochemistry, and type of biopsy. The median age of the patients was 54 years (range: 29–70 years). The median delay from tissue shipping to receipt of CupPrint result was 11 days (range: 1–26 days). The most common tissues of origin identified were lung cancer (22%) and colorectal cancer (17%). Of note, primary cancers which would not be adequately treated by an empiric chemotherapy regimen currently recommended in CUP (such as cisplatin-gemcitabine or carboplatin-paclitaxel) was identified in about half of the patients: kidney cancer (1), hepatocarcinoma (1), colorectal cancer (3), head and neck cancer (2), cholangiocarcinoma (1) (table 1).

At least two clinical cases suggested the potential implication of adjusting treatment according to the suggested primary. The first patient, a 50-year-old woman (patient 1, table 1), presented with a swollen left inguinal lymph node. The biopsy specimen revealed an undifferentiated carcinoma, ck7–, ck20–, RE–, PS100–, EMA– (07/2008). The CupPrint suggested the diagnosis of breast cancer, melanoma, or oral carcinoma. She underwent an inguinal dissection that showed a metastasis from melanoma (PS100 and vimentin positive). The dermatological examination revealed a lesion of the skin on the left knee. Surgical excision and histology confirmed the diagnosis of a superficial melanoma, SSM level 2, 0.44 mm deep. The patient is still in remission.

The second case, a 63-year-old woman, presented with swollen left supra-clavicular, mediastinal and axillary lymph nodes (patient 2, table 1). The axillary lymph node dissection revealed an epidermoid carcinoma (ck7+, 20–). The CupPrint

Table 1. Suggested primary diagnosis (5 preliminary diagnoses and final considered diagnosis) with CupPrint for the 18 patients (pt)

Pt	1st tumor type	2nd tumor type	3rd tumor type	4th tumor type	5th tumor type	final suggested diagnosis
1	melanoma	breast cancer	breast cancer	small cell lung cancer		breast cancer
2	hypopharyngeal cancer	hypopharyngeal cancer	hypopharyngeal cancer	non small cell lung cancer	urothelial carcinoma	hypopharyngeal cancer
3	urothelial carcinoma	urothelial carcinoma	non-small cell lung cancer	non-small cell lung cancer	hepatocarcinoma	urothelial carcinoma
4	breast cancer	hypopharyngeal cancer	urothelial carcinoma	urothelial carcinoma	non-small cell lung cancer	urothelial carcinoma
5	cholangiocarcinoma	cholangiocarcinoma	cholangiocarcinoma	hepatocarcinoma	hepatocarcinoma	pancreatic carcinoma
6	non-small cell lung cancer	pancreatic carcinoma	cholangiocarcinoma	adenocarcinoma of the colon/rectum	urothelial carcinoma	non-small cell lung cancer
7	hepatocarcinoma	hepatocarcinoma	hepatocarcinoma	hepatocarcinoma	hepatocarcinoma	adenocarcinoma of the colon/rectum
8	cholangiocarcinoma	pancreatic carcinoma	non-small cell lung cancer	pancreatic carcinoma	cholangiocarcinoma	cholangiocarcinoma
9	adenocarcinoma of the colon/rectum	adenocarcinoma of the colon/rectum	adenocarcinoma of the colon/rectum	breast cancer	small bowel carcinoma	adenocarcinoma of the colon/rectum
10	non-small cell lung cancer	non-small cell lung cancer	non-small cell lung cancer	non-small cell lung cancer	meningioma	non-small cell lung cancer
11	small cell lung cancer	small cell lung cancer	small cell lung cancer	non-small cell lung cancer	squamous cell carcinoma of the skin	small cell lung cancer
12	hypopharyngeal cancer	hypopharyngeal cancer	oral cavity carcinoma	cervix squamous cell carcinoma	cervix squamous cell carcinoma	hypopharyngeal cancer
13	renal cell cancer	renal cell cancer	renal cell cancer	urothelial carcinoma	renal cell cancer	renal cell cancer
14	mesothelioma	mesothelioma	mesothelioma	mesothelioma	mesothelioma	mesothelioma
15	ovarian carcinoma	ovarian carcinoma	endometrial carcinoma	ovarian carcinoma	ovarian carcinoma	ovarian carcinoma
16	hepatocarcinoma	hepatocarcinoma	hypopharyngeal cancer	non-small cell lung cancer	non-small cell lung cancer	hepatocarcinoma
17	non-small cell lung cancer	non-small cell lung cancer	hypopharyngeal cancer	urothelial carcinoma	adenocarcinoma of the colon/rectum	non-small cell lung cancer
18	adenocarcinoma of the colon/rectum	adenocarcinoma of the colon/rectum	adenocarcinoma of the colon/rectum	adenocarcinoma of the colon/rectum	non-small cell lung carcinoma	adenocarcinoma of the colon/rectum

suggested that the diagnosis was a hypopharyngeal carcinoma. Treatment with 4 cycles of cisplatin, 5-fluorouracil, and cetuximab resulted in a partial response (12/2008). Then she received concomitant radio-chemotherapy which led to tumor regression. The patient is still in complete remission (07/2009).

In the present study, we showed that CupPrint diagnosis is feasible in daily practice without delaying treatment, and may change the chemotherapy regimen and clinical management in more than 50% of patients. This new diagnosis tool could improve the prognosis in CUP patients, as suggested by Bridgewater et al. [5]. The feasibility of integrating a gene expression analysis allowed us to elaborate the GEFCAP-04

trial. This randomized phase III trial is planned to compare therapy based on gene expression-suspected primary cancer versus empiric chemotherapy. The main objective will be to demonstrate a benefit in progression free survival in patients receiving 'individualized' treatment, including chemotherapy and targeted therapies.

Disclosure Statement

None of the authors declares a conflict of interest.

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