European Journal of Cancer 94 (2018) 179–186

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Comprehensive analysis of cancers of unknown primary for the biomarkers of response to immune checkpoint blockade therapy

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Received 1 December 2017; received in revised form 16 February 2018; accepted 20 February 2018

KEYWORDS
Cancer of unknown primary (CUP);
Targeted therapy;
Immune checkpoint inhibitors

Abstract ***Background:*** Cancer of unknown primary (CUP) accounts for approximately 3% of all malignancies. Avoiding immune destruction is a major cancer characteristic and therapies aimed at immune checkpoint blockade are in use for several specific cancer types. A comprehensive survey of predictive biomarkers to immune checkpoint blockade in CUP were explored in this study.

Methods: About 389 cases of CUP were analysed for mutations in 592 genes and 52 gene fusions using a massively parallel DNA sequencing platform (next-generation sequencing [NGS]). Total mutational load (TML) and microsatellite instability (MSI) were calculated from NGS data. PD-L1 expression was explored using immunohistochemistry (with 5% cutoff value).

Results: High TML was seen in 11.8% (46/389) of tumours. MSI-high (MSI-H) was detected in 7/384 (1.8%) of tumours. Tumour PD-L1 expression was detected in 80/362 CUP (22%). A small proportion of CUP cases harboured genetic alterations of negative predictive biomarkers to immune checkpoint inhibitors (predictors to hyperprogression) including *MDM2* gene amplification (2%) and loss of function *JAK2* gene mutations (1%). Amplifications of *CD274 (PD-L1)* and *PDCD1LG2 (PD-L2)* genes were also rare (1.4% and 0.8%, respectively). The most frequently mutated genes were *TP53* (54%), *KRAS* (22%), *ARID1A* (13%), *PIK3CA* (9%), *CDKN2A* (8%), *SMARCA4* (7%) and *PBRM1, STK11, APC, RB1* (5%, respectively).

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https://doi.org/10.1016/j.ejca.2018.02.021

Conclusions: Using a multiplex testing approach, 28% of CUP carried one or more predictive biomarkers (MSI-H, PD-L1 and/or TML-H) to the immune checkpoint blockade, providing a novel option for treatment in patients with CUP.

1. Introduction

Cancer of unknown primary (CUP) are a heterogeneous group comprising approximately 3–5% of all malignancies and are associated with poor prognosis [1–3]. Usually, extensive tumour sample investigations are performed to identify the presumed tissue of origin [3–5], but in true CUP, by definition, the diagnosis of the primary cancer cannot be verified. Recently, we [6] and others [7–10] have identified numerous genetic alterations in common cancer pathways [11] in CUP, providing an opportunity to administer pathway-specific (targeted) therapies in CUP. All these studies identified at least one clinically targetable genetic alteration in CUP. In contrast to the previous studies, we utilised an extended next-generation sequencing (NGS) panel composed of 592 genes and used Archer Panel to explore the gene fusions.

In the last couple of years, a dramatic improvement in advanced cancers therapy has been achieved with immune checkpoint blockade. To date, five immune checkpoint inhibitors (pembrolizumab, nivolumab, atezolizumab, avelumab and durvalumab) targeting either programmed death 1 (PD-1) or its ligand (PD-L1) have received the US Food and Drug Administration (FDA) approval (https://www.fda.gov/) and caused a paradigm shift in treatment of various cancer types including melanoma, non–small cell lung carcinoma, renal cell carcinoma, advanced bladder carcinoma, Merkel cell carcinoma, gastroesophageal junction adenocarcinoma and classical Hodgkin lymphoma [12–23]. Several predictive biomarkers for immune checkpoint inhibitors have been proposed (PD-L1 status in tumour and inflammatory cells, tumour mutational load and microsatellite instability [MSI] status) and some have achieved companion diagnostics status (e.g. PD-L1 immunohistochemistry in certain cancer lineages and MSI status in all tumours regardless of a lineage). In addition, recent breakthrough studies revealed several predictors of hyperprogression after the therapy with the immune checkpoint inhibitors (e.g. *JAK1/2, MDM2* and *EGFR*) [24–26]. A comprehensive molecular profiling (biomarkers) of CUP with regard to immune checkpoint inhibitors has not been conducted so far. Therefore, we decided to explore a comprehensive survey of predictive biomarkers to immune checkpoint inhibitors in a large cohort of CUP profiled at a single institution.

2. Results

2.1. Patients and histopathologic characteristics

Three hundred eighty-nine patients (53% female and 47% male) were included in the study cohort. The average patient's age was 62.7 years. No clinically recognised primary tumour site was identified in any of the patients tested (Table 1) [3].

Histologically, CUP were classified as adenocarcinomas (n = 175, 45%), carcinomas not otherwise specified (n = 120, 31%), squamous cell carcinomas (n = 30, 8%) or other subtypes (n = 64, 16%) (Table 1). Referring laboratories' immunohistochemical analyses for markers of tissue of origin (e.g. wide-spectrum cytokeratins [AE1/AE3, Cam5.2], CK7, CK20, PSA, oestrogen receptor, progesterone receptor, CDX2, TTF1, napsin-A, thyroglobulin, calcitonin, neuroendocrine markers: NSE, chromogranin, synaptophysin) were non-conclusive in all analysed cases (i.e. more than one possible site of origin was considered) [3]. Board-certified pathologists reviewed all cases and selected appropriate slides for molecular profiling.

2.2. Predictive biomarkers to immune checkpoint inhibitors

Fig. 1 (Venn diagram) summarises total mutational load (TML), PD-L1 status and MSI status for the subgroup of CUP tumours that had PD-L1, MSI and TML information available (n = 362).

In the complete cohort of 389 tumour analysed, TML-high was seen in 11.8% (46/389) of CUPs, similar to the rate observed in common cancers profiled at Caris (Non-small cell lung cancer (NSCLC), bladder carcinoma, Fig. 3). In contrast to other common cancers, MSI-high (MSI-H) rate was detected in 7/389 (1.8%) of CUP cases (Fig. 3). Subsequent immunohistochemistry (IHC) analysis of MSI-H cases showed combined loss of expression of MSH2 and MSH6 or MLH1 and PMS2 mismatch repair proteins in five cases and isolated PMS2 loss in one case, while one case was not evaluable (Table 2). In addition, 12 microsatellite stable cases by NGS were also confirmed by IHC as mismatch repair proficient (no loss of expression of mismatch repair proteins).

Expression of PD-L1 (on ≥5% cancer cells) was seen in 22.5% (82/365) of tumours, while the presence of PD-1