Plasma circulating tumor DNA assessment reveals KMT2D as a potential poor prognostic factor in extranodal NK/T-cell lymphoma

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Abstract

Background: The early detection of tumors upon initial diagnosis or during routine surveillance is important for improving survival outcomes. Here, we investigated the feasibility and clinical significance of circulating tumor DNA (ctDNA) detection for Extranodal NK/T-cell lymphoma, nasal type (ENTKL).

Methods: The plasma ctDNA assessment was based on blood specimens collected from 65 newly diagnosed patients with ENKTL in the hematology medical center of Xinqiao Hospital. Longitudinal samples collected under chemotherapy were also included. The gene mutation spectrum of ENKTL was analyzed via next generation sequencing.

Results: We found that the most frequently mutated genes were KMT2D (23.1%), APC (12.3%), ATM (10.8%), ASXL3 (9.2%), JAK3 (9.2%), SETD2 (9.2%), TP53 (9.2%) and NOTCH1 (7.7%). The mutation allele frequencies of ATM and JAK3 were significantly correlated with the disease stage, and mutated KMT2D, ASXL3 and JAK3 were positively correlated with the metabolic tumor burden of the patients. Compared with the tumor tissue, ctDNA profiling showed good concordance (93.75%). Serial ctDNA analysis showed that treatment with chemotherapy could decrease the number and mutation allele frequencies of the genes. Compared with PET/CT, ctDNA has more advantages in tracking residual disease in patients. In addition, patients with mutated KMT2D had higher expression compared with those with wild type, and mutated KMT2D predicted poor prognosis.

Conclusion: Our results unveil the mutation spectrum of ENKTL patients’ plasma, which can be used to monitor the disease status of the patients exactly, and KMT2D is the most frequently mutated gene with prognosis prediction value. The application of ctDNA sequencing can provide precision treatment strategies for patients.

Trial registration: This study is registered with chictor.org (ChiCTR1800014813, registered 7 February, 2018- Retrospectively registered).

Keywords: ENKTL, Circulating tumor DNA, Mutation allele frequency, Minimal residual disease, Prognosis

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Introduction
Extranodal NK/T-cell lymphoma, nasal type (ENTKL), is an aggressive extranodal lymphoma of NK-cell or T-cell lineage, which is highly aggressive and heterogeneous disease, with predominance in males [1–4]. Current treatment strategies (such as the combination of chemotherapy, radiotherapy, targeted therapy) can improve the complete remission of patients, but most will ultimately relapse and progress [5–7]. For these patients, standard monitoring methods, including computed tomography (CT) and serum protein markers analysis upon initial diagnosis and during routine surveillance, are pivotal for therapeutic response evaluation; however, these tests have limitations of low sensitivity and specificity or false positivity. Therefore, there is an urgent need for a highly sensitive, standardized and noninvasive assay that can be used for detecting early relapse and/or progression of disease.

Current treatment response criteria for non-Hodgkin lymphoma (non-HL) rely on CT scans or positron emission tomography (PET) scans. Imaging scans upon initial diagnosis or recurrence can provide macro overviews of the tumor volume and location, but CT scans have some limitations due to cost and radiation exposure, and PET lacks specificity [8–10]. Moreover, imaging scans cannot monitor dynamic tumor response and the clonal evolution in time. Therefore, clinically validated technology is needed to overcome the current monitoring treatment response limitations. One potential biomarker for detection is circulating cell-free DNA (ctDNA), which comes from dying cells that release DNA fragments into circulation, and in tumor patients, some of the ctDNA primarily originates from apoptosis and necrotic cancer cells, which carry tumor-specific alterations (termed circulating tumor DNA, ctDNA) [11–14]. ctDNA sequencing is a promising tool for the real-time monitoring of tumor progression, and its application has been explored in multiple solid tumors [15], ctDNA can reflect the temporal evolution of tumors. Current studies have shown that ctDNA detection can be used to monitor minimal residual disease and track recurrence after treatment [16, 17]. In addition, ctDNA sequencing is a noninvasive and tumor-specific assay, and ctDNA in circulation has a half-life of less than 2 h, which is more sensitive than protein biomarkers and more specific than routine surveillance imaging through CT scans or PET scans [18]. Thus, dynamic tracking of ctDNA would be of higher value in guiding personalized management strategy.

The clinical application of ctDNA has been performed for multiple types of lymphoma, such as diffuse large B-cell lymphoma (DLBCL), classical HL, follicular lymphoma, multiple myeloma and peripheral T-cell lymphoma [19–25]. ctDNA assessment acts as a dynamic marker of burden during treatment based on immunoglobulin next-generation sequencing (Ig-NGS), T-cell receptor gene next-generation sequencing (TCR-NGS) and cancer personalized profiling sequencing (CAPP-Seq). Recent evidence concerning ctDNA assessment in peripheral T-cell lymphoma by TCR-NGS suggested that ctDNA detection in T-lineage lymphoma is feasible [26]. In the present study, we thoroughly determined the molecular spectrum of ctDNA mutations in ENKTL and characterized the correlation between ctDNA mutations and clinical factors. Paired tumor tissues and plasma samples were collected to compare the concordance between tumor DNA and ctDNA. Moreover, dynamic ctDNA mutation alterations during treatment were also observed.

Materials and methods
Study design and patient selection
In this prospective cohort study, 65 patients newly diagnosed with ENKTL in the hematology medical center of Xinqiao Hospital from February 2017 to December 2019 were enrolled (ClinicalTrials identifier: ChiCTR1800014813). All consecutive patients who were deemed appropriate for this study during the study period were included without selection. Histological diagnoses were established independently by at least two experienced senior pathologists according to the WHO classification of Tumors of Hematopoietic and Lymphoid tissue criteria. All patients underwent baseline staging using laboratory, radiographic, and bone marrow examinations upon diagnosis. The Eastern Cooperative Oncology Group (ECOG) performance status was also assessed. The stage was evaluated in accordance with the Ann Arbor staging system. The International Prognostic Index (IPI) was calculated based on the serum lactate dehydrogenase, stage, extranodal status and performance status. Patient characteristics and treatment regimens of each therapy cycle were collected from each patient. Three healthy individuals were also recruited as control participants to test the accuracy of our sequencing platform for ctDNA profiling. All participants provided informed written consent before undergoing any study-related procedures in accordance with the Declaration of Helsinki. This study was approved by the China Ethics Committee of Registering Clinical Trials (ChiECRCT-20,180,005).

After the initial stage assessment, all patients were given 6 to 8 cycles of pegaspargase-based CHOP like regimens. Patients were reviewed routinely by a combination of clinical assessment and CT or fluorodeoxyglucose-PET (FDG-PET) before the administration of chemotherapy. FDG-PET was often used as an interim scan, and the metabolic tumor volume (MTV) was determined from the initial and interim PET images using PET Edge software
sequence alignment software. Samtools version 1.3 was used for single nucleotide variant (SNV)/indel calling and filter workflow.

Statistical analysis

Statistical analysis was conducted using SPSS software (Version 18.0, LEAD Corp). Descriptive statistics were used to analyze the clinical, demographic and genetic test result characteristics. Concentrations of ctDNA were expressed in haploid genome equivalents per mL (hGE/mL) and were calculated by multiplying the mean ctDNA mutant allele frequencies by the input concentration of ctDNA in pg/mL as determined by fluorometry and then dividing by 3.3 [20]. The correlation of gene mutation, ctDNA concentration and clinicopathologic features of patients were conducted using the Pearson χ² tests. The overall survival (OS) measured the proportion of patients who were alive at a specific time after diagnosis. Survival estimates were obtained using the Kaplan–Meier method, and comparisons were made using a log-rank test. Unpaired Student’s t-test for two groups was used in this study. A COX proportional regression model was used to calculate the survival hazard ratio (HR). Statistical differences were considered significant if the P value was less than 0.05.

Results

Patient characteristics

To examine the feasibility of ctDNA detection in plasma, from February 2017 to December 2019, 65 newly diagnosed ENKTL patients were recruited, and initial and longitudinal plasma specimens were obtained from patients undergoing chemotherapy (Fig. 1). The median age at first blood draw was 45.2 years (range, 40 to 55 years), and 45 patients were men. At disease onset, 26 patients (40%) exhibited B symptoms. According to the Ann Arbor staging criteria, 14 patients were identified as stage I, 20 patients were identified as stage II, 13 patients were identified as stage III, and 18 patients were identified as stage IV (Table 1). ctDNA was successfully extracted from all patients and healthy individuals. The average concentration of the ctDNA was 3.79 hEG/ml (range, 0 to 5.39 hEG/ml).

ctDNA mutation spectrum of newly diagnosed ENKTL

Of the 65 patients recruited in this study, to exclude hematopoietic clonal mutations, somatic mutations observed in both plasma and PBMC were excluded, mutation were detected in the plasma of 49 (75.3%) patients with a median of 1.7 per sample (range, 0 to 19) (Fig. 2, upper panels). The range of mutant allele frequencies in each gene of the samples was shown in Table S1. The most frequently mutated genes were KMT2D (23.1%),
APC (12.3%), ATM (10.8%), ASXL3 (9.2%), JAK3 (9.2%), SETD2 (9.2%), TPS3 (9.2%) and NOTCH1 (7.7%) (Fig. 2, middle panels). Consistent with the somatic SNV spectrum in other tumors, we found that C > T/G > A was a preferred alteration (Fig. 2, lower panels).

**Correlation of detectable ctDNA with the clinical characteristics of ENKTL patients**

To assess the concordance of the ctDNA test with the tissue NGS test, 6 paired tumor biopsies were genotyped. Compared with the results of the plasma ctDNA spectrum, most of the genes overlapped, the average biopsy-confirmed detection ratio was 93.75% (range 75–100%) (Fig. 3a). In 4 patients, biopsy-confirmed tumor mutations were all detectable in ctDNA samples (Fig. 3b), but for patient #10, TRAF3 variation was not detected in tumor DNA. For patient #8, STAT3 could not be detected in tumor ctDNA (Fig. 3c). These differences might be caused by tumor anatomical heterogeneity or tumor-associated stromal tissue infiltration.

To validate the potential clinical utility of the mutations detected in the plasma ctDNA, we investigated the correlation between clinical factors and ctDNA levels. As MTV measured by PET/CT could quantitatively reflect the tumor burden, we found that the plasma ctDNA concentration was significantly correlated with the MTV ($P = 0.04$) (Fig. 4a), suggesting that ctDNA could be identified as a promising biomarker of the tumor load. In patients with stage III-IV disease, the mutation frequencies of KMT2D (11/33), ATM (5/33), and JAK3 (3/33) were higher than that of patients with stage I-II disease. Additionally, mutation allele frequencies of KMT2D, APC and ASXL3 were not correlated with disease stage, but the mutation allele frequencies of ATM and JAK3 showed significant differences (Fig. 4b). Furthermore, the correlation of the mutation status of each gene with the metabolic tumor burden was investigated. We found that patients with mutated KMT2D, ASXL3, and JAK3 showed significantly higher MTV than patients with

**Table 1 Patients’ demographic and clinical characteristics**

| Prognostic variables | No. | Proportion(%) |
|----------------------|-----|---------------|
| **Gender**           |     |               |
| Male                 | 45  | 69.23         |
| Female               | 20  | 30.77         |
| **Clinical stage**   |     |               |
| I                    | 14  | 21.54         |
| II                   | 20  | 30.77         |
| III                  | 13  | 20.00         |
| IV                   | 18  | 27.69         |
| **B symptoms**       |     |               |
| With                 | 26  | 40.00         |
| Without              | 39  | 60.00         |
| **IPI Scores**       |     |               |
| 1                    | 22  | 33.85         |
| 2                    | 24  | 36.92         |
| 3                    | 14  | 21.54         |
| 4                    | 5   | 7.69          |
| **Final Recurrence status** | | |
| With                 | 9   | 13.85         |
| Without              | 56  | 86.15         |
| **Ki67 index**       |     |               |
| Low                  | 33  | 50.77         |
| High                 | 32  | 49.23         |
wild type (Fig. 4d), suggesting that these genes might be positively correlated with the disease malignancy. Recently, plasma EBV-DNA has served as a valuable biomarker of the tumor load and prognostic factor, thus, we also evaluated the correlation between the plasma EBV-DNA and clinical characteristics, and found that the pre-treatment plasma EBV-DNA was positively correlated with MTV (Fig. 4c), but the correlation between EBV-DNA levels and the ctDNA concentration were not significant different (Fig. 4e).

Serial ctDNA detection during therapy could complement the response assessment of the patients, and patients with mutated KMT2D and ATM predicted poor prognosis.

We further investigated the potential role of plasma ctDNA in the therapeutic monitoring of patients...
Fig. 3 Concordance of the ctDNA assessment with the tissue NGS test. 

a The consistent gene mutation spectrum and mutation allele frequency detected in the plasma ctDNA and tumor tissue of the patients. 
b Venn diagram summarizing the detailed mutations discovered in both plasma ctDNA (gray) and tumor DNA (blue). 
c For each patient, the fraction of tumor biopsy-confirmed mutations that were detected in plasma ctDNA is shown. The gray portion of the bar marks the part of the tumor biopsy-confirmed mutation that was not found in the plasma ctDNA.

Fig. 4 Correlation of the ctDNA assessment with the patient clinical characteristics. 

a Linear regression of the plasma ctDNA concentration with metabolic tumor volume. 
b Mutation allele frequencies of KMT2D, APC, ATM, ASXL3 and JAK3 in patients with different tumor stages. 
c The correlation between plasma EBV-DNA levels and metabolic tumor volume. 
d The general metabolic tumor volume in patients with different mutation statuses of KMT2D, APC, ATM, ASXL3, JAK3 and TP53. 
e The correlation of the EBV-DNA level with the ctDNA concentration.
undergoing chemotherapy. Ten patients were recruited, and sequential plasma samples upon disease diagnosis, before treatment cycle 4 (C4), and before cycle 8 (C8) were collected. In total, the mutated gene number and mutation allele frequencies decreased with the chemotherapy administration, 7 patients could achieve CR at C4, in these patients, only 2 patients didn’t detect ctDNA mutations at C4, suggesting that ctDNA assessment was more sensitive than PET/CT scans, all patients couldn’t detect plasma EBV-DNA when achieved CR. In addition, with more cycles of chemotherapy, all patients could achieve complete molecular remission (Fig. 5). In these 10 patients, we also observed one relapsed, and the relapsed mutation spectrum was similar to the original spectrum. All these results suggested that ctDNA detection could be regarded as a molecular response evaluation marker, and could complement the response assessment of the patients.

As KMT2D was the most frequently somatic mutations, the expression of KMT2D was investigated, and we found that the KMT2D expressions of patients with mutations were higher than that of patients with wild type (Fig. 6a). Then, prognosis value of the plasma ctDNA mutation status and EBV-DNA level was also investigated. Kaplan-Meier analysis estimated that patients with mutated KMT2D and ATM had a shorter OS (Fig. 6b, c), but no significant difference was observed in patients with other mutated genes (Fig. 6d-g), which might be due to the small sample recruited in our cohort. In addition, patients with high EBV-DNA levels showed significantly poorer prognosis (Fig. 6h). The results of univariate and multivariate analysis for risk factor of OS in patients were summarized in Table 2. Univariate analysis of the factors revealed that the stage, IPI scores, recurrence status, EBV-DNA levels, KMT2D mutation and ATM mutation were independent prognostic indicators of the overall survival of patients, and multivariate analysis showed that the EBV-DNA level, KMT2D mutation and ATM mutation were solely prognostic factors. All these results suggested that the dynamic detection of ctDNA during therapy could better reflect the disease status than PET/CT scan, and patients with mutated KMT2D, and ATM predicted poor prognosis.

**Discussion**

This study was the first to determine the plasma ctDNA gene mutation spectrum of ENKTL and demonstrated that ctDNA sequencing could be considered a promising biomarker for monitoring the minimal residual disease (MRD) of patients. The most frequently mutated genes in plasma ctDNA were KMT2D (23.1%), APC (12.3%), ATM (10.8%), ASXL3 (9.2%), JAK3 (9.2%), SETD2 (9.2%), TP53 (9.2%), and NOTCH1 (7.7%). The ctDNA concentration was positively correlated with tumor stage and
MTV. In terms of consistency with tumor tissue, almost all of the gene mutations detected in the plasma ctDNA could be found in tumor DNA, and further analysis demonstrated that a decreased number and MAF of certain genes in plasma ctDNA could complement the therapeutic response of the patients, and patients with mutated KMT2D and ATM had poor prognosis, suggesting that the gene mutations in plasma could serve as promising biomarkers for use in the diagnosis or monitoring of ENKTL disease courses.

Tumor biopsies can only offer information about lymphoma at one specific time in a specific area and can be accompanied with complications, and PET/CT may lead to false negatives due to technology limitations or false positive caused by tumor flares or pseudoprogression [27]. ctDNA detection is an optimal noninvasive and specific technique by which to overcome these traditional monitoring method limitations. A rapidly growing body of evidence has established that ctDNA detection during curative therapies can identify patients for whom there remains evidence of residual, radiographically occult cancer. In DLBCL, ctDNA can be used to predict the tumor load and treatment outcome of newly diagnosed patients, and patients with a pretreatment ctDNA level above the median had a significantly poorer prognosis than those with levels below the median [28]. Later, Kurtz et al. reported that serial ctDNA measurements of 125 patients were an effective tool for prognostication, patients with 2-log or greater decreases in ctDNA had a better event-free survival (EFS) after one cycle of therapy, and patients with 2.5-log or greater reduction in ctDNA had a significantly better EFS after two cycle treatments. These studies suggested that serial ctDNA detection could be used to assess the therapy

Fig. 6 Prognostic value of frequently mutated genes for patients with ENKTCL. a Immunohistopathology staining of KMT2D in patients with mutated KMT2D and wild type, scale bar, 25 μm. Kaplan-Meier estimated OS with the plasma KMT2D (b), ATM (c), APC (d), ASXL3 (e), JAK3 (f), SETD2 (g) mutation status in patients with ENKTL, and plasma EBV-DNA levels (h).
response dynamically and guide personalized target therapy. In another study, Daigle et al. found that mutation of CREBBP in R/R DLBCL had the greatest association with tazemetostat treatment (an EZH2 inhibitor), and patients with mutations in PIM1, BCL6, HIST1H1E and TP53 lacked response to treatment [29]. In HL, Valeria et al. determined the molecular mutation spectrum using ctDNA measurement. The most frequently mutated genes were STAT, TNFAIP3 and ITPKB, and detection sensitivity was 87.5%, suggesting that ctDNA could well reflect the spectrum of tumor tissue mutation. Compared with newly diagnosed and refractory HL mutation profiles, most mutations overlapped, and mutations of STAT, TNFAIP3, GNA13 and ITPKB might be an ancestral clone persisted throughout the disease course [23]. Although only a few studies have focused on assessing ctDNA in T-cell lymphoma, preliminary evidence has also demonstrated that ctDNA measurement in TCL is feasible [26, 30]. Our study is the first to determine the mutation spectrum of ctDNA in ENKTL. The most commonly mutated genes are KMT2D, APC, ATM, ASXL3, JAK3, SETD2, TP53 and NOTCH1. Our results revealed that ctDNA could reflect the MTV, and by longitudinally profiling patients treated with chemotherapy. In addition, we provided evidence that serial ctDNA assessment could monitor the disease status, with the results able to partially reflect which mutated gene was sensitive to chemotherapy drugs. However, only one of the 10 recruited patients relapsed. For relapsed patients, whether primary mutation clone recurrence or new mutation occupies the main role needs to be further investigated. In addition, future trials should include more patients and long-term follow-up to explore the plasma gene clone evolution process.

Plasma cfDNA includes tumor cell-derived plasma ctDNA and normal cell apoptosis-derived cfDNA, which are DNA fragments. ctDNA could be a more comprehensive reflection of gene mutations in all tumors than conventional biopsies [31, 32]. Therefore, ctDNA-based detection methods are critical for identifying which mutation are tumor-specific. As sensitivities increase with the development of new methods and because age-related somatic mutations could also lead to hematopoietic clonal expansion, the possibility of false-positive results might occur [33, 34]. Recently, Razavi et al. showed that clonal hematopoiesis was the major contributor to plasma variants using a high-intensity sequencing assay of matched plasma DNA, tumor tissue and white blood cells, emphasizing that matched cfDNA-white blood cells were critical for accurately screening somatic mutations [35]. In the present study, to decrease the bias caused by clonal hematopoiesis associated mutations, the age range of the included patients was narrow, matched ctDNA-PBMC sample was applied, and a sequencing panel was conducted according to a previous gene-expression profiling study on ENKTL. Zhao et al. confirmed that the most frequently mutated genes in NKTCL were the RNA helicase gene DDX3X, tumor suppressors (TP53 and MGA), JAK-STAT pathway molecules (STAT3 and STAT5b) and epigenetic modifiers (MLL2, ARID1A, EP300 and ASXL3) [36, 37]. In addition to these recurrent

| Table 2 Univariate and multivariate analyses of the overall survival of patients |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Prognostic variables            | Univariate analysis | Multivariate analysis |
|                                 | HR (95% CI)     | p value         | HR (95% CI)     | p value         |
| Gender (female vs. male)        | 0.712 (0.279–1.812) | 0.476          | 1.159 (0.253–5.311) | 0.849          |
| Stage (I-II vs. III-IV)         | 2.625 (1.043–6.604) | 0.04           | 2.066 (0.843–5.078) | 0.112           |
| B symphony (with vs. without)   | 1.234 (0.512–2.973) | 0.64           | 3.466 (1.364–8.204) | 0.008           |
| IPI                             | 2.595 (1.073–6.277) | 0.034          | 0.565 (0.118–2.712) | 0.476           |
| Final recurrence status         | 3.346 (1.364–8.204) | 0.008          | 2.705 (0.941–7.776) | 0.065           |
| MTV                             | 2.066 (0.843–5.078) | 0.112          |                 |                 |
| ctDNA concentration             | 2.975 (0.984–8.997) | 0.053          |                 |                 |
| EBV-DNA level                   | 3.672 (1.396–9.664) | 0.008          | 3.174 (1.091–9.231) | 0.034          |
| KMT2D mutation (without vs. with) | 0.289 (0.119–0.702) | 0.006          | 0.266 (0.086–0.828) | 0.022          |
| APC mutation (without vs. with) | 0.550 (0.16–1.895) | 0.344          |                 |                 |
| ATM mutation (without vs. with) | 0.271 (0.098–0.752) | 0.012          | 0.212 (0.064–0.700) | 0.011          |
| ASXL3 mutation (without vs. with) | 2613 (0.347–19.68) | 0.351          |                 |                 |
| JAK3 mutation (without vs. with) | 0.569 (0.166–1.949) | 0.369          |                 |                 |
| SETD2 mutation (without vs. with) | 0.804 (0.184–3.507) | 0.771          |                 |                 |
| TP53 mutation (without vs. with) | 0.615 (0.179–2.115) | 0.441          |                 |                 |
| NOTCH1 mutation (without vs. with) | 0.410 (0.120–1.406) | 0.156          |                 |                 |
mutations, JAK3, KMT2D, EZH2, NOTCH1 and TET2 were also identified by different groups [38–41]. Based on these findings combined with the most frequent gene mutation spectrum in Chinese individuals, we selected 41 mutated genes for our study. Interestingly, we found that KMT2D alterations possessed a higher frequency (23.1%) and which were all non-synonymous mutations (p.R2068L, p.D4378E, p.R755W, p.P2382S, p.V160L, p.L2316V). We also found some mutation existed in both plasma and PBMC, such as MGA (p.T716S, p.P1523A), ASXL3 (p.N954S), EZH2 (p.D185H), TET2 (p. P29R), P53 (p.P72R), APC (p.V1822D), KDM6A (p.T726K), et al. KMT2D belongs to a family of mammalian histone H3 lysine 4 (H3K4) methyltransferase. It is frequently mutated in Kabuki syndrome and various cancers, and it acts as a scaffold protein within the complex depending on the cell type and stage of differentiation [42]. In acute myeloid leukemia, follicular lymphoma and DLBCL, KMT2D could inhibit tumorigenesis and metastasis [43, 44], but in solid cancer, such as prostate cancer, KMT2D is critical factor for promoting tumor cell proliferation [45]. In our study, we found that the KMT2D expression of patients with the mutation was higher than that of patients with wild type, suggesting that KMT2D might be an oncogene in NKTL lymphoma. However, its underlying significance in NKTL has not been explored to date, and further studies regarding the function of KMT2D mutation in NKTL lymphomagenesis are urgently recommended.

Conclusions
To our knowledge, this is the first study to prospectively evaluate the potential utility of ctDNA analysis for NKTL patients. We have demonstrated that ctDNA assessment could predict the therapy response in NKTL, and the highest mutation frequencies were in KMT2D, APC, ATM, ASXL3, JAK3, SETD2, TP53, NOTCH1. Our results could define the optimal strategy for patient follow-up.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s40364-020-00205-4.

Additional file 1: Table S1. The mutant allele frequencies in each gene of each sample.

Additional file 2: Table S2. Correlation between KMT2D mutation status and clinicopathological features of ENKTL.

Additional file 3: Table S3. Correlation between ATM mutation status and clinicopathological features of ENKTL.

Abbreviations
ENTKL: Extranodal NK/T-cell lymphoma, nasal type; CT: Computed tomography; Non-NHL: Non-Hodgkin lymphoma; cfDNA: Circulating cell-free DNA; ctDNA: Circulating tumor DNA; DLBCL: Diffuse large B-cell lymphoma; MTV: Metabolic tumor volume; ADAM3A: ADAM Metallopeptidase Domain 3A; APC: Adenomatous Polyposis Coli Protein; ARID1A: AT-Rich Interaction Domain 1A; ARID1B: AT-Rich Interaction Domain 1B; ARID2: AT-Rich Interaction Domain 2; ASXL3: ASXL Transcriptional Regulator 3; ATM: Ataxia Telangiectasia Mutated; BCL6: BCL6 Corepressor; BCOR: BCL6 Corepressor Like 1; CHD8: Chromodomain Helicase DNA Binding Protein 8; CRB9BP: CRB Binding Protein; DDX3X: DEAD-Box Helicase 3 X-Linked; DNMT3A: DNA Methyltransferase 3 Alpha; EP300: E1A Binding Protein P300; EZH2: Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit; FYN: Src Family Tyrosine Kinase; IDH2: Isocitrate Dehydrogenase (NADP (+)) 2, Mitochondrial; IL2RCS: Interleukin 2 Receptor Subunit Gamma; JAK1: Janus Kinase 1; JAK3: Janus Kinase3; KDM6A: Lysine Demethylase 6A; KMT2A: Lysine Methyltransferase 2A; KMT2D: Lysine Methyltransferase 2D; MGA: MAX Dimersation Protein; NF1: Neurofibromin 1; NOTCH1: Notch Receptor 1; POM1: Positive Regulatory Domain 1-Binding Factor 1; PTPN1: Protein Tyrosine Phosphatase Non-Receptor Type 1; RHOC: Ras Homolog Family Member A; SETD2: SET Domain Containing 2; SOCS1: Suppressor of Cytokine Signaling 1; STAT3: Signal Transducer and Activator of Transcription 3; STAT5B: Signal Transducer and Activator of Transcription 5B; STAT6: Signal Transducer and Activator of Transcription 6; TET1, TET2, TET3: Tet Methylcytosine Oxidase; TETRAFR3A: TNF Receptor Superfamily Member 14; TET3: Tumor Protein P53; TETRAF3: TNF Receptor Associated Factor 3; ZAP608: Zeta Chain Of T Cell Receptor Associated Protein Kinase 608; MAF: Mutated allele frequency; SNV: Single nucleotide variant

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Authors’ contributions
Jun Rao, Yao Liu and Xi Zhang conceived and designed the study. Jun Rao, Wei Zhang and Qiong Li developed the methodology and analyzed and interpreted the data. Jun Rao and Qiong Li wrote the manuscript. All authors reviewed and revised the manuscript. The author(s) read and approved the final manuscript.

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Availability of data and materials
All data generated and analyzed during this study are included in this article and its supplementary information files.

Ethics approval and consent to participate
The study was approved by China Ethics Committees of Registering Clinical Trials (ChiECRCT-2018005).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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