Research paper

Circulating tumor DNA predicts response in Chinese patients with relapsed or refractory classical hodgkin lymphoma treated with sintilimab

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ABSTRACT

Background: Blood-based biomarker such as circulating tumor DNA (ctDNA) has emerged as a promising tool for assessment of response to immunotherapy in solid tumors; But in hematological malignancies, evidences are still lacking to support its clinical utility. In current study the feasibility of ctDNA for prediction and monitoring of response to anti-PD-1 therapy in Chinese patients with relapsed or refractory classical Hodgkin lymphoma (r/r cHL) was assessed.

Methods: A total of 192 plasma samples from 75 patients with r/r cHL were collected at baseline and upon therapeutic evaluation. ctDNA were sequenced by targeting panels capturing frequently mutated genes in cHL and other hematological malignancies and then quantified. Analysis on: 1) Gene mutation profile and association of the gene mutations with progression-free survival; 2) Association of pre- and post-treatment ctDNA variant allelic frequencies with clinical outcome; (3) Correlation of the mutated genes with treatment resistance; were performed.

Findings: Somatic mutations were detected in 50 out of 61 patients by ctDNA genotyping. The mutations of CHD8 was significantly higher in patients with PFS ≥ 12 months. Baseline ctDNA was significantly higher in responders and a decrease of ctDNA ≥ 40% from baseline indicated superior clinical outcome. Strong agreement between ctDNA dynamic and radiographic response change during therapy was observed in majority

Abbreviations: HL, Hodgkin lymphoma; cHL, classical Hodgkin lymphoma; r/r, relapsed or refractory; HRS, Hodgkin Reed-Sternberg; ctDNA, circulating tumor DNA; VAF, variant allelic frequency; PD-1, programmed cell death-1; ORR, objective response rate; TTR, time to response; PFS, progression-free survival; PET/CT, positron emission tomography and computed tomography; MRI, magnetic resonance imaging; CR, complete remission; PR, partial remission; PD, progressive disease; SD, stable disease

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1. Introduction

Hodgkin lymphoma (HL) accounts for 50% of all lymphomas in children and young adults in the Western world [1] and 8–6% of all lymphomas in mainland China [2]. This disease is a B-cell lymphoid malignancy characterized by a scarcity of malignant Hodgkin Reed-Sternberg (HRS) cells (i.e., only ~1% of all cells in the tumor environment) among the abundance of inflammatory/immune cells [3]. The pathogenesis of the disease involves amplification of chromosome 9p24.1, which leads to the overexpression of programmed cell death ligand 1 (PD-L1) and PD-L2 and constitutive activation of the JAK-STAT, NF-κB, and NOTCH signaling pathways. Approximately 5–10% of the patients with HL are refractory to first-line treatment, and 10–30% will relapse after attaining complete remission (CR) [4]. Two anti-PD-1 antibodies, nivolumab and pembrolizumab, have been approved to treat relapsed/refractory classical HL (r/r cHL) in the US. In China, another anti-PD-1 antibody, sintilimab was recently approved by the National Medical Products Administration to treat r/r cHL. All three agents achieve a high objective response rate (ORR) exceeding 60%.

Despite this robust ORR, some patients do not respond to anti-PD-1 treatment or have progressive disease (PD) after a short initial response. In recent years, some studies have investigated possible biomarkers that are potentially correlated with response to anti-PD-1 treatment in patients with r/r cHL. These are tissue biopsy-based biomarkers, which include the expression levels of PD-L1, PD-L2, major histocompatibility complex (MHC) class I and class II in tumor cells [5–7]. However, the overall attempts to explore the potential biomarkers are limited and until recently, no reproducible, well-validated biomarker is available.

Blood-based biomarker is an emerging attractive tool for disease diagnosis and treatment assessment because of the minimally invasive sampling and thus high patient compliance. More importantly, blood is a easy-to-access source which allows for serial sampling for monitoring disease progress during treatment. Circulating tumor DNA (ctDNA) in peripheral blood contains DNA fragments that are derived from apoptotic or necrotic cancer cells [8] and, thus, deep next-generation sequencing (NGS) of this DNA can provide valuable information of cancer. Use of ctDNA as a dynamic biomarker for treatment response in solid tumor [9] and hematological malignancies [10] is becoming more common. In cHL, the ctDNA quantity is low in peripheral blood due to the scarcity of HRS cells in the tumor, which brings challenges to the analysis of ctDNA. Despite this, progress has been made in using ctDNA as a biomarker for multiple clinical applications such as the assessment of genomic imbalances, prognosis, minimal residual disease, therapeutic response to chemotherapy, and clonal evolution in cHL [11,12]. However, to date, no study has explored the correlation between ctDNA and clinical outcome of anti-PD-1 treatment in this disease.

In this study, we utilized ctDNA to examine the genetic features of Chinese patients with r/r cHL and assess the predictive value of ctDNA for response to sintilimab immunotherapy in a large cohort [13].

Evidence in context

**Evidence before this study**

Circulating tumor DNA (ctDNA) has been used for multiple clinical applications such as the assessment of genomic imbalances, subtyping, prognosis, minimal residual disease, monitoring therapeutic response and clonal evolution in hematological malignancies. We searched Pubmed for articles published before Oct 01, 2019 using the terms, “circulating tumor DNA”, “Classical Hodgkin lymphoma”, “immunotherapy”, with no limitation to clinical trials or other types of studies. Only one article titled “Circulating tumor DNA reveals genetics, clonal evolution, and residual disease in classical Hodgkin lymphoma” (published in May of 2018 on journal of “blood”) was found. In this study, only five patients treated with anti-PD-1 blockade were included and there is no report on the association between ctDNA and clinical outcome of these patients. To our knowledge, there were no published studies that evaluated the value of ctDNA for predicting or monitoring the response to immunotherapy in patients with relapsed or refractory classical Hodgkin lymphoma (cHL) before our study.

**Added value of this study**

Genetic profiling of Chinese patients with relapsed or refractory cHL was delineated. Our study showed that baseline ctDNA was predictive for response to immunotherapy in these patients. Patients with a ctDNA decrease of ≥ 40% from baseline achieved superior clinical outcome than those with < 40% and patients with a tumor area decrease of ≥ 60% showed a more substantial ctDNA decline than those with tumor area decrease of < 60%. Strong agreement between ctDNA dynamic and radiographic response change during therapy was also observed in majority of the patients. Furthermore, the mutations of B2M, TNFRSF14 and KDM2B were found to be associated with acquired resistance to anti-PD-1 therapy.

**Implications of all the available evidence**

There is no validated biomarker available for assessment of response to immunotherapy in patients with relapsed or refractory cHL. Imaging is the standard approach for therapeutic response assessment and disease monitoring. However, imaging has its limitation as it measures the size of the tumor mass including inflammatory component, which is often seen in patients under immunotherapy. ctDNA may reflects the actual tumor burden, therefore, it could be complement to imaging for the comprehensive assessment of immunotherapy efficacy. We proved the concept that ctDNA could be a valuable biomarker for predicting or monitoring the response to immunotherapy in patients with relapsed or refractory cHL. Besides, we also proved that ctDNA could be a reliable source for detection of gene mutations, which could provide valuable information for further understanding the pathogenesis and clone evolution of cHL, as well as mechanism of resistance to immunotherapy.
2. Materials and methods

2.1. Participants and sample collection

Of the 96 Chinese patients with r/r cHL enrolled in the ORIENT-1 study (NCT03114683), 75 agreed to participate in this biomarker study and provided informed consent. Participants were enrolled from April to November 2017, and the data cutoff was December 29, 2018 after a median follow-up time of 16.7 months. The characteristics of participants in this study are listed in Table 1 and additional details of individual cohorts are in supplementary Table 1.

From the 75 enrolled patients, 192 longitudinal samples were collected and analyzed for ctDNA. A flowchart of blood samples for ctDNA analysis is shown in Fig. 1. Treatment response was assessed by positron emission tomography and computed tomography (PET/CT), enhanced CT scan (preferred), or magnetic resonance imaging at baseline, at weeks 6, 15, and 24, every 12 weeks from weeks 24 to 48, and every 16 weeks beyond week 48, until commencement of a new therapy, disease progression, death, or withdrawal of consent, as described previously [13]. The PET/CT scan was assessed by an independent radiological review committee (IRRC) and then the therapeutic response was defined. The study was conducted in accordance with the Declaration of Helsinki. The protocol, amendments, and patient informed consent were approved by the independent ethics committee at each participating site before the study began.

2.2. Sample processing and DNA extraction

Peripheral blood was collected in cell-free DNA collection tubes (Cat# 218962, Streck Inc., Omaha, NE) and processed within 24 h. After separation of plasma, the cell pellet was resuspended to remove peripheral blood mononuclear cells using Ficoll-Paque Premium solution (Cat# GE17-5442/C02, GE Healthcare, RRID:SCR_000004), and the remaining high-density peripheral blood cells were collected. Cell-free DNA and genomic DNA were extracted from plasma and high-density peripheral blood cells using the QIAamp circulating nucleic acid kit and QIAamp DNA blood mini kit (Cat# 55114 and Cat# 51106, Qiagen, RRID:SCR_008539), respectively, according to the manufacturer’s instructions. DNA concentration was measured using a Qubit fluorometer and the Qubit dsDNA high sensitivity assay kit (Cat# Q32854, Invitrogen, RRID:SCR_008539). The size distribution of plasma DNA was assessed using an Agilent 2100 bioanalyzer and the DNA high sensitivity kit (Cat# 5067-4626, Agilent Technologies, RRID:SCR_013575).

2.3. Sequencing library construction and target enrichment

Before library construction, 1 μg of each genomic DNA was sheared to 300-bp fragments with a Covaris S2 ultrasonicator (Covaris, Woburn, MA). For library construction, 20–80 ng of cell-free DNA were used. Indexed Illumina libraries were prepared for genomic and cell-free DNA using the KAPA DNA library (Cat# KK2602, Table 1

| Characteristic | Number (Ratio) |
|---------------|----------------|
| Median age (range), years | 35 (19–70) |
| Female | 29 (38.7%) |
| Male | 46 (61.3%) |
| Cell-free DNA samples available | 192 |
| Pre-treatment/Baseline* | 61 |
| Cycle 3, week 6 | 45 |
| Cycle 6, week 15* | 41 |
| Cycle 9, week 24* | 31 |
| Cycle 13, week 36* | 11 |
| Cycle 17, week 48* | 1 |
| Others | 2 |
| The best response | |
| Complete remission | 24 (32.0%) |
| Partial remission | 39 (52.0%) |
| Stable disease | 10 (13.3%) |
| Progressive disease | 2 (2.7%) |
| Number of patients by different analysis | |
| Baseline ctDNA analysis | 61 |
| ctDNA decline after 2 treatment cycles | 29 |
| Serial ctDNA dynamics analysis | 34 (including the 29 above) |
| Drug resistance mechanism | 13 |
| Median follow-up time, month | 16.7 (3.7–18.9) |

Abbreviations: ctDNA, circulating tumor DNA.
* Peripheral blood samples (10 mL) were collected and then plasma was isolated for ctDNA analysis for each participant at baseline and immediate before each subsequent treatment.

Fig. 1. Scheme of blood samples for circulating tumor DNA (ctDNA) analysis. A flowchart showing the blood samples of participants analyzed in the study.
Kapa Biosystems, Wilmington, MA). Target enrichment was performed with a custom SeqCap EZ library (Cat# 5634253001, Roche NimbleGen, RRID:SCR_008571). To explore the comprehensive genetic profiling of cHL, two capture probes were chosen. One was designed based on -2.1-Mb genomic regions of 619 genes that are frequently mutated in cHL and other common lymphoma and hematologic malignancies. The other probe was designed based on -2.4-Mb genomic regions of 659 genes, which is an updated version of a 619-gene panel with 42 T-cell lymphoma-related genes included and two RNA genes removed. Capture hybridization was carried out according to the manufacturer's protocol. Following hybrid selection, the captured DNA fragments were amplified and pooled to generate multiplex libraries.

2.4. NGS sequencing

Cell-free and genomic DNA libraries were sequenced using the HiSeq Sequencing System (Illumina, San Diego, CA) with paired-end reads supported by a commercial vendor (Geneplus-Beijing, China).

2.5. Sequence data analysis

After removal of terminal adapter sequences and low-quality data, reads were mapped to the reference human genome (hg19) and aligned using BWA version 0.5.9 (Broad Institute) [14]. Sequencing data from paired tumor-germline samples were used to identify somatic mutations. Single-nucleotide variants were identified using MutTect (version 1.1.4) and NChot [15,16]. Small insertions and deletions were determined using GATK [17]. Copy number variations were detected using the CONTRA tool (2.0.8) [18]. An in-house algorithm was used to identify split reads and discordant read pairs to identify gene fusion. At least five supporting reads were required for true fusion. All final candidate variants were manually verified with the integrative genomics viewer browser.

2.6. ctDNA analysis

ctDNA was quantified by determining the allelic fraction of cfDNA fragments that harbor cancer-associated somatic mutations [9]. The variable variant allelic frequencies (VAFs) in different mutations from ctDNA implied variable clonality of different mutations. To explore the subclonal architecture of cHL by ctDNA sequencing, we used PyClone [19] to infer the cancer cell fraction of each mutation in each ctDNA sample. Mutations were then clustered by corresponding cancer cell fraction, and the subclonal architecture of ctDNA from cHL was subsequently inferred. The overall ctDNA VAF was defined as the 100-fold average VAF of mutations from the major clones (the cluster with the greatest cancer cell fraction).

2.7. Statistics

Time to response (TTR) was measured from the date of first sintilimab treatment to the date of first radiological remission. Progression-free survival (PFS) was measured from the date of first sintilimab treatment to the date of progression (event) or last follow-up (censoring). The significance of mutation genes was computed using Fisher’s exact test between different PFS groups. Differences in ctDNA between two groups were analyzed using the Mann-Whitney test. Survival and response analysis was performed using the Kaplan–Meier method with the log-rank test. All statistical analysis was conducted using IBM SPSS software and GraphPad Prism. All tests were two-sided and p-value < 0.05 was considered as statistically significant.

2.8. Data availability

The supplementary materials provide all reported mutations. All other relevant data can be obtained from the corresponding author.

3. Results

3.1. Genomic profiling of r/r cHL at baseline

Genomic profiling of ctDNA was performed on baseline plasma samples from 61 patients with r/r cHL (Suppl. Table 1) at an average sequencing depth of 1260 × (range, 484–2231 ×) (Suppl. Table 2). DNA from granulocytes of the same patient was sequenced as the germline control (Suppl. Table 3). Nonsynonymous somatic mutations were observed in 50 out of 61 patients. A median of 15 somatic mutations per sample (range, 1–234) were identified, for a total of 1416 (Suppl. Table 4). The median allele frequency of ctDNA mutations was 3.15% (range, 0.49–60.15%), and 97.46% of the mutations had an allele frequency > 1%. The 25 genes found to be mutated in more than 10% of patients (Suppl. Fig. 1) included many commonly reported in studies of purified HRS cells in cHL (7), such as STAT6 (34.43%), TNFAIP3 (31.15%), SOCS1 (24.59%), and B2M (22.95%), indicating the reliability of the non-invasive genotyping using ctDNA as a source. Two mutations, PCLO (22.95%) and LRPIB (22.95%), appear to be unique to Chinese patients.

Next, we assessed whether mutations in individuals were associated with progression-free survival (PFS). The patients were divided into two groups, PFS < 12 months vs. PFS ≥ 12 months, and baseline mutation profiles were compared. CHD8 mutation frequencies was significantly different between the two groups (Fig. 2), detected only in the PFS ≥ 12 months group (Fisher p = 0.0287). However, comparison of the mutations between responders and non-responders was limited due to the small sample size of the non-responders (Suppl. Fig. 2).

3.2. Association between baseline ctDNA and clinical outcome

To investigate whether ctDNA could be used to predict immunotherapy response, we first tested the association between baseline ctDNA and best radiographic response (Suppl. Table 5). Varied ctDNA VAF were observed among patients, with a median value of 6.21% (95% CI, 3.86–10.57). The median ctDNA VAF was significantly higher in the responder group (CR + PR, n = 41, median = 8.72%) than the non-responder group (SD+ PD, n = 9, median = 2.9%) (Fig. 3a, p = 0.0070 Mann-Whitney test). Consistently, using receiver operating characteristic (ROC) analysis, the median baseline ctDNA VAF could distinguish the different objective response groups (Fig. 3b; AUC = 0.7832 (95% CI: 0.6383 to 0.9281); p = 0.0083 Mann-Whitney test). Then, we divided the ctDNA-positive patients into two groups based on the median ctDNA VAF (high, n = 25; and low, n = 25). Patients with higher baseline ctDNA VAF had a shorter TTR (Fig. 3c). However, the baseline ctDNA VAF could not predict PFS (Fig. 3d).

3.3. Correlation between ctDNA change and efficacy of immunotherapy

To determine whether an early ctDNA change was associated with the best radiographic response to sintilimab treatment, ctDNA analysis was conducted on serial samples collected both at the baseline and immediate before the third treatment from 29 patients. Patients achieving CR and PR had a greater decline in ctDNA VAF after two treatment cycles than those who relapsed (Fig. 4a, b). When 40% ctDNA reduction was selected as the cutoff in our cohort, 79.31% (23/29) of patients showed agreement between the change in ctDNA and the best radiographic response, with CR or PR achieved in 90% (18/20) patients who showed a ctDNA decrease >40% (Fig. 4a). TTR analysis further showed that patients with a ctDNA decrease >40% achieved first response significantly earlier (median = 71 days) than
the others (median = 216 days) (Fig. 4c; \( p = 0.0238 \) Mann-Whitney test). However, the ctDNA reduction could not predict PFS (data not shown).

The trend of change in ctDNA VAF in 34 patients (including the above 29 patients, Supplementary Table 6) with serial evaluable samples from baseline to different cycles posttreatment was also compared. Patients with tumor area decrease \( \geq 60\% \) (\( n = 17 \)) showed a more substantial ctDNA decline than those with tumor area decrease \( <60\% \) (\( n = 17 \)) (Fig. 4d).

Next, we examined the agreement between ctDNA dynamics with radiographic response changes (reflected by PET/CT scan) during treatment in these 34 patients. A strong agreement during part or all
of the treatment cycle was observed in most of the patient (94.12%, 32/34) (Suppl. Table 6) and representative cases are shown in Fig. 5A-C. For example, patient 7002 whose radiographic response was PR, demonstrated a posttreatment decrease in ctDNA (Fig. 5a). Seven patients initially responded to the therapy but later relapsed and progressed; as shown for patient 3018, the ctDNA VAF dropped at the beginning of therapy but subsequently increased during later cycles, again demonstrating consistency between ctDNA dynamics and radiographic response (Fig. 5b). While in patient 19,002 and patient 23,010 (Suppl. Table 6), who did not respond to immunotherapy or had SD initially but soon progressed later, respectively, both the ctDNA VAF and tumor burden increased constantly during therapy.

3.4. Potential resistance mechanism to immunotherapy in cHL

In patients 8003 and 23,002, the B2M loss-of-function mutation was shown at baseline and reoccurred in the progressive disease cycle (Table 2). In the plasma samples from these two patients after relapse, a homozygous nonsense mutation B2M in patient 23,002 was identifi ed based on the adjusted variant allele frequency calculated according to the method described by Shin DS[20] (Suppl. Table 7). The adjusted variant allele frequency was greater than 85%, which is the threshold proposed and validated in a study of anti-PD-1 therapy in melanoma [21].

In addition, several potential resistance-related genes were only found in the relapsed patients (Table 2). There was no report on the association of these genes with resistance to anti-PD-1 immunotherapy. Of note, two of the genes, TNFRSF14 and KDM2B, were identifi ed in more than one resistant patient, and the association of these two genes with resistance will need to be confirmed in further study.

4. Discussion

Dynamic sampling of ctDNA provides genetic information pre- and post-treatment which allows disease subtyping, study of genome evolution patterns, and real-time monitoring of response or resistance to therapy in cancers including hematological malignancies [12,22–24]. Here, in a cohort of Chinese patients with r/r cHL treated with sintilimab, we report that ctDNA can serve as a clinically informative biomarker for (1) genotyping; (2) response prediction and monitoring; and (3) resistance to PD-1 blockade immunotherapy.

To our knowledge, this is the fi rst study to genotype r/r cHL in Chinese patients. The most frequently mutated genes revealed by ctDNA genotyping in the current study (Suppl. Fig. 1) are consistent with those observed in purified HRS cells [25] and in ctDNA from Western patients with newly diagnosed cHL [12]. Therefore, our results validated the utility of ctDNA as an alternative source for disease investigation, especially given the challenges in obtaining rare malignant
HRS cells from HL patients. Besides, we also identified other frequently mutated genes in Chinese patients, PCLO and LRP1B, neither of which have been previously reported in non-Chinese patients. It is possible that PCLO and LRP1B were detected due to clonal evolution, which was also reported previously during the treatment of cHL [12] and diffuse large B-cell lymphoma (DLBCL) [23], since patients included in the study cohort have experienced heavy treatment regimens before enrollment in the trial. However, further investigation will be needed to elucidate the potential roles of these genes in the pathogenesis of cHL.

The mutation frequencies of CHD8 was significantly higher in patients with PFS ≥ 12 months than those with PFS < 12 months, indicating that the mutation could serve as predictive biomarkers for clinical benefit. There are few reports on the correlation of CHD8 with cancer. One study showed that CHD8 suppresses p53-mediated apoptosis during early embryogenesis [26]. It is likely the CHD8 mutation may suppress the function of TP53 and thus mediate the therapeutic effect of anti-PD-1 treatment.

Besides serving as an easily accessible and reliable source for genotyping, ctDNA could be used to predict response, monitor response, and study resistance mechanisms to anti-PD-1 therapy. First, we found that baseline ctDNA VAF were strongly correlated with response, including with TTR. This could be because ctDNA is a better measurement of tumor cell turnover, which reflects real-time tumor cell mortality, rather than tumor mass [27]. High baseline ctDNA VAF could mirror high tumor antigen release, leading to strong immune response against tumors which is blocked by the PD-1 pathway. Under these conditions drugs blocking PD-1 action might be more effective.

Further, an association between ctDNA dynamics and clinical outcome was observed. A decrease of ctDNA VAF ≥40% after two cycles was found to be associated with superior radiographic response (i.e., CR or PR). This observation is similar to that reported in a small cohort of cHL patients (n = 24) treated with chemotherapy, which showed that a decrease in ctDNA after two cycles of ABVD of at least 2-log was associated with more favorable clinical outcome than those with a smaller ctDNA reduction [12]. We also observed a strong agreement between ctDNA dynamics and radiographic response during therapy, consistent with that reported in DLBCL [10], indicating that ctDNA could be used as a biomarker for the assessment of immunotherapy efficacy. The current standard for therapeutic response assessment and disease monitoring for cHL is imaging. While it is an essential clinical tool, imaging has its limitations, such as low...
Fig. 5. Correlation of ctDNA response to radiographic response during sintilimab therapy. Plasma levels of ctDNA (red line) and measurements of radiographic tumor area (mm²) (blue line) are plotted for three representative patients. (a) Patient 7002, a 36-year-old woman achieved persistent ctDNA response and radiographic response after treatment. (b) Patient 3018, a 33-year-old man who achieved ctDNA and radiographic response after 2 treatment cycles, and then progressed after 12 cycles. (c) Patient 21,003, a 26-year-old woman who showed an increase of ctDNA VAF and radiographic progression after 2 cycles, showed ctDNA decrease with radiographic responses after 8 cycles. ctDNA and radiographic measurements for the remaining 31 patients in the study are presented in Supplementary Table 6.
sensitivity/specificity when tumor size is < 10 mm and difficult to distinguish disease progression from treatment effects, infection, or inflammatory changes upon immunotherapy. In this circumstance, as ctDNA reflects mainly malignant cell burden, it could serve as an important dynamic biomarker to complement standard imaging for monitoring response during anti-PD-1 immunotherapy.

The mechanisms of resistance to PD-1 therapy are very complex, involving factors both intrinsic and extrinsic factors [28]. To date, previous studies have demonstrated that loss-of-function mutations of B2M, JAK1/2, or PTEN were associated with resistance to immunotherapy [20, 21, 29-31]. Subsequent efforts have confirmed that mutations in B2M and JAK1/2 are associated with primary or acquired resistance to immunotherapy in solid tumors [32]. In cHL, however, the mechanisms of resistance to checkpoint inhibitor-based immunotherapy remain largely unknown [33]. In this study, using ctDNA to probe the gene mutations that might be involved in mediating resistance to sintilimab therapy, we identified B2M, TNFRSF14 or KDM2B mutation in 2 of 12 patients with acquired resistance, respectively. Noticeably, the B2M mutation in patient 23,002 is homozygous, with an adjusted VAF% over 85%, the threshold validated for homozygous mutation in melanoma treated with anti-PD-1 blockade [20]. Unfortunately, we were unable to determine whether the B2M mutation in patient 8003 is homozygous or heterozygous because of insufficient tumor DNA quantity derived from ctDNA extraction.

No association between TNFRSF14 or KDM2B mutation with resistance of immunotherapy has been previously reported. TNFRSF14 is expressed ubiquitously in multiple tissues, with a relatively high expression in peripheral blood T cells, B cells, and monocytes. Although it is well known that TNFRSF14 stimulates T-cell immune responses, the biological role of this protein in cancer cells might be distinct from that in immune cells. TNFRSF14 mutation was reported to be associated with inferior outcome in follicular lymphoma and studies suggested that TNFRSF14 acts as a tumor suppressor [34, 35], which might contribute to the resistance to anti-PD-1 treatment. KDM2B also acts as a tumor suppressor by controlling ribosome biogenesis in cancer cells [36, 37], which supports our findings in this study. Overall, our results are preliminary and further investigation is anticipated to validate the roles of these genes in mediating resistance to immunotherapy.

Study limitations include the lack of matched tumor biopsy, short duration of follow-up and small sample size of ctDNA kinetics. The study is also somewhat preliminary in that the patients are still being followed up, and the association between ctDNA and clinical outcome will be reanalyzed. Additionally, our study population may be biased because patients who did not have detectable ctDNA at baseline were excluded. It is worthy to mention that all the preliminary findings reported in the current study will be further investigated in a larger patient populations recruiting in an ongoing phase III study (ORIENT-21, NCT04044222).

In summary, we demonstrated in this r/r cHL cohort of Chinese patients that ctDNA can be a reliable biomarker for predicting and monitoring response to sintilimab, and a tool for exploring treatment resistance mechanisms. ctDNA could be incorporated into clinical practice to complement PET/CT scan for monitoring early response to anti-PD-1 treatment. Furthermore, our results suggest future research directions for studying genes that might be involved in the pathogenesis of cHL and resistance to anti-PD-1 treatment.

Author contributions

YSh was the lead principal investigator and contributed to study design and conception, data analysis, and interpretation. All authors were involved in patient recruitment and data acquisition. YX, HZ, XW, and BP completed the literature search, data analysis and provided writing support. XDu did the ctDNA sequencing, analyzed data and generated figures. All authors had full access to all study data, contributed to writing and reviewing the manuscript, and approved the submitted version.

Declaration of Competing Interest

Yan Xiong, Hui Zhou, Xiong Wang, and Bo Peng are employees of Innovent Biologics (Suzhou) Co., Ltd. XinHua Du is an employee of Geneplus-Beijing. All other authors declare no competing interests.

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Data sharing

There are currently no plans to share additional data beyond what is included in this paper.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2020.102731.

Table 2

| Patient ID | The best response | Resistance | PFS (day) | Gene | Mutations | COSMIC ID |
|------------|-------------------|------------|-----------|------|-----------|-----------|
| 19,002     | PD                | Primary    | 41        |      |           |           |
| 21,003     | PD                | Primary    | 42        |      |           |           |
| 8003       | PR                | Acquired   | 164       | B2M  | c.261C>A  (p.Y87*)|           |
| 23,002     | PR                | Acquired   | 439       | B2M  | c.371G>G  (p.R71*)| 144,525  |
| 10,010     | SD                | Acquired   | 107       | TNFRSF14 | c.216(C>A) (p.T73S) |           |
| 7001       | PR                | Acquired   | 252       | TNFRSF14 | c.256dupACCTCTT (p.V246E) |           |
| 23,003     | SD                | Acquired   | 168       | KDM2B | c.880C>T (p.R294*) | 1,586,272 |
| 23,010     | SD                | Acquired   | 169       | KDM2B | c.2375G>A (p.W1092*) |           |
| 3013       | PR                | Acquired   | 252       |      |           |           |
| 3018       | PR                | Acquired   | 251       | S1PR2* | c.1019G>C (p.G3400*) |           |
| 13,002     | PR                | Acquired   | 168       | NFKB2* | c.1408G>C (p.Y473F) |           |
| 13,004     | PR                | Acquired   | 100       | RELN* | c.1019G>C (p.G3400*) |           |
| 14,002     | PR                | Acquired   | 103       |      |           |           |
| 26,002     | SD                | Acquired   | 166       |      |           |           |

Abbreviations: PD, progressive disease; PFS, progression-free survival; PR, partial remission; SD, stable disease.

* Potential resistance-related genes, which have not been previously reported in an immunotherapy study.
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