Translational Oncology
www.transonc.com

Volume 11 Number 3 June 2018 pp. 779–785 779

# High Serum Level of Soluble Programmed Death Ligand 1 is Associated With a Poor Prognosis in Hodgkin Lymphoma

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## Abstract

Blockade of the programmed cell death 1-programmed cell death ligand 1 pathway is a new and promising therapeutic approach in Hodgkin lymphoma (HL). To our knowledge, the impact of soluble programmed cell death ligand 1 (sPD-L1) serum levels on HL patient prognosis has not yet been investigated. In this study, the prognostic value of sPD-L1 was assessed in patients with HL. We measured serum sPD-L1 levels and identified their prognostic value in 108 newly diagnosed HL patients using an enzyme-linked immunosorbent assay (ELISA). We found higher serum sPD-L1 concentrations in HL patients than in healthy controls. The best sPD-L1 cutoff value for predicting disease progression risk was 25.1674 ng/ml. The 4-year progression-free survival (PFS) rates for the high-sPD-L1 and low-sPD-L1 groups were 78.8% and 93.3%, respectively. Multivariate survival analysis showed that advanced stage and higher sPD-L1 levels (>25.1674 ng/ml) were independent prognostic factors for shorter PFS. In addition, higher sPD-L1 levels were positively correlated with advanced stage and negatively correlated with peripheral blood monocyte number. The serum sPD-L1 level is an independent prognostic factor for PFS in HL patients and may allow identification of a subgroup of patients who require more intensive therapy and who may benefit from anti-PD-1 agents.

*Translational Oncology (2018) 11, 779–785*

## Introduction

Hodgkin lymphoma (HL) is a rare cancer that originates from B lymphocytes and accounts for approximately 11% of all lymphoma cases and 0.5% of all cancers [1]. Standard treatment of newly diagnosed HL often involves a combination of multi-agent chemotherapy and radiotherapy, tailored to the stage of disease and the risk of relapse; this

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Received 21 November 2017; Revised 14 March 2018; Accepted 19 March 2018


1936-5233
https://doi.org/10.1016/j.tranon.2018.03.012

treatment can cure approximately 80% of patients [2]. Unfortunately, 20% of HL patients still relapse or develop refractory HL, for which effective treatment options are limited [3,4]. Second-line salvage with high-dose chemotherapy (HDC) and autologous stem cell transplantation (auto-SCT) has become the standard care for refractory/relapsed HL, leading to long-lasting responses in approximately 50% of patients [5]. However, disease recurrence or progression after auto-SCT is associated with a very poor prognosis. Thus, alternative therapies, such as antibody-drug conjugates (anti-CD30) [6] and immune checkpoint blockade drugs (anti-PD-1 and anti-PD-L1) [7,8] may be necessary. Furthermore, identification of patients with high risk of relapse is crucial in HL treatment.

Cancer cells have been shown to escape immune surveillance by up-regulating surface molecules that directly induce T-cell suppression [9]. These mechanisms are known as immune checkpoint pathways. Programmed cell death-1 (PD-1), an immune checkpoint expressed on the surface of T-, B- and natural killer (NK) cells, is indicative of this phenotype, and signaling through its ligands, programmed cell death ligand-1 (PD-L1) and programmed death ligand-2 (PD-L2), can attenuate signaling through the T-cell receptor (TCR) and lead to anergy/apoptosis and contribute to immune escape [10,11]. Recent clinical trials have shown that PD-1-blocking antibodies can enhance immunity in solid tumors and several hematologic malignancies, resulting in durable clinical responses [12–16]. Nivolumab and pembrolizumab, PD-1-blocking antibodies, both received breakthrough therapy designation from the FDA for HL patients [17–19].

Previous studies have indicated that PD-L1 overexpression was associated with poor survival in most solid tumors and hematopoietic malignancies [20,21]. However, the value of PD-L1 as a prognostic factor remains controversial [22]. There is an association between PD-L1 protein expression and relative genetic alterations in classical HL (cHL). For example, progression-free survival (PFS) has been shown to be significantly shorter for patients with 9p24.1 amplification, which up-regulates PD-L1 expression [23]. PD-L1 expression can be detected on the surface of tumor and immune cells by immunohistochemistry (IHC) [24] and in blood samples by enzyme-linked immunosorbent assay (ELISA) [25]. Serum sPD-L1 levels are reportedly higher in patients with malignant cancer than in healthy individuals, and high sPD-L1 was found to be a poor prognostic factor for hematopoietic malignancies in recent studies [26]. However, no investigations have assessed the relationship between serum sPD-L1 levels and HL patient prognosis. Therefore, the present study was conducted to address this issue. In addition, we also explored the correlation between serum sPD-L1 levels and the clinicopathological characteristics and immunologic features of HL patients.

## Materials and Methods

### *Patients*

In total, 108 consecutive patients diagnosed with HL and treated in Sun Yat-Sen University Cancer Center between May 2005 and April 2015 were enrolled in our study. The criteria included a primary diagnosis of HL, serum at diagnosis was available, and complete follow-up information. This study was approved by the Sun Yat-Sen University Cancer Center Research Ethics Board and informed consent for use of patient samples and publication was obtained from all patients.

### *Treatments and Response Evaluation*

Patients were clinically staged according to the Ann Arbor staging system and treated with risk-adapted treatment strategies. First-line treatment involved ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) or COPP (cyclophosphamide, vincristine, procarbazine and prednisone) chemotherapy, and some advanced stage patients underwent BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) chemotherapy in standard doses. The treatment courses, which comprised four to eight cycles, were based on the chemotherapy response. Radiotherapy was conducted depending on patients' age, risk group, residual tumor and response to chemotherapy. Treatment response was evaluated after every two cycles based on the World Health Organization (WHO) evaluation criteria. Routine follow-up imaging analyses were performed every 3 months for the first 2 years, every 6 months for the next 3 years, and annually (or whenever clinically indicated) thereafter.

### *Soluble PD-L1 Measurement*

Patient serum was collected at diagnosis before treatment from all 108 patients and from 15 healthy individuals matched for sex and age with enrolled patients and stored as 500 ml aliquots at –80°C. sPD-L1 was measured using an enzyme-linked immunosorbent assay (PDCD1LG1 ELISA kit, USCN Life Science, catalogue: SEA788Hu) according to the manufacturer's instructions. The minimum detectable concentration of sPD-L1 was 0.057 ng/ml. Each sample was analyzed in duplicate. The intra-assay and inter-assay coefficients of variation were below 20%. Briefly, samples and standards were added to a microplate precoated with a PD-L1-specific monoclonal antibody. After enzyme reagent and any unbound antibody were removed by washing, a substrate solution was added to the wells, Stop Solution was used to terminate color development, and the absorbance value was read at 450 nm using a spectrophotometer (Tecan, Mannedorf, Switzerland). The sPD-L1 concentrations were calculated using a standard curve, which was constructed using the standards provided in the kit.

### *Statistical Analysis*

Receiver operating characteristic (ROC) curve analysis was performed to determine the best cutoff value for the sPD-L1 concentration [27]. In this ROC curve, the point with the maximum sensitivity and specificity was selected as the cutoff value. Correlations between sPD-L1 concentration and various clinicopathological parameters were assessed using a Mann-Whitney *U* test or Wilcoxon-matched test, and a chi-squared test or Fisher's exact test was used for categorical values. Overall survival (OS) was defined as the time between the first day of diagnosis and the date of death from any cause; the follow-up of surviving patients was censored at their latest follow-up date. PFS was defined as the time between the first day of diagnosis and the date of disease relapse or progression; the follow-up of surviving patients was censored at their latest follow-up date. OS or PFS was analyzed using Kaplan-Meier curves, which were compared using log-rank tests. Multivariate prognostic analyses of OS or PFS were performed using the Cox proportional hazards regression model. All statistical analyses were performed using SPSS version 21.0. The results were considered statistically significant when $P < .05$.

## Results

### *Patient Characteristics and Baseline Serum sPD-L1 Levels*

*Patient Characteristics.* In total, 108 HL patients were enrolled in our study. The median age at diagnosis was 34.6 years of age (range, 4~76 years), and the study included more male patients (68 cases) than