Serum CD163 and TARC as Disease Response Biomarkers in Classical Hodgkin Lymphoma.

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**Statement of Translational Relevance:**

The challenge in treating classical Hodgkin Lymphoma (cHL) remains to tailor therapies to eradicate malignancy with minimal side-effects, and to simultaneously identify those patients in whom alternate strategies should be instituted early. There are currently no circulating disease response biomarkers for HL. Blood biomarkers may assist interpretation of PET/CT, and can also be performed at each consultation to assess disease response and detection of early relapse. We perform the first comparison of HRS-specific (TARC) and tumor-infiltrate specific (CD163) serum markers in cHL and demonstrate that both serum CD163 and serum TARC by ELISA are cHL disease response biomarkers in early and advanced stage disease. Furthermore, functional analysis suggests that CD163+ monocyte-mediated immunosuppression of T-cells occurs in cHL, providing potential targets for novel therapeutics.
Abstract:

Purpose: Candidate circulating disease response biomarkers for classical Hodgkin lymphoma (cHL) might arise from Hodgkin-Reed-Sternberg (HRS) cells or non-malignant tumor-infiltrating cells. HRS cells are sparse within the diseased node, whereas benign CD163+ M2 tissue associated macrophages (TAMs) are prominent. CD163+ cells within the malignant node may be prognostic, but there is no data on serum CD163 (sCD163). The HRS-specific serum protein sTARC shows promise as a disease response biomarker. Tumor-specific and tumor-infiltrating circulating biomarkers have not previously been compared.

Experimental Design: We prospectively measured sCD163 and sTARC in 221 samples from 47 HL patients and 21 healthy participants. Blood was taken at five fixed time-points prior, during and after first-line therapy. Results were compared with radiological assessment and plasma Epstein-Barr virus DNA (EBV-DNA). Potential sources of circulating CD163 were investigated, along with immunosuppressive properties of CD163.

Results: Pre-therapy, both sCD163 and sTARC were markedly elevated compared to healthy and complete remission samples. sCD163 better reflected tumor burden during therapy whereas sTARC had greater value upon completion of therapy. sCD163 correlated with plasma EBV-DNA, and associated with B symptoms, stage and lymphopenia. Circulating CD163+ monocytes were elevated in patients, indicating sCD163 are likely derived from circulating and intra-tumoral cells. Depletion of cHL CD163+ monocytes markedly enhanced T-cell proliferation, implicating monocytes and/or TAMs as potential novel targets for immunotherapeutic manipulation.

Conclusion: The combination of circulating tumor-infiltrate (sCD163) and tumor-specific (sTARC) proteins are more informative than either marker alone as disease response biomarkers in early and advanced disease during first-line therapy for cHL.
**Introduction:**
Classical Hodgkin lymphoma (cHL) is associated with high cure rates (1). Those with a rapid response to initial treatment have the best outcomes, and may benefit from truncated treatment regimens. Paradoxically, there remains a significant minority with refractory disease in whom prolonged exposure to first-line agents induce chemo-resistance and unnecessary toxicity. The challenge remains to tailor treatments to eradicate malignancy with minimal side-effects, and to simultaneously identify those patients in whom alternate strategies should be instituted early.

Radiological imaging modalities such as computed tomography (CT) or positron emission tomography (PET) scans are typically used to assess treatment efficacy. Although combined functional-anatomical imaging modality (PET/CT) has a high negative predictive value, its positive predictive value is more modest (2, 3). Outside of the context of a clinical trial, PET/CT scanning is typically available only prior to and at the end of, but not during, therapy. Unlike blood tests, it is impractical to perform PET/CT prior to each follow-up visit (4). Furthermore, PET/CT interpretation can be confounded by concurrent inflammation or infection, and influenced by the timing of prior therapy.

Ideal biomarkers must be specific, sensitive, easy to assay and interpret, with rapid turnaround, high reproducibility and comparability between laboratories. Serum protein estimation by ELISA exploits an established technology used in the diagnostic laboratory. Furthermore, whilst tissue markers could be applied routinely to the diagnostic biopsy to provide prognostic information, they have no applicability in monitoring disease response. By contrast, blood based biomarkers have potential to provide information on disease response and detection of early relapse. It is known that indirect blood markers of biological activity, such as erythrocyte sedimentation rate, lymphocyte count, hemoglobin and albumin have prognostic value at baseline. However, once therapy has commenced their lack of specificity precludes their clinical value as a measure of disease response. A validated circulating measure of tumor burden, that is informative in the majority of patients, and accurately reflects disease response during first-line therapy would be of great benefit.
In cHL, the malignant Hodgkin and Reed-Sternberg (HRS) cells account for a minority of the diseased nodal cell population. The affected node is primarily composed of non-neoplastic, tumor-infiltrating cells which are important in pathogenesis (5). It is yet to be determined whether disease response in patients with cHL will be best reflected by HRS-specific or tumor-infiltrate specific biomarkers. Biomarkers that originate from HRS cells hold the promise of high specificity, whereas those that reflect the benign tumor-infiltrate may be more sensitive. The relative kinetics of circulating tumor-specific versus tumor-infiltrating biomarkers during therapy have not been compared.

The CC thymus and activation related chemokine (TARC, CCL17) is elevated in HRS cell-lines, primary cHL tissues and pre-therapy cHL patient serum (irrespective of EBV-tissue status) and holds promise as a tumor-specific disease response marker in cHL (6-11). A recent study of 60 cHL patients with serial samples throughout therapy found that sTARC reflected disease response in both early and advanced stage cHL and in relapsed disease (11).

cHL nodes are enriched with anti-inflammatory M2 macrophages. CD163 is an M2 macrophage marker that is also expressed in a subset of monocytes, and is highly expressed within tumor-infiltrating cells of the malignant node. Tissue levels of CD163 within the cHL node may be associated with survival, but results are conflicting (12-18). A study of serum CD163 (sCD163) in stage I/II melanoma patients found that pre-therapy sCD163 was an independent predictor of survival (19). To our knowledge, no study of serum CD163 (sCD163) in HL has been performed.

We prospectively evaluated sCD163 and sTARC in HL patients (43 cHL and 4 nodular lymphocyte predominant: NLPHL) at five fixed time-points; pre-therapy, during (immediately prior to the second and the third cycles), one month and six months after completion of therapy. Results were compared with disease response as determined by radiological assessment. In patients with EBV-related cHL, plasma cell-free EBV-DNA was quantified. cHL tissue CD163 immunohistochemistry was correlated to matched serum samples. Potential sources of circulating CD163 were investigated, along with the immunosuppressive properties of CD163 on global T-cell proliferation.
Materials and methods:

Patients

Forty-seven newly diagnosed HL patients were prospectively enrolled. One patient with atopic dermatitis was excluded from analysis and will be discussed separately (excluded from Table 1). Of the remaining 46 patients, 42 had cHL and 4 were NLPHL patients. Patient characteristics are provided in Table 1. NLPHL and cHL patients were analyzed separately. Exclusion criteria were limited to HIV positivity, active Hepatitis B or C infection. All patients were enrolled before commencement of therapy and serial blood samples were taken at five fixed time-points: pre-therapy, immediately pre-second and pre-third therapy, one month and six months post-therapy. Peripheral blood mononuclear cells (PBMC), plasma and sera were cryopreserved, thawed and tested in batches as previously outlined (20). Tissue from diagnostic tumor biopsies was tested when available. Clinical parameters including the Hasenclever prognostic score were prospectively recorded (21, 22). Early stage disease was defined according to the Southwest Oncology Group (SWOG) and Cancer and Leukemia Group B (CALGB) previously published definition (Ann Arbor stage I or II without any B symptoms, infradiaphragmatic presentations or mediastinal masses greater than one third the maximum thoracic diameter) (23).

This was a multi-center, Australia-wide, observational (non-interventional) study conducted under the auspices of the Australasian Leukaemia & Lymphoma Group (ALLG). Therapy was applied as per clinician’s preference (Table 1). The majority of our patient cohort (85%) was treated with ‘ABVD’ [adriamycin, bleomycin, vinblastine, dacarbazine] combination chemotherapy (24). Other regimens used were ‘BEACOPP’ [bleomycin, etoposide, adriamycin, cyclophosphamide, procarbazine and prednisolone] (25), ABVD followed by BEACOPP and ‘ChlVPP’ [chlorambucil, procarbazine, prednisolone and vinblastine] (26).

Initial staging and re-staging one month after completion of therapy was by PET and CT scans. Interim disease response (generally after the third cycle of therapy), and re-staging at the three-six months after completion of therapy was assessed by CT, typically in combination with PET (70% had interim treatment restaging PET). CR and partial response (PR) were defined as per the
International Harmonization response criteria (27) or, when applicable (i.e. patients that had interim treatment CT scans only), the International Working Group response criteria was used (28). Refractory disease was defined as patients with persistent HL at one month post-therapy, which was either residual disease (if there was stable or reduced tumor burden as compared to pre-therapy), or progressive disease (if there was an increase in tumor burden). Relapse was defined as HL reappearing in a patient who had previously been in CR at the interim therapy or one month post-therapy time-point. Relapsed/refractory disease was based on clinical and radiological assessment and in some cases by repeat biopsy.

Twenty-one healthy age and gender matched participant blood samples were used. This study conformed to the Declaration of Helsinki and written informed consent was provided by all participants and was approved by all participating hospitals/research institute Human Research Ethics Committees.

**Serum ELISA Analysis**

CD163 and TARC were quantified in 221 serum samples using Quantikine® Human CD163 ELISA kits (R&D Systems) and RayBio® Human TARC ELISA kits (RayBiotech) as per manufacturer’s instructions. A 1:20 dilution was used for sCD163.

**Tissue mRNA quantification**

RNA was extracted from all available formalin-fixed, paraffin-embedded (FFPE) tumor biopsies (36 HL in total: 32 cHL and 4 NLPHL; 29 of the 32 cHL had paired slides for IHC staining available) using RecoverAll™ kit (Ambion) as per manufacturer’s instructions. RNA was stored at -80°C. Superscript® III reverse transcriptase (Invitrogen) was used to produce cDNA primed by random hexamer as per manufactures instructions. TARC, CD163, CD68 and Beta-2-microglobulin (β2M) mRNA levels were quantified using real-time PCR (TARC forward primer: 5’-CTTCTTCAGCAGCATCCAC-3’, TARC reverse primer: 5’-AGTACTCCAGGCGAG-3’, CD68 forward primer: 5’-CCACACAGGGGTCTTGT-3’, CD68 reverse primer: 5’-TGATGAGGGCAGCAAGATG-3’, CD163 forward primer: 5’-CAATGGGG-
TGGACTTACCTG-3', CD163 reverse primer: 5' - AACCAGTCTGGGTTCCCTGT -3', β2M forward primer: 5'-ACTCTCTCTTTCTGCGCGCATGAG-3', β2M reverse primer: 5'-CATTCTCTGCTGGATGACGTGAG-3'). All primers were synthesized by Sigma-Aldrich and used at 0.2 μM. Rotorgene 3000 real-time PCR machine (Corbett Research) was used to run 20 μl reactions with A&B SYBR® Green (Applied Biosystems). Results were normalized to β2M and comparative quantification was used for analysis. Healthy participant PBMC cDNA was used as the standard control for CD68 and CD163 and the HL cell line HDML2 cDNA was used for TARC.

**Circulating plasma EBV-DNA quantification**

EBV-tissue positivity was determined by EBV encoded RNA in situ hybridization (EBER-ISH) in conjunction with hematoxylin and eosin staining (29). EBV-DNA was quantified in plasma of EBV-related cHL patients by real-time PCR using primers for the single copy EBV gene BALF5 as previously described (20). A threshold of 200 EBV genomes/ml was used.

**Immunohistochemistry**

CD163 (mouse anti-human CD163 clone: 10D6, 10mg/ml, Biocare Medical) staining was performed on 36 HL tumor biopsy FFPE Superfrost Plus Adhesive slides (32 cHL, 4 NLPHL; 29 of the 32 cHL had paired FFPE tumor slices for RNA extraction available). CD163 positivity was scored by two independent hematopathologists (C.K, L.A.S) using a standardized protocol and the mean value recorded. Samples were delineated as high or low based on a previously published cut-off of 25% (16-18). In addition, dual staining of CD163 and CD68 (mouse anti-human CD68 clone: PG-M, 40 ug/ml, DAKO) was done on 12 samples to confirm CD163 specificity. The number of HRS cells per 5 fields at 40x magnification (hematoxylin and eosin staining) was quantified in the 32 available cHL slides, and tested for correlation with sTARC.

**CD163 Flow Cytometry**

CD163+CD14+ monocytes and monocytic myeloid-derived suppressor cells (mo-MDSC, defined as CD14+HLA-DRlow-/) were quantified in PBMC from 10 healthy
participant and 10 cHL patient at two time-points: pre-therapy and six months post-therapy. Briefly, PBMC were surface stained for CD14-PE (Miltenyi), CD163-Alexa-647 (Biolegend), HLA-DR-PerCP (BD Biosciences) and intracellular stained for CD68-FTIC (Invitrogen). Samples were run on FACSCanto II (BD Biosciences) and analyzed using FlowJo version 9.2 (Treestar).

**Effect of monocyte-depletion on global T-cell proliferation**

Eight pre-therapy cHL patient (3 Nodular Sclerosing, 1 Mixed Cellularity, 1 Lymphocyte Rich, and 3 cHL unspecified) and 6 healthy participant samples were randomly selected from our cohorts. PBMC were carboxyfluorescein succinimidyl ester (CFSE) stained (1.5µM), washed, and then divided into two. One half was depleted of CD14+ cells using Easysep® Human CD14 positive selection kit (Stem Cell Technologies), as per manufacturer's instructions. 2.5x10^5 cells from both the depleted and non-depleted populations were surface stained for CD14-PerCP Cy5.5 (BD Pharmingen) and CD163-Alexa-647 (Biolegend) and the proportions of CD163+CD14+ cells determined by flow cytometry.

To account for a higher proportion of T-cells in the depleted population, depleted and non-depleted cells were cultured at a concentration of 2.0x10^6 cells/ml and 2.5x10^6 cells/ml respectively. Both populations were stimulated with Dynabeads® Human T-activator CD3/CD28/CD137 (1:10, Invitrogen) and expanded for seven days in RPMI 1640 with penicillin/streptomycin, 10% fetal bovine serum and 20U/ml IL2. On day seven, cells were surface stained for CD3-APC-Cy7, CD4-APC and CD8-PerCP (BD Pharmingen). Samples were run on FACSCanto II (BD Biosciences) and analyzed using FlowJo version 9.2 (Treestar).

**Statistics**

Paired T-tests were used to compare matched serum samples. Wilcoxin matched pairs tests were used to compare matched monocyte-depleted and non-depleted T-cell expansion samples. Otherwise the unpaired T-test was used and Welch’s correction was included when variances were significantly different. Correlations were determined using the Pearson test with the exception of plasma EBV-DNA (which had non-Gaussian distribution), in which the Spearman
test was used. Receiver Operating Curve (ROC) analysis was used to determine sensitivity and specificity of sCD163 and sTARC. Statistical analysis was performed using Graphpad Prism 5.0 (Graphpad Software Inc, California).
Results:

Patient Characteristics

Forty-seven HL patients were accrued (mean age: 36 years, range: 18-79; female: male ratio 21:26; 42 cHL, four NLPHL, one cHL with atopic dermatitis). Post-therapy samples were available for 37 of the 42 cHL patients. Of these 37 cHL patients, 32 remained in CR by the six month post-therapy time-point, while five had relapsed/refractory disease. Two of the five had biopsy proven relapsed/refractory disease, while three were confirmed by clinical and radiological assessment. In addition, 21 healthy participant blood samples were used (mean age: 36 years, range: 22-68; female: male ratio 9:12).

Specificity and sensitivity of sCD163 and sTARC as biomarkers in cHL

Firstly, we performed ROC analysis to determine the sensitivity and specificity of sCD163 and sTARC at defined time-points. We found that both sCD163 and sTARC are highly sensitive and specific markers for delineating pre-therapy cHL from healthy participants (sCD163: AUC 0.8776, P<0.0001, 95% C.I. 0.7877 to 0.9674; sTARC: AUC 0.9138, P<0.0001, 95% C.I. 0.8440 to 0.9837; Supplementary Fig. S1A,C). Pre-therapy sCD163 and sTARC cHL samples were also highly sensitive and specific when compared to CR samples at six months post-therapy (sCD163: AUC 0.7333, P=0.001, 95% C.I. 0.6072 to 0.8594; TARC: AUC 0.8793, P<0.0001, 95% C.I. 0.7492 to 0.9613; Supplementary Fig. S1B,D). Cut-off levels were defined by two criteria; a value greater than the healthy participant mean plus one standard deviation and a value with greater than 80% sensitivity and specificity from the ROC analysis of pre-therapy versus healthy participant values. A cut-off level of 500ng/ml for sCD163 resulted in 86% sensitivity and 81% specificity at pre-therapy and 62% sensitivity and 75% specificity at six months post-therapy. For sTARC, the cut-off value was defined as 300pg/ml with 90% sensitivity and 86% specificity at pre-therapy and 70% sensitivity and 84% specificity at six months post-therapy.

sCD163 and sTARC as disease response biomarkers in cHL

Secondly, we performed a match-paired analysis of sCD163 and sTARC at all time-points in all cHL patients attaining CR and remaining in CR at the six month
post-therapy time-point. Results were compared with healthy control participants. Both sCD163 and sTARC were elevated in pre-therapy cHL patients compared to healthy participants (sCD163 P<0.0001 and sTARC P<0.0001, Fig. 1A,B). In addition, both sCD163 and sTARC were significantly elevated in pre-therapy cHL patients compared to paired samples taken one month post-therapy (sCD163 P=0.0008, sTARC P<0.0001) and six month post-therapy (sCD163 P=0.0005, sTARC P<0.0001). Levels of sTARC was equivalent to healthy participants by pre-second therapy while levels of sCD163 approached but did not reach equivalence by six months post-therapy. These results demonstrate that sCD163 and sTARC are disease response biomarkers in cHL patients attaining and remaining in CR.

Next, we analyzed patients with relapsed/refractory disease. One patient had biopsy proven refractory disease at one month post-therapy (Fig. 2A), another patient who had resolution of disease at the interim time-point relapsed (confirmed by biopsy) at one month post-therapy, (Fig. 2B) and three patients had clinical and radiological confirmed relapse after one month post-therapy (Fig. 2C-E). The time course of sCD163 and sTARC are shown in Fig. 2, in association with the tumor burden. In three patients, sTARC was well above threshold at time of refractory/relapsed disease (Fig. 2A,C,E). In one of these cases (Fig. 2C) both sCD163 and sTARC were elevated, whilst in two of these cases (Fig. 2A,E) sCD163 was not (in the case shown in Fig. 2A, sCD163 was not elevated pre-therapy). In the remaining other two cases (Fig. 2B,D) both serum biomarkers were elevated at pre-therapy, but neither were elevated at time of diagnosis of low volume relapsed/refractory disease.

We then analyzed whether sCD163 and/or sTARC were useful in the setting of equivocal PET scans (i.e. low-grade avidity of uncertain clinical significance). Out of our cHL cohort, four patients had persistent clinical CR despite an equivocal restaging PET result. We will discuss each of these patients in turn. The first patient was not informative as both sCD163 and sTARC were below threshold prior to therapy and remained below during and after therapy (data not shown). In the remaining three patients (Fig. 2 F-G), the HRS-specific marker sTARC remained below threshold consistent with ongoing CR. However values of the tumor-infiltrate specific marker sCD163 ranged from either modestly above or
borderline below threshold, indicating that sTARC was more informative in patients with equivocal restaging PET scans that remain in clinical CR.

**sCD163 levels decline gradually in cHL patients, whilst sTARC levels decline rapidly.**

Figure 1A-C illustrates that sCD163 levels decline gradually during treatment. In contrast, levels of the tumor-specific biomarker sTARC dropped rapidly, with levels pre-second therapy comparable to healthy participants. Interestingly, a similar pattern to sTARC was observed for plasma EBV-DNA in the EBV-related cHL patients (Fig. 1C). Circulating cell-free EBV-DNA is an established tumor-specific biomarker in EBV-related cHL (20, 29-31).

Our next aim was to determine the association between each serum biomarker and the interim radiological assessment. The serum sample taken closest to the interim radiological assessment (typically pre-third therapy) was compared to its paired pre-therapy sample. Interestingly, for sCD163 levels there was no significant difference between pre-therapy samples and those in PR at the interim time-point. However, there was a significant difference for sCD163 between pre-therapy and those in CR at the interim time-point (P=0.0152, Fig. 1D). At pre-therapy, 21% of patients were below the sCD163 threshold, rising to 33% and 48% in those attaining PR and CR respectively by the interim time-point.

In contrast, there was a significant difference between pre-therapy sTARC levels and both PR and CR samples at the interim time-point (P=0.0001 for both, Fig.1E). For sTARC at pre-therapy, 14% of patients were below threshold, and the proportions below threshold in those attaining PR and CR were similar to each other at 75% and 86% respectively by the interim time-point. This is consistent with a more rapid decline in the tumor-specific circulating biomarker as compared to the tumor-infiltrate biomarker.

We then determined whether the kinetics of the biomarker (stratified by CR/PR) at the interim time-point was associated with outcome once chemotherapy was completed. All patients in CR at the interim time-point maintained ongoing CR at one and six months post-therapy. Interestingly, of those PR patients below the sCD163 threshold at the interim time-point, all achieved and maintained ongoing
CR, while four of the eight patients above the sCD163 threshold went on to have refractory/relapsed disease. However, for sTARC, the results were less clear-cut. There were four patients above the sTARC threshold at the interim therapy time-point. Two remained in ongoing CR, whereas one had refractory disease and one relapsed by six months post-therapy. Similarly, two (of the nine) patients below the sTARC threshold had relapsed disease by six months post-therapy.

**sCD163 levels correlate with tissue CD163 protein levels.**

Biopsy tissue was available in 32 cHL patients from our cohort. Tissue CD163 protein levels (quantified by immunohistochemistry) were modestly correlated to matching pre-therapy sCD163 levels (r= 0.4005, 95% C.I 0.05373 to 0.6611, P= 0.0256). The number of HRS cells did not correlate with pre-therapy sTARC levels (P=NS). Tissue CD163 protein levels were associated with the Hasenclever prognostic score (P=0.0332, Score <3: 31.3% ± 3.9%, ≥3: 51.4% ± 6.5%) and with advanced stage disease (P=0.0025, early: mean 21.2% ± SEM 2.5, advanced: mean 37.8% ± SEM 4.3) (21-23). No other associations were found between tissue CD163 protein levels and any other clinical characteristics. In addition to measuring CD163 protein levels, real-time RT-PCR was used to measure tissue CD163, CD68 and TARC mRNA levels. Tissue CD163 mRNA levels weakly correlated with tissue protein levels (r=0.3898, 95% C.I 0.02703 to 0.6618, P=0.0366), however, neither CD163 nor TARC tissue mRNA levels correlated with paired pre-therapy serum protein levels and TARC tissue mRNA levels did not correlate with number of HRS cells. No associations were found between any of the clinical parameters and mRNA levels of CD163, CD68 and TARC. CD163 and CD68 mRNA levels moderately correlated (r=0.3851, 95% C.I 0.04202 to 0.6470, P=0.0295). Twelve cHL tissue samples were dual stained for CD163 and CD68. In these, CD163 cells were universally positive for CD68 whereas the proportion of CD68 cells that were CD163 positive was variable.

**cHL patients have a higher proportion of total circulating CD163+CD14+ monocytes, suggesting not all of the elevated circulating CD163 is tumor-derived.**
Pre-therapy cHL patients (n=10) had a higher proportion of circulating CD14+ monocytes within the mononuclear gate compared to ten healthy control participants (P= 0.0006, Fig. 3A). The proportion of circulating CD163+CD14+ cells were also higher in cHL patients compared to healthy control participants (P= 0.0437, Fig. 3B). All these patients remained in CR at six months post-therapy. They continued to have an elevated proportion of total monocytes and CD163+ monocytes (P= 0.0010, Fig. 3A and P= 0.0332, Fig. 3B, respectively). Levels of sCD163 did not correlate with the proportion of circulating CD163+CD14+ cells in cHL. mo-MDSC were not elevated in cHL compared to healthy control participants.

**sCD163 levels are associated with B symptoms, Ann Arbor stage, lymphocyte count and plasma EBV-DNA in cHL**

Pre-therapy sCD163 and sTARC levels were associated with patient characteristics as outlined in Table 2. Higher sCD163 levels were significantly associated with B symptoms (P=0.0351), Ann Arbor stage (P=0.0328), lower lymphocyte counts (P=0.0297) and (in EBV-related cHL) with higher plasma EBV-DNA (P=0.0197). sTARC levels were inversely associated with age (P=0.0250). We then performed a correlation analysis between plasma EBV-DNA and sCD163 and sTARC. sCD163 was strongly correlated with plasma EBV-DNA (r=0.791, P=0.001), whereas sTARC was not.

**Depletion of CD163+ monocytes enhances proliferation of T-cells in cHL.**

To further investigate the inverse association between sCD163 and lymphocyte count, we assessed global CD4+ and CD8+ T-cell proliferation in the presence or absence of CD163+ monocytes. We randomly selected 8 cHL patients and 6 healthy participants. As the majority of CD14+ monocytes are CD163+ (pre-therapy cHL patient mean= 65%), we compared *in-vitro* proliferation of CD14+-depleted PBMC with non-depleted PBMC in the pre-therapy cHL and healthy participants. CFSE staining was used to quantify CD4+ and CD8+ T-cell proliferation following stimulation by T-cell proliferation beads. Total percent proliferation and extent proliferation (top ten percentile) were compared. *In-vitro* depletion of monocytes from pre-therapy cHL samples led to enhancement
of CD4+ and CD8+ T-cell proliferation, indicating monocyte-dependent T-cell immunosuppression occurs in cHL patients (total proliferation CD4+ P=0.0078, CD8+ P=0.0078, extent of proliferation CD4+ P=0.0078, CD8+ P=0.0078, Fig. 4). Monocyte depletion from healthy participants also enhanced proliferation; however, this only reached significance for CD8+ T-cell total proliferation (P=0.0313).

**CD163 but not TARC levels are elevated in the serum of patients with NLPHL**

TARC is expressed by the HRS cells of cHL, as well as the thymus, subsets of dendritic cells, endothelial cells, and activated PBMC (6, 32). HRS cells are not present in NLPHL and it is known that TARC is not expressed by primary NLPHL tissues (6, 10). Consistent with this, sTARC was not elevated in all four pre-therapy NLPHL patients compared to healthy participants and were significantly lower than pre-therapy cHL samples (P<0.0001). This indicates that sTARC is specific for HRS cells and is not a useful circulating biomarker for NLPHL. Using the previously published cut-off, CD163 protein was present at low levels in three of the four NLPHL tissues (mean 17%, range 12-31%) (16-18). Interestingly, sCD163 levels were elevated in all four pre-therapy NLPHL patients compared to healthy participants (P=0.001) at a similar level compared to pre-therapy cHL samples. Further investigation into the use of sCD163 as a disease response biomarker for NLPHL is warranted.

**sTARC is not a disease response biomarker in cHL patients with concomitant severe allergic disease**

One patient with stage IVb EBV-related nodular sclerosing cHL in the setting of long standing methotrexate therapy for severe atopic dermatitis was censored from all analysis. The dermatitis remained poorly controlled for the duration of the study. This patient was treated with ABVD which resulted in radiological PR at the interim time-point and CR at the end of therapy. This patient’s sCD163 was elevated (646ng/ml) at pre-therapy, and borderline at the interim time-point (493ng/ml). EBV-DNA was present pre-therapy, but could not be detected at any subsequent time-point. At one month post-therapy, sCD163 fell below the cut-off
to 340ng/ml. Interestingly, sTARC was grossly elevated at all time-points with the mean over 10x our threshold value (mean 3163pg/ml, range 2383-3920pg/ml). Serum TARC level is known to be elevated in a range of allergic disease such as atopic dermatitis (32, 33).
Discussion

We present the first study comparing tumor-infiltrate versus tumor-specific circulating disease response biomarkers in HL patients. Both sCD163 and sTARC are sensitive and specific disease response biomarkers in the majority of cHL. However, when results are stratified by interim radiological assessment, sCD163 appears to more accurately reflect disease response than sTARC during therapy in cHL. Reflecting the relative abundance of tumor-infiltrating cells compared to HRS cells, sCD163 levels were 350-fold higher at pre-therapy compared to sTARC, and then declined less rapidly (than sTARC and plasma EBV-DNA) once chemotherapy had commenced. However, in patients completing first-line therapy who went on to have persistent clinical CR despite low-grade PET avidity of uncertain significance at restaging, sCD163 was frequently (albeit only modestly) above threshold, whereas sTARC values remained below threshold. sTARC appeared more frequently elevated than sCD163 in those with relapsed/refractory disease. However, in a patient with active atopic dermatitis sTARC was persistently elevated despite attaining CR. Thus the combination of sCD163 and sTARC appear more informative than either biomarker alone. Finally, we found that cHL patients had increased total circulating CD163+CD14+ monocytes, suggesting that the diseased node is not the only source of sCD163. Depletion of circulating CD163+CD14+ monocytes in cHL enhanced both CD4+ and CD8+ T-cell proliferation.

Tissue associated macrophages (TAMs) are potentially one of the most powerful independent markers for clinical prediction in cHL, (12-18) as well as potentially representing a novel therapeutic target (34). The best markers of TAMs are still a matter of debate (35). Results from studies are conflicting, with some, but not all, finding that high protein expression (by immunohistochemistry) of the monocyte/macrophage markers CD68 and CD163 correlated with adverse outcomes (12-15). Although our study was not designed to test the prognostic ability of tissue CD163, we did find (in line with some but not all prior reports), that values were associated with both Hasenclever score and the presence or absence of early stage disease. The inconsistency between reported studies may in part reflect the different populations tested and their retrospective nature, and also the intrinsic variability that is inherent in quantifying protein by IHC. By
contrast, quantification of proteins by ELISA is readily standardized between laboratories. We observed a modest but significant correlation between tissue and sCD163, and also (consistent with previous studies of tissue CD163) that sCD163 correlated with B symptoms and Ann Arbor stage (13, 14).

Previous studies have implied that EBV+ HRS cells modulate the tumor microenvironment (36-38). Intriguingly, we demonstrate a strong positive correlation with sCD163 and plasma EBV-DNA levels, suggesting a possible interaction with EBV proteins in HRS cells and CD163+ monocyte/macrophages. Monocytes have been shown to enhance proliferation and LMP1 expression of tumor cells in nasal natural killer/T-cell lymphoma (NNKTL), which is also an EBV latency type II associated malignancy (39). Consistent with this, four studies (with sample sizes ranging from approximately 100 to 300 tissues) have found that tissue CD163 protein does correlate with the presence of EBV-positive HRS cells (13-16). Cell-free EBV-DNA was not tested in these studies. Although we found no association between tissue CD163 protein levels and EBV-related cHL, our results are likely underpowered to show this association. Further studies are required to investigate the mechanistic basis for the association between CD163 and EBV in cHL.

Interestingly, sCD163 correlated with low lymphocyte count. At present, it is not fully understood how HRS cells recruit monocyte/macrophages, and once recruited to the tumor microenvironment, how these cells impact host systemic circulating immunity. HRS cells may result in increased CD163+ monocytes/macrophages. These, in turn, may mediate impaired T-cell proliferation, resulting in the inverse association between sCD163 and circulating lymphocyte count numbers that we observed. It has been shown in diffuse large B-cell lymphoma and follicular lymphoma that mo-MDSC inhibit T-cell proliferation (40). Notably, we found that mo-MDSC were not elevated in cHL, whereas circulating CD163+CD14+ monocytes were high compared to healthy participants. In functional assays, we demonstrate that monocyte depletion enhances T-cell proliferation in both cHL and healthy participants, albeit to a greater extent in cHL. Understanding why monocytes are elevated in cHL and the mechanism by which monocytes limit T-cell proliferation may be critical to understanding the pathogenesis of cHL and have therapeutic
implications, particularly as inhibition of TAMs with blocking molecules has been shown to slow tumor growth in other cancers (34). The elevated circulating CD163+CD14+ monocytes in cHL suggest that the diseased node is not the only source of sCD163. Consistent with this, although sCD163 was greatly reduced at six months post-therapy in CR cHL patients, sCD163 remained modestly elevated compared to healthy participants. Circulating CD163+CD14+ monocytes also remained elevated at six months post-therapy in CR cHL compared to healthy participants, possibly accounting for the remaining sCD163 levels.

This is the first study of sCD163 as a marker of disease response and the first to compare tumor-specific and tumor-infiltrate markers in cHL. Both sCD163 and sTARC were markedly elevated in pre-therapy cHL samples and significantly declined in complete responders. Our sTARC results are comparable to the recent study by Plattel et al. (11). In both cases, sTARC was elevated pre-therapy and dropped rapidly to normal levels following the first cycle of therapy in the majority of responding patients. A similar trend was also observed in a phase II study of patients receiving panobinostat (41). In both the Platell et al. study and our study (11), there was little difference between CR and PR sTARC levels at the mid-treatment time point. In our study, the gradual decline of sCD163 throughout treatment and elevated levels of sCD163 in PR versus CR at the mid-treatment time point, suggests that the tumor-infiltrate marker may have higher specificity during treatment. However, although our numbers of relapsed/refractory patients were limited, sCD163 was elevated in only one of the five patients at time of relapse/refractory disease. In contrast, sTARC was elevated in three of the five, suggesting sTARC may be a more sensitive marker of relapse. Notably, the two relapsed patients with non-elevated levels of sTARC had low tumor volume, one with ongoing disease confined to the bone. The work of Platell et al. found that sTARC was elevated in all 12 of their relapsed cHL patients, however, sCD163 was not measured (11). The different levels of sTARC and sCD163 may reflect a change in the relative proportions of tumor and tumor-infiltrating cells in the diseased nodes of refractory/relapsed patients. Biopsies were not taken at refractory/relapse presentation, otherwise a comparison of
tumor composition between biopsies taken at initial presentation and at relapse may have been informative.

We demonstrate for the first time that sCD163 is elevated in cHL patients. Circulating CD163 appears to arise from both tumor tissue and CD163+ monocytes. sCD163 correlated with cell-free EBV-DNA, and was associated with B symptoms, stage and low lymphocyte count. Depletion of CD163+ monocytes enhanced T-cell proliferation, implicating monocytes and/or TAMs as potential novel targets for immunotherapeutic manipulation. Finally, our results suggest that the combination of tumor-infiltrate (CD163) and tumor-specific (TARC) serum proteins are more informative than either marker alone as disease response biomarkers in early and advanced stage disease during first-line therapy for cHL. It is likely that circulating disease response biomarkers will vary according to the regimen and dose schedule. Future studies are needed to evaluate the differences in kinetics of serum disease response biomarkers in patients treated with commonly used first-line regimens such as ABVD, escalated BEACOPP and BEACOPP-14.

Acknowledgements

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References


Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>No. Patients (Total N=46†)</th>
<th>% Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age median (range)</td>
<td>35.6 (18-79)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>54%</td>
</tr>
<tr>
<td>Ann Arbor Stage III-IV</td>
<td>22</td>
<td>48%</td>
</tr>
<tr>
<td>Serum Albumin, less than 40g/L</td>
<td>32</td>
<td>70%</td>
</tr>
<tr>
<td>Haemoglobin, less than 105g/L</td>
<td>9</td>
<td>20%</td>
</tr>
<tr>
<td>Lymphocyte Count, at least 0.6x10^9/L</td>
<td>42</td>
<td>91%</td>
</tr>
<tr>
<td>White blood cell count, at least 15x10^9/L</td>
<td>7</td>
<td>15%</td>
</tr>
<tr>
<td>Hasenclever Score, at least 3</td>
<td>16</td>
<td>35%</td>
</tr>
<tr>
<td>B symptoms</td>
<td>17</td>
<td>37%</td>
</tr>
<tr>
<td>EBER-ISH Positive</td>
<td>12</td>
<td>26%</td>
</tr>
<tr>
<td>EBER-ISH Unavailable</td>
<td>3</td>
<td>7%</td>
</tr>
<tr>
<td>*Early stage</td>
<td>10</td>
<td>22%</td>
</tr>
<tr>
<td>Advanced stage</td>
<td>36</td>
<td>78%</td>
</tr>
</tbody>
</table>

**Histological Subtype**

- Nodular Sclerosing: 26 (57%)
- Mixed-cellularity: 4 (9%)
- Lymphocyte-rich: 4 (9%)
- Lymphocyte depleted: 1 (2%)
- cHL, unspecified: 7 (15%)
- NLPHL: 4 (9%)

**Treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Patients (Total N=42†)</th>
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<tr>
<td>ABVD</td>
<td>36</td>
</tr>
<tr>
<td>BEACOPP</td>
<td>2</td>
</tr>
<tr>
<td>ABVD + BEACOPP</td>
<td>2</td>
</tr>
<tr>
<td>CHVPP</td>
<td>2</td>
</tr>
</tbody>
</table>

† excluded patient with atopic dermatitis.

*Early stage is defined Ann Arbor stage I or II without any B symptoms, infradiaphragmatic presentations or mediastinal masses greater than one third the maximum thoracic diameter. Patients not fulfilling this criteria were defined as advance stage.
Table 2. cHL patient characteristics and pre-therapy serum protein levels

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>Total (N=42)</th>
<th>% Total</th>
<th>Mean ± SEM Serum CD163 (ng/ul)</th>
<th>CD163 P-value</th>
<th>Mean ± SEM Serum TARC (pg/ul)</th>
<th>TARC P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 45 years</td>
<td>30</td>
<td>71%</td>
<td>725.9 ± 52.61</td>
<td></td>
<td>2442 ± 295.4</td>
<td></td>
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<tr>
<td>At least 45 years</td>
<td>12</td>
<td>29%</td>
<td>816.8 ± 137.7</td>
<td></td>
<td>816.8 ± 137.7</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>48%</td>
<td>731.9 ± 53.66</td>
<td></td>
<td>1912 ± 376.6</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>52%</td>
<td>770.1 ± 91.57</td>
<td></td>
<td>2240 ± 354.1</td>
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<tr>
<td>Ann Arbor Stage</td>
<td></td>
<td></td>
<td></td>
<td>0.0328</td>
<td></td>
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<tr>
<td>I-II</td>
<td>20</td>
<td>48%</td>
<td>632.6 ± 61.04</td>
<td></td>
<td>1748 ± 327.4</td>
<td></td>
</tr>
<tr>
<td>III-IV</td>
<td>22</td>
<td>52%</td>
<td>860.3 ± 80.92</td>
<td></td>
<td>2389 ± 383.4</td>
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<tr>
<td>B symptoms</td>
<td></td>
<td></td>
<td></td>
<td>0.0351</td>
<td></td>
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<tr>
<td>No</td>
<td>26</td>
<td>62%</td>
<td>647.8 ± 43.18</td>
<td></td>
<td>1968 ± 325.3</td>
<td></td>
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<tr>
<td>Yes</td>
<td>16</td>
<td>38%</td>
<td>921.0 ± 112.4</td>
<td></td>
<td>2272 ± 424.4</td>
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</tr>
<tr>
<td>Hasenclever Score</td>
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<td></td>
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<tr>
<td>At least 3</td>
<td>27</td>
<td>64%</td>
<td>670.8 ± 45.55</td>
<td></td>
<td>1996 ± 330.6</td>
<td></td>
</tr>
<tr>
<td>Less than 3</td>
<td>15</td>
<td>36%</td>
<td>897.8 ± 120.1</td>
<td></td>
<td>2242 ± 411.8</td>
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<tr>
<td>Lymphocyte Count</td>
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<td>At least 1x10⁹/L</td>
<td>15</td>
<td>36%</td>
<td>933.8 ± 110.1</td>
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<td>2621 ± 467.6</td>
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</tr>
<tr>
<td>Less than 1x10⁹/L</td>
<td>27</td>
<td>64%</td>
<td>650.8 ± 48.73</td>
<td></td>
<td>1785 ± 293.1</td>
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<td>Stage</td>
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<td></td>
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<tr>
<td>Early</td>
<td>9</td>
<td>21%</td>
<td>609.8 ± 86.72</td>
<td></td>
<td>1763 ± 554.9</td>
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<tr>
<td>Advanced</td>
<td>33</td>
<td>79%</td>
<td>790.6 ± 63.15</td>
<td></td>
<td>2171 ± 291.3</td>
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</tr>
<tr>
<td>Histology</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Nodular sclerosing</td>
<td>26</td>
<td>62%</td>
<td>793.6 ± 67.99</td>
<td></td>
<td>2140 ± 326.9</td>
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<tr>
<td>Other specified cHL</td>
<td>9</td>
<td>21%</td>
<td>651.0 ± 95.64</td>
<td></td>
<td>1141 ± 517.8</td>
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<tr>
<td>cHL, unspecified</td>
<td>7</td>
<td>17%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EBER-ISH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>27</td>
<td>64%</td>
<td>808.1 ± 66.62</td>
<td></td>
<td>2357 ± 314.4</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>12</td>
<td>29%</td>
<td>611.4 ± 103.1</td>
<td></td>
<td>1286 ± 464.1</td>
<td></td>
</tr>
<tr>
<td>Unavailable</td>
<td>3</td>
<td>7%</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Plasma EBV-DNA†</td>
<td></td>
<td></td>
<td></td>
<td>0.0197</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least 200 genomes/ml</td>
<td>5</td>
<td>12%</td>
<td>344.7 ± 74.42</td>
<td></td>
<td>583.3 ± 326.6</td>
<td></td>
</tr>
<tr>
<td>Less than 200 genomes/ml</td>
<td>7</td>
<td>17%</td>
<td>801.9 ± 127.5</td>
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<td>1788 ± 724.8</td>
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</tr>
<tr>
<td>NA</td>
<td>29</td>
<td>69%</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

NS, not significant; SEM, standard error of the mean; Other specified cHL includes mixed-cellularity, lymphocyte-rich and lymphocyte-depleted cHL subtypes.

†In patients with EBV-related cHL
Figure Legends

Figure 1. sCD163 and sTARC as biomarkers in cHL. (A-C) Comparison of sCD163, sTARC and plasma EBV-DNA levels in cHL patients with CR by one month post-therapy and who remained in CR at six months post-therapy. (A) sCD163 levels, (B) sTARC levels, (C) Plasma EBV-DNA levels. (D,E) Comparison of interim-therapy treatment response. cHL patients, restricted to those with paired interim samples that matched interim radiological assessment. (D) sCD163 levels show a significant difference between paired pre-therapy and CR interim-therapy (P=0.0152) while no significant difference was seen between paired pre-therapy and PR interim-therapy (P=NS). (E) sTARC levels show a significant difference between paired pre-therapy versus CR interim-therapy (P=0.0001) and paired pre-therapy versus PR interim-therapy (P=0.0001). Error bars represent mean with SEM. P<0.001=***, P<0.01=**, P<0.05=*, P>0.05=NS.

Figure 2. Kinetics of serum CD163 and TARC in relapsed/refractory cHL and in patients in ongoing CR with equivocal restaging PET.

The dashed grey lines represent sTARC and the solid black lines sCD163. The dotted grey and black lines are the sTARC and sCD163 thresholds respectively. The arrows represent the timing of the radiological assessment. Limited small volume disease was defined as disease confined to a single site and ≤2cm. Patient A was a 31 year old female with EBV non-related nodular sclerosing cHL stage IIIA and a Hasenclever score of 3, with refractory disease at one month following completion of ABVD. sTARC but not sCD163 was elevated at the one month post-therapy time-point. The patient died of progressive disease at four months post-therapy. Patient B was a 22 year old female with EBV non-related nodular sclerosing cHL stage IVB and a Hasenclever score of 3, with resolution of disease by interim PET/CT prior to third cycle of BEACOPP. One month following completion of BEACOPP limited small volume bony disease was present by PET/CT. Neither sCD163 nor sTARC were above threshold at the one month post-therapy time-point. The patient went on to have salvage chemotherapy and no further research blood samples were obtained. Patient C was a 54 year old male with EBV non-related nodular sclerosing cHL stage IVB with a Hasenclever score of 5 who entered CR at one month following completion of ABVD, but had a
PET/CT confirmed relapse at five-months post-therapy. Both sCD163 and sTARC were elevated at one month post-therapy. Patient D was a 27 year old male with EBV non-related nodular sclerosing cHL stage IIIA with a Hasenclever score of 3 who entered CR at one month following completion of ABVD, but small volume relapse at six months post-therapy confirmed by PET-CET. Neither sCD163 nor sTARC were elevated at this time-point. Patient E was a 34 year old male with EBV non-related nodular sclerosing cHL stage III with a Hasenclever score of 2 who entered CR at one month following completion of ABVD, but relapsed with extensive PET/CT positive disease at six months post-therapy. sTARC, but not sCD163 was elevated at six months post-therapy. Patients F-H had persistent clinical CR despite low-grade PET avidity of uncertain significance at restaging.

**Figure 3. Comparison of total circulating CD163+CD14+ monocytes in cHL patients versus healthy participants.** Error bars represent mean with SEM. (A) Percentage of monocytes (defined as CD14+) within the total population of viable mononuclear cells (pre-therapy cHL versus healthy P=0.0006, post-therapy cHL versus healthy P=0.0010). (B) Percentage of CD163+ monocytes within the total population of viable mononuclear cells (pre-therapy cHL versus healthy P=0.0437, post-therapy cHL versus healthy P=0.0332).

**Figure 4. Monocyte depletion enhances T-cell proliferation in cHL patients.**
Histograms show (A) CD4+ and (B) CD8+ T-cell proliferation from eight cHL patients (labeled I-VIII). T-cell proliferation from CD14+ cell-depleted PBMC in grey and from non-depleted PBMC in black. (C) CD4+ and (D) CD8+ T-cell proliferation from cHL patient and healthy participant CD14+ cell-depleted PBMC and non-depleted PBMC represented as total percent proliferation above background. Error bars represent mean with SEM. P<0.01=** P>0.05=NS.
Figure 1

A

CD163 (ng/ul of Serum)

B

TARC (pg/ul of Serum)

C

EBV Genomes/mL of Plasma

D

CD163 (ng/ul of Serum)

E

TARC (pg/ul of Serum)

pre therapy

pre 2nd

pre 3rd

1M post

6M post

healthy

pre therapy

pre 2nd

pre 3rd

1M post

6M post

healthy

pre therapy

interim
cR

interim

P R

pre therapy

interim
cR

interim

P R

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited. Author Manuscript Published OnlineFirst on December 5, 2012; DOI: 10.1158/1078-0432.CCR-12-2693
I. Relapsed/Refractory patients

Patient A
- Refractory enlarging lymphadenopathy
- Deceased 4 months post

Patient B
- Relapsed small volume bone disease
- Sample N/A

Patient C
- CR
- Relapse extensive disease sample N/A

Patient D
- PR
- CR
- Relapse small volume disease

Patient E
- PR
- CR Sample N/A
- Relapse extensive disease

II. Patients in ongoing clinical CR with equivocal restaging PET

Patient F

Patient G

Patient H
% CD14+ of total mononuclear cells

A

% CD14+ of total mononuclear cells

B

% CD163+CD14+ of total mononuclear cells

pre-therapy chL
post-therapy chL
healthy

Figure 3

Research.
on April 14, 2017. © 2012 American Association for Cancer Research.
Figure 4

A

CD4$^+$ T-cells

% of Total

0 10 20 30 40 50 60 70 80 90 100

CFSE

PBMC CD14$^+$ cell-depleted

B

CD8$^+$ T-cells

% of Total

0 10 20 30 40 50 60 70 80 90 100

CFSE

PBMC CD14$^+$ cell-depleted

C

CD4$^+$ T-cells

% Proliferation

0 20 40 60 80 100

cHL CD14$^+$ depleted

CD8$^+$ T-cells

% Proliferation

0 20 40 60 80 100

cHL CD14$^+$ depleted

D

CD4$^+$ T-cells

% Proliferation

0 20 40 60 80 100

Healthy CD14$^+$ depleted

Healthy PBMC

Author Manuscript Published OnlineFirst on December 5, 2012; DOI: 10.1158/1078-0432.CCR-12-2693
Serum CD163 and TARC as Disease Response Biomarkers in Classical Hodgkin Lymphoma

Kimberley L Jones, Frank Vari, Colm Keane, et al.

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