Programmed cell death protein-1 (PD1) expression in the microenvironment of classical Hodgkin lymphoma is similar between favorable and adverse outcome and does not enrich over serial relapses with conventional chemotherapy

We read with interest the recent paper published in Haematologica by Sasse *et al*. examining programmed cell death protein – 1 (PD1) expression in paired pre-treatment/relapse samples for patients receiving PD1 inhibitor therapy in classical Hodgkin lymphoma (CHL).[1] Although the authors comment on an increase in PD1^{+} lymphocyte numbers in cases relapsing after PD1 inhibitor therapy, this difference did not reach statistical significance. The possibility that PD1^{+} lymphocytes might be enriched post PD1 inhibitor therapy is interesting, and raises a number of questions. We examined the expression of PD1 and Programmed Cell Death Ligand – 1 (PDL1) in a cohort of 123 CHL patients and in 35 paired diagnosis and relapse specimens in patients receiving conventional chemotherapy and found no evidence of enrichment. Should the enrichment of PD1^{+} lymphocytes seen by Sasse *et al*. be validated it would therefore suggest a difference in the nature of disease relapse post immunotherapy. This would be plausible as recent work by Spina *et al*. hints at fundamental differences in lymphoma cell dynamics under the influence of immunotherapy; they observed cycling of clones rather than the outgrowth of individual chemo-resistant clones seen with conventional chemotherapy.[2] This is likely to be a consequence of PD1 inhibition causing broad T-cell activation improving immunosurveillance, hence we would expect to observe marked changes within the microenvironment.

Whilst the findings of Sasse *et al*. are potentially interesting, it is important to recognise the limitations of this analysis. Firstly, as the authors note, the finding did not reach statistical significance. Additionally, all but two of the relapse biopsies represented small tissue fragments which may affect the quality of the data. Finally, most comparisons of PD1 expression in this study are made either across tissue types (e.g., between soft tissue and lymph node (LN) or LN and bone marrow) or across a change in CHL histological subtype. Cross tissue-type comparison is problematic as the baseline cellular composition is different. Cross-histological subtype composition also presents a problem: the histology at relapse in one case in the cohort by Sasse *et al*. changed to the rare lymphocyte-rich (LR) subtype. We have found PD1 expression in LR cases to be significantly higher than other subtypes (Figure 1). Further validation of their results is therefore essential before any conclusions can be drawn.

We conducted a similar analysis to Sasse *et al*., but in the setting of relapse post conventional chemotherapy, and our findings were different. This provides a useful comparator to their results.

We have previously published that PD1 expression in diagnostic LN biopsies of CHL is low.[3] This is a paradoxical finding given the sensitivity of CHL to PD1 inhibition, so we assessed whether changes in PD1 expression between the diagnostic and relapsed settings could explain this. We did not find evidence of enrichment for PD1 positive cases within the diagnostic biopsies of patients who went on to relapse (Figure 2). This is despite reports by our group and others of PD1 expression being associated with an increased risk of relapse in CHL.[3-5] This apparent discrepancy may be due to the fact that each of these studies associated infrequent PD1hi cases with poor outcome. Hence, although rare PD1hi cases may be preferentially enriched, this does not necessarily imply an overall difference in PD1 expression between groups. PD1 enrichment in the LR subtype does not explain the increased relapse rate in PD1^{hi} cases as this entity is associated with improved outcome.[6]

Given this finding, we considered whether PD1 or PDL1 expression might increase at relapse in paired cases

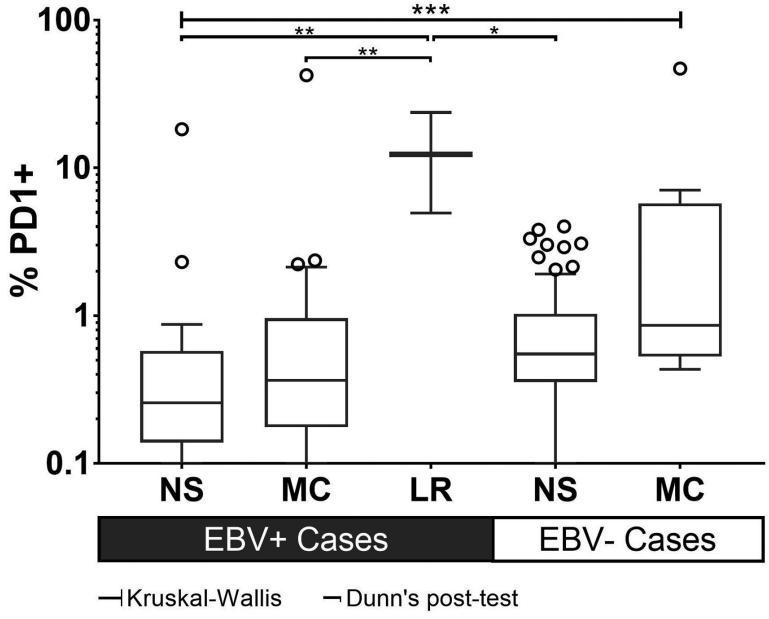

Figure 1. Frequency of PD1^{+} lymphocytes by histological subtype of CHL. Significant enrichment of PD1^{+} lymphocytes seen in LR cases when compared to EBV positive MC, EBV positive NS or EBV negative NS. No significant difference was observed between other groups. Box and whiskers in Tukey style, log 10 scale. Kruskal-Wallis test (P=0.006) with Dunn's multiple comparison. ns : non-significant; *:P<0.05; **:P<0.01, *** :P<0.001; MC: mixed cellularity; NS: nodular sclerosing; LR: lymphocyte rich; EBV+NS n=20, EBV+MC n=22, EBV+LR n=3, EBV-NS n=87, EBV-MC n=8. EBV: Epstein-Barr virus.

due to the selective effects of chemotherapy. We compared 35 paired LN biopsies at presentation and first relapse and found no evidence of a shift in expression of PD1 or PDL1 (Wilcoxon two-tailed, P=0.84 and P=0.6 respectively) (Figure 3A-B). Marked changes in PD1 expression were observed in some cases, but these did not reflect a consistent increase or decrease. Analysis by histological subtype and Epstein-Barr virus (EBV) status did not resolve this. We further assessed 9 cases where serial relapses were available. Here, again, no enrichment or depletion of PD1$^+$ cells or PDL1 expression was observed (one-way ANOVA P=0.47, P=0.68 respectively) (Figure 3C-D). No correlation was observed between PD1 and PDL1.

In summary, we did not observe net selection for PD1 positivity when comparing presentation biopsies of patients who subsequently relapsed to those who did not, despite the role of PD1 as an adverse prognostic

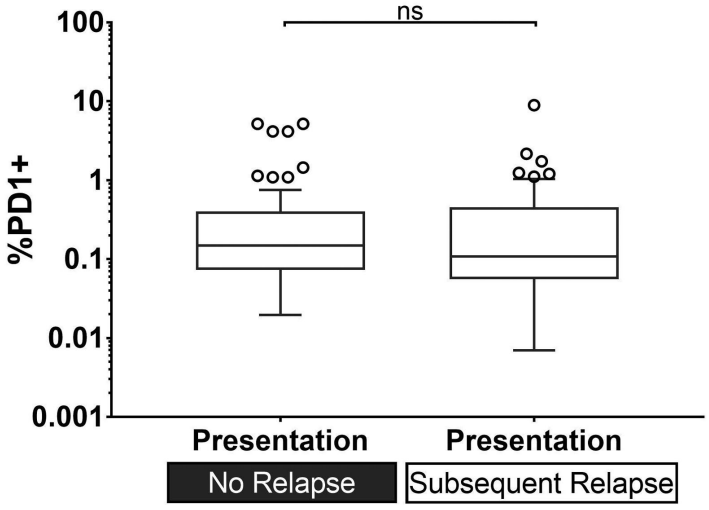

Figure 2. Frequency of PD1$^+$ lymphocytes on presentation biopsies by subsequent relapse status. No significant enrichment of PD1$^+$ lymphocytes between presentation biopsies comparing patients who subsequently relapsed (n=43) to those who remained in remission (n=80). Box and whiskers in Tukey style, log 10 scale. Mann-Whitney test P=0.49. ns: non-significant

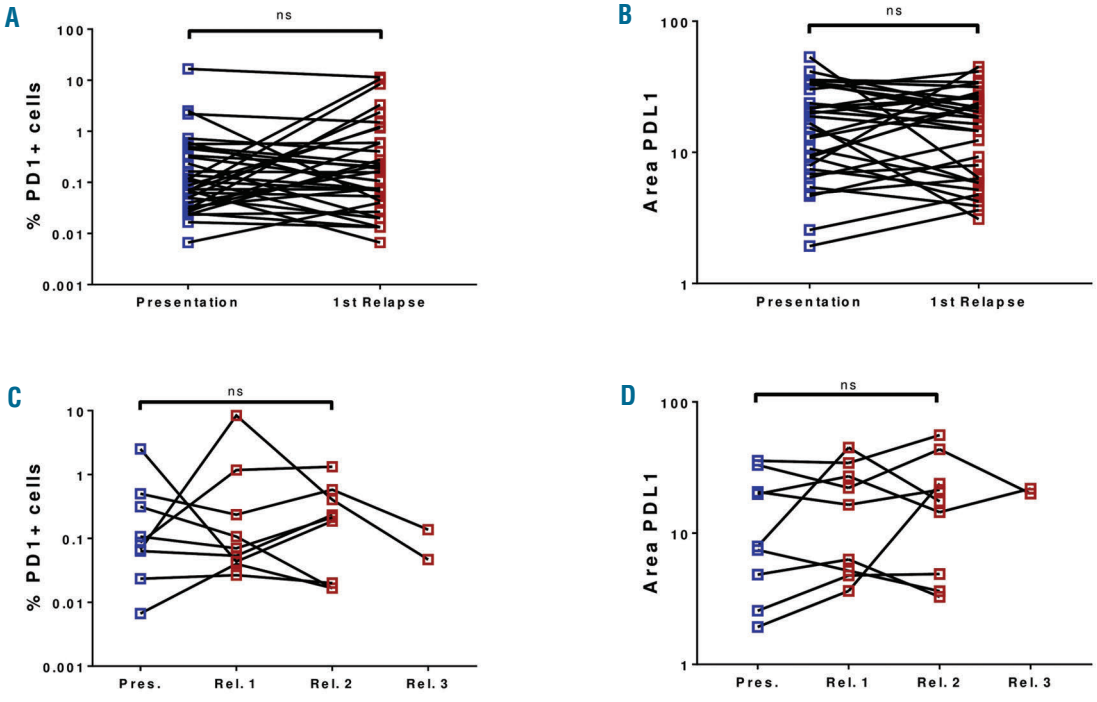

Figure 3. Changes PD1$^+$ lymphocyte frequency and PDL1 expression between presentation and relapse. A) Number of PD1$^+$ lymphocytes do not significantly enrich between paired biopsies at presentation and first relapse in CHL. Comparison by Wilcoxon rank P=0.8 (n=35) B) PDL1 expression does not significantly enrich between paired biopsies at presentation and first relapse in CHL. Comparison by Wilcoxon rank P=0.6 (n=35) C and D) No enrichment of PD1 or PDL1 is seen across serial relapses. Comparison by Friedman test, P=0.5 and 0.7 respectively (n=9). Pres.: Presentation biopsy (pre-therapy); Rel. : Relapse biopsy; ns : non-significant

marker. This suggests that the adverse predictive power of PD1 stems from a subgroup of PD1hi patients as opposed to a generalized effect. Additionally, our finding that PD1 and PDL1 do not enrich in paired biopsies over serial relapses suggests that these mechanisms are not selected for by conventional treatment. These observations appear reassuring for the incorporation of PD1 inhibitors into front-line therapy for CHL as they suggest that the encouraging responses in relapse are not due to selection for PD1 positivity. However, this may not prove relevant as the role that PD1$^+$ cells play in PD1 inhibitor activity is unclear. PD1 and PDL1 are independent prognostic factors in CHL and PD1 does not correlate with PDL1 expression.[4,5,7] Lymphoma cell PDL1 expression, but not PD1$^+$ lymphocyte infiltration, is associated with response to PD1 inhibition.[8,9] This suggests that PD1 and PDL1 expression may be driven by independent processes and that PDL1 is more important to PD1 inhibitor activity.

If the findings of Sasse *et al*. are validated, these data would suggest a biological difference between relapse post immunotherapy *versus* conventional chemotherapy. Interpreting this finding would be challenging as our understanding of cellular dynamics within the microenvironment during immunotherapy is limited. It is likely that the T landscape will differ significantly to that seen in the absence of PD1 inhibition, and PD1$^+$ cells in this context may be playing different roles. It will be difficult, therefore, to distinguish signatures of appropriate T-cell activation from those of hyperactivation or PD1 resistance. The increase in PD1$^+$ cells observed by Sasse *et al*. may represent activated, exhausted or even follicular helper T cells. Given that PD1 inhibitor therapy reverses T-cell exhaustion, it would seem counter-intuitive if these cells were exhausted, but this would be possible if hyperactivated cells were exhausted in the context of treatment failure, a described mechanism of resistance to PD1 inhibitor therapy.[10] It is interesting to note that patients 2, 7, 8 and 9 in their study showed the most marked increase in PD1$^+$ lymphocytes and are alive, two with stable disease, which would go against this hypothesis. A second alternative might be increased PD1 expression as a marker of widespread T activation. This might represent an appropriate response to PD1 inhibition in the presence of lymphoma. This is a possible explanation as the relapsed biopsies in the study by Sasse *et al*. comprised cases of both relapse and stable disease. A further explanation might be increased differentiation of T follicular helper-like cells, although these are usually scarce in the CHL microenvironment. This might fit with the author's hypothesis of a PD1-PDL1 axis crosstalk required for HRS growth.

The analysis by Sasse *et al*. raises the possibility of an interesting finding which differs to our observations in the setting of conventional chemotherapy and deserves to be repeated when a larger cohort becomes available.

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Funding: this project was supported by a Wellcome Trust Clinical Research Fellowship

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doi:10.3324/haematol.2018.204818

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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