PD-1/PD-L1 expression is frequent and correlated with lymphocyte density in Erdheim-Chester disease

In patients with Erdheim-Chester disease (ECD), a rare histiocytosis of the L group of the 2016 revised classification, the accumulation of foamy histiocytes leads to multisystemic disease with the involvement of various organs.[1] The detection of *BRAF*^{V600E} mutation in up to 70% of ECD tissue samples tested has led to the reclassification of ECD as a myeloid neoplasm, which has already considerably improved the treatment of adults with histiocytoses, whether wild-type or carrying *BRAF*^{V600E} mutations.[2,3,4] In November 2017, the BRAF inhibitor vemurafenib was approved by the US Food and Drug Administration (FDA) for the treatment of *BRAF*^{V600E}-mutant ECD. The MEK inhibitor cobimetinib will probably follow this year in the US. Vemurafenib has an orphan drug designation for *BRAF*^{V600E}-mutant ECD in Europe, but the therapeutic options for multisystem and refractory ECD, and for other histiocytic neoplasms, may be limited in Europe and elsewhere due to the current lack of access to targeted therapies for such indications. Moreover, further improvements to ECD treatment are required, as targeted therapies can cause morbidity and late treatment effects, and patients almost always experience relapses when these therapies are stopped.[5]

Over the last 10 years, immune checkpoint inhibitors, such as programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) inhibitors, have proven remarkably effective for the treatment of several hematological and solid-organ cancers.[6–8] This high efficacy has led to their approval for use in diverse indications being fast-tracked by the US FDA.

In 2015, Gatalica *et al.* reported a high expression of PD-L1 (≥2+/≥5%) in three of four ECD cases tested, all of which presented *BRAF*^{V600E} mutations.[9] Shortly after the publication of this article, we decided to analyze a larger case series of patients, to see if PD-L1 expression could provide a rationale for the addition of immune checkpoint inhibitors to treatment regimens for multisystemic and/or refractory histiocytoses. Goyal *et al.* recently reported conflicting results for an additional three cases of ECD, which displayed low levels of PD-L1 expression on IHC (14-15%).[10] This led us to extend our series analysis further.

We included 54 ECD patients in our study and biopsy samples were reviewed for all patients (Table 1). Lymphocyte and plasma cell densities were evaluated and classified as low (+), intermediate (++), or high (+++) on hematoxylin and eosin (H&E) staining (Figure 1). Immunostaining was performed to detect PD-L1 (QR1Clone) in histiocytes and PD-1 (NAT105 clone) in lymphocytes. PD-L1 levels were assessed as the percentage of histiocytes positive for this molecule. The combined positivity score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100, is a predictive marker for response to the therapy with inhibitors of immune checkpoints in various types of cancer. In our study, the percentage of PD-L1+ histiocytes was used rather than the CPS because no distinction is possible between tumoral and inflammatory histiocytes in ECD. Patients were PD-L1-positive if ≥ 5% of the histiocytes expressed this molecule. PD-1 immunostaining was evaluated and classified as weak (+), moderate (++), or strong (+++). Patients were PD1-positive if PD-1 immunostaining was moderate or strong. PD-1/PD-L1 expression and the density of lymphocyte and plasma cell infiltration assessment was performed subjectively and classified in three categories by comparing slides with reference patterns as presented in Figure 1.

C-reactive protein levels were assessed at diagnosis, at the time of the biopsy.

Table 1. Clinical and biological characteristics of ECD patients.

Variables	ECD patients (N=54)
Age, yr (IQR)	62 (55-70)
Male, N (%)	42 (78)
Mutation status, N (%)	
WT	15 (28)
BRAF^{V600E}	27 (50)
MAP2K1	5 (9)
NRAS	2 (4)
Not determined	5 (9)
Langerhans cell histiocytosis, N (%)	4 (7)
PD-1^{+}, N (%)	31 (57)
- +	21 (39)
- ++	10 (18)
PD-L1^{+}, N (%)	22 (40)
- 5 - 50 %	13 (23)
- >/= 50 %	9 (17)
PD-L1^{+} / PD-1^{-}, N (%)	4 (7)
PD-L1^{-} / PD-1^{+}, N (%)	13 (24)
PD-L1^{+} / PD-1^{+}, N (%)	18 (33)
PD-L1^{-} / PD-1^{-}, N (%)	19 (35)
Lymphocyte/plasma cell density, N (%)	
Low (+)	34 (63)
Intermediate (++)	12 (22)
High (+++)	8 (15)
High C-reactive protein (> 5 mg/L) at diagnosis *, N (%)	23 (74)

*% calculated for a denominator of 31 patients. ECD: Erdheim-Chester disease; yr: years; IQR: interquartile range; WT: wild-type; PD-1: programmed death-1; PD-L1: programmed death ligand 1.

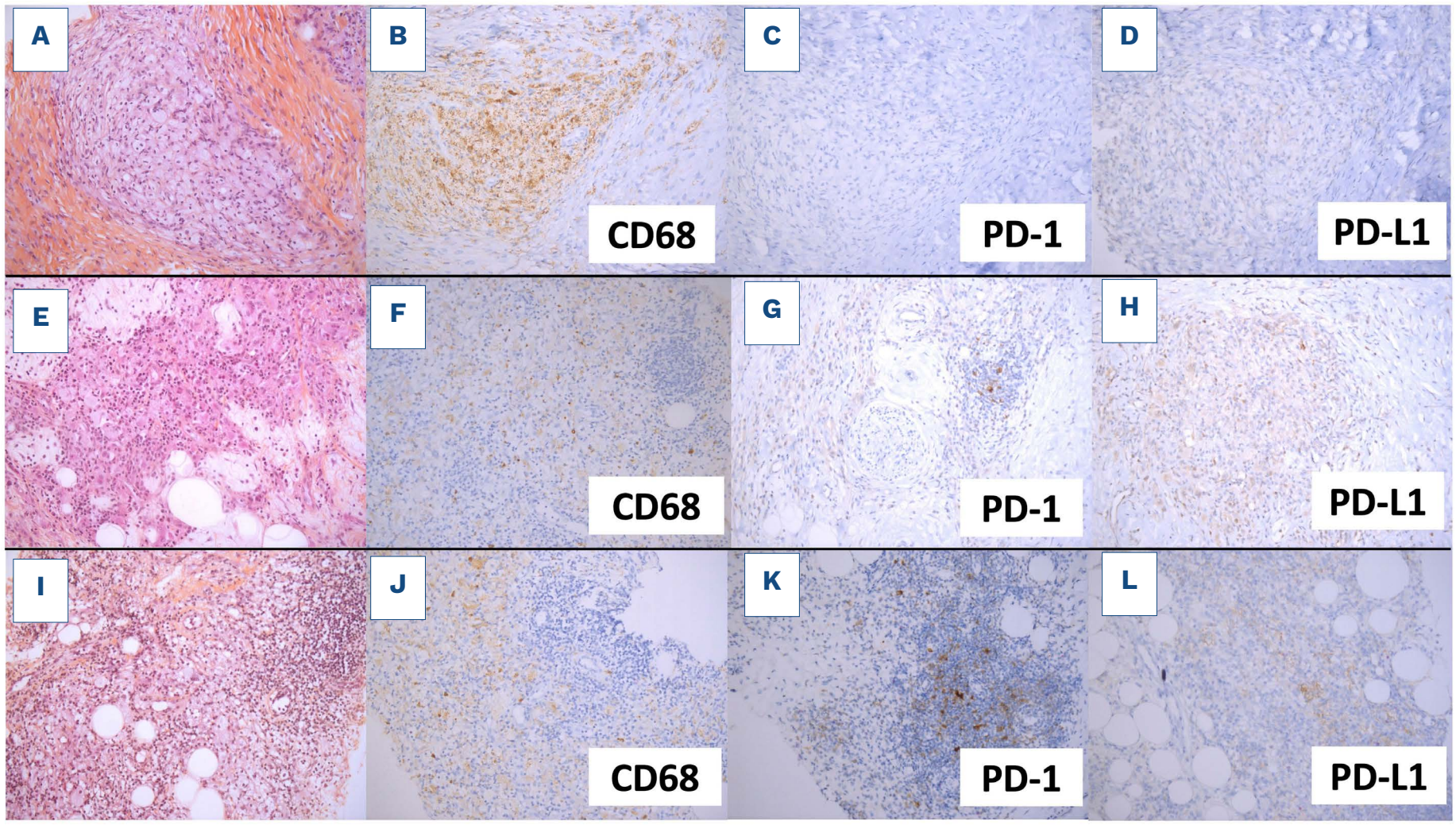

Figure 1. Histopathological images displaying all types of staining over three cases (three *BRAF*^{V600E} mutated patients). (A) In this case, there is only weak lymphocyte infiltration (+) (hematoxylin and eosin [H&E] x200 magnification). (B) the histiocytes are CD68 positive. (C and D) PD-1 and PD-L1 are negative (immunoperoxidase, x200). (E) In this case, the lymphocyte density is moderate (++) (H&E x200); F) the histiocytes are CD68 positive. (G) PD-1 expressed by lymphocytes was evaluated as mild (+), and H) PD-L1 is expressed by 30% of histiocytes (immunoperoxidase, x200). (I) In this case, the lymphocyte density is marked (+++) (H&E x200); J) the histiocytes are CD68-postive (immunoperoxidase, x200). (K) PD-1 expressed by lymphocytes was evaluated as moderate (++), and L) PD-L1 is expressed in 50% of histiocytes (immunoperoxidase, x200).

Continuous variables are expressed as the mean and standard deviation, and categorical variables are expressed as numbers and percentages.

The significance of differences between groups of patients was evaluated in Student's *t*-tests for continuous data and Pearson's chi-squared tests with Yates' continuity correction for categorical data. We used RStudio (Version 1.1.456) for analyses.

The patients had a mean age of 62 years, and 42 (78%) patients were male. *BRAF*^{V600E} mutation was detected in 27 patients (50%), *MAP2K1* mutation in five (9%) and *NRAS* mutation in two (4%). Four of the 54 ECD patients also had Langerhans-cell histiocytosis (LCH) (Table 1; Figure 2).

Overall, 22 patients were positive for PD-L1 (40%), 31 were positive for PD-1 (57%) and 18 were positive for both (33%). Lymphocyte/plasma cell infiltration density was low in 34 (63%) patients, moderate in 12 patients (22%) and of high in eight patients (15%) (Figure 2).

We found a strong association between PD-1 status and lymphocyte/plasma cell density: density was intermediate-to-high in 18 (58%) PD-1-positive patients *versus* in only two PD-1-negative patients (9%) (P<0.001).

A similar association was found concerning PD-L1 status: lymphocyte/plasma cell density was intermediate-to-high in 15 (68%) PD-L1-positive patients whereas it was intermediate-to-high in only five (16%) PD-L1-negative patients (P<0.0003).

PD-L1 positivity was negatively associated with *BRAF*^{V600E} mutation status: five (25%) PD-L1-positive patients were *BRAF*^{V600E}-mutated, whereas 22 (76%) PD-L1-negative patients had the mutation (P=0.001).

We found no association between PD-1 positivity and *BRAF*^{V600E} mutation (P=0.39).

PD-1 status and PD-L1 status were significantly associated with one another: 37 (69%) patients were either PD-1^{-}/PD-L1^{-} or PD-1^{+}/PD-L1^{+}, 19 (35%) were PD-1^{-}/PD-L1^{-}, and 18 (33%) PD-1^{+}/PD-L1^{+} (P=0.006).

Nine (75%) PD-L1^{-}/PD-1^{+} patients had *BRAF*^{V600E} mutations. By contrast, none of the three PD-L1+/PD-1- patients had *BRAF*^{V600E} mutations.

Patients with *BRAF*^{V600E} mutations had significantly lower levels of lymphocyte/plasma cell infiltration, with intermediate-to-high cell density detected in only seven (26%) patients with mutations, *versus* 13 (59%) wild-type (WT) patients (P=0.04).

We report the largest study to date exploring PD-1 status

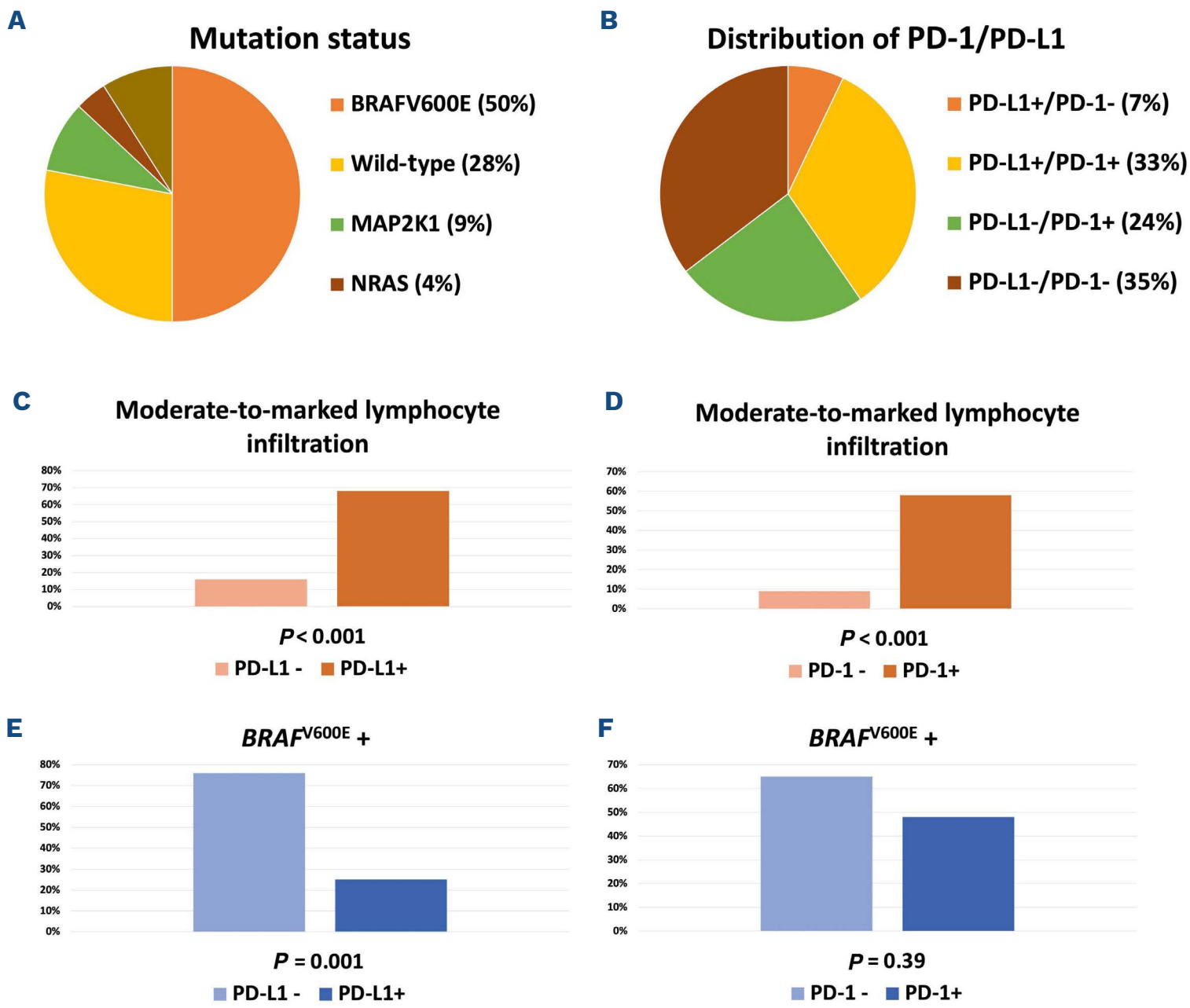

Figure 2. Mutation and PD-1/PD-L1 status. (A) Distribution of *BRAF*^{V600E}, *NRAS* and *MAP2K1* mutations. (B) Distribution of PD-1/PD-L1 C). (D to F) Associations of *BRAF*^{V600E} and lymphocyte infiltration with PD-1/PD-L1 status. PD-1: programmed death-1; PD-L1: programmed death ligand 1.

and PD-L1 status in ECD. We found that PD-1 and/or PD-L1 were frequently expressed in ECD. Positivity for PD-L1 was significantly associated with an absence of *BRAF*^{V600E} mutation, and intermediate-to-high lymphocyte/plasma cell density. Our data suggest that they may be two phenotypes, one combining WT *BRAF* status with intermediate-to-high lymphocyte density and positivity for PD-L1 (+/- PD-1), and the other combining a *BRAF*^{V600E} mutated phenotype with a low lymphocyte/plasma cell density and negativity for PD-L1 (+/- PD-1).

Sengal *et al.*[11] previously performed a phenotypic analysis of LCH lesions and reported an association between *BRAF*^{V600E} expression and PD-L1 expression that we do not find in our series of ECD samples. We evaluated lymphocyte and plasma cell density, but did neither analyze T cells nor dendritic cells (DC). Furthermore, there are subtle but profound differences between LCH and ECD. LCH cells belong to the DC lineage, whereas ECD cells have phenotype of macrophages. Regarding the mechanistic effects of the expression PD-1 and PD-L1 on histiocyte proliferation and lymphocyte activity, it is still unknown whether ECD cells do proliferate or if a proliferation occurs in mutated monocytes seeding the tissues. Single cell transcriptomic data will probably help address these questions.

The recent success of immune checkpoint blockade therapy for many different types of hematological and solid-organ cancers, and the demonstration of immune checkpoint antigen expression in the tissues of patients with ECD suggest that such therapies could be tested for the treatment of patients with multisystemic and refractory ECD, particularly those with a contraindication for MEK inhibitors.

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Disclosures

No conflicts of interest to disclose.

Contributions

FC, FC-A, J-FE and JH designed the study; FC, L-DA and JH collected the data; L-DA and JH conducted the statistical analysis; L-DA, FC-A, L-DA, ZA, J-FE, and JH analyzed and interpreted the data; FC, FC-A, L-DA and JH wrote the manuscript. All the authors critically reviewed and approved the final version of the manuscript.

Data-sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding authors (JH) on reasonable request.

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