Combined ipilimumab and nivolumab in previously treated patients with cancer of unknown primary: Results of the CheCUP trial

Cancer of unknown primary (CUP) has a dismal prognosis. Second-line therapy following progression to platinum-based treatment leads to response rates of 0-19% and a median progression-free survival (PFS) of only 2-4 months. 10-23% of CUP patients demonstrate a high tumour mutation burden (TMB), which predicts response to immunotherapy in several cancer types.

**METHODS**
After failure of platinum-based therapy, patients were stratified based on TMB (high vs low, cut-off 12 mutations/Mb) (allowing comprehensive genomic profiling and treated with combined ipilimumab (1 mg/kg every 6 weeks) and nivolumab (240 mg biweekly) until disease progression. PFS was defined as the primary endpoint and overall survival (OS), overall response rate (ORR), duration of clinical benefit and safety objectives were secondary endpoints. Parallel, serum plasma samples collected at baseline and every response assessment were analysed regarding circulating cell-free (cfDNA) and circulating tumor DNA (ctDNA) using capture-base targeted sequencing to monitor treatment response. Clinical trial identification: EuDraCT 2018-004562-33

**RESULTS**
In 31 patients were stratified as either TMB high (N=15) or TMB low (N=16, Table 1). Demographic and clinical characteristics are shown in Table 1. Safety data is summarized in Table 2. No treatment-related mortality was reported.

**EFFICACY**
Dual checkpoint blockade led to two complete (6.5%) and three partial remissions (9.7%) according to RECIST v1.1, resulting in an ORR of 16.2%. Stable or progressive disease was found in one (3.3%) and twelve cases (38.7%) respectively, while 13 additional patients suffered rapid clinical progression already before first response assessment. Three of five patients (60%) with high TMB and two of 26 patients (7.7%) with low TMB reached an objective response. Median PFS was 2.53 and OS 3.8 months for the overall population (95% CI 1.77-3.32 and 3.3-8.8 respectively). High TMB showed a trend for better PFS (A) and OS (B), however without reaching statistical significance. Patients with a low number of affected organs (C) and a good performance status (D) reached significantly longer median PFS and OS, while PD-L1 expression (E) and the number of previous therapy lines (F) did not affect survival.

**Correlation of ctDNA levels with tumor burden**
CUP patients (N=355) showed significantly higher ctDNA concentrations compared to healthy controls (N=19) (median 4.5 vs 1.7 ng/ml plasma; p<0.0001), with a wide range (0.9-305.9 ng/ml plasma) (A). ctDNA was detected at baseline in 26 patients. Using a tissue-informed approach, 65 of 77 known mutations could be identified in ctDNA. Change in ctDNA levels at week 12 correlated with radiologic response to treatment (B).

**Liquid biopsies can predict the long-term benefit of immunotherapy**
CUP patients with a poor or stable (C) were more likely to benefit from combination immunotherapy than patients with a PR or SD at first response assessment (B). Among patients responding with a PR or SD at first response assessment, a concurrent decrease in ctDNA levels predicted for durable remissions under continued immunotherapy (C).

**CONCLUSIONS**
Results of the CheCUP trial demonstrate an ORR of 16% to combined ipilimumab/nivolumab in patients relapsed or refractory to platinum-based chemotherapy. With the limitations of a small sample size, high TMB showed a trend for better PFS and OS. Good performance status and a low number of affected organs emerged as prognostic factors for survival, while the number of previous therapy lines did not affect efficacy. ctDNA levels at baseline correlated with tumor burden, while ctDNA dynamics at week 12 identified patients benefiting from continued treatment and achieving long-term remissions under immune checkpoint inhibition.