**RESEARCH ARTICLE**

# Clinical and Pathologic features of North American/Caribbean Adult T-cell leukemia/lymphoma (ATLL), the Brooklyn, NY experience

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**Abstract**

Adult T-cell leukemia/lymphoma (ATLL) is a rare, highly aggressive, T-cell malignancy caused by human T-cell leukemia/lymphoma virus type 1(HTLV-1) infection.1 It is estimated that there are at least 5-10 million HTLV-1 carriers worldwide. Most of the HTLV-1 infection is thought to be acquired from mother-to-child transmission through breastfeeding. Sexual transmission and blood transfusion are other means of infection. Only a small number (2-5%) of HTLV-1 infected patients develop ATLL, usually after a long latency period (30-50 years after infection), suggesting additional genetic and epigenetic events are required for HTLV-1 infected cells to transform to ATLL. 2-3ATLL is endemic in certain regions where HTLV-1 infection is prevalent, including southwestern Japan, the Caribbean basin, areas of South America and tropical Africa.4 ATLL is rare in North America and the majority of the patients are immigrants from Caribbean basin, and rarely, of US-born African Americans. Studies have shown that HTLV-1 infection is prevalent in the black, predominantly Caribbean population of central Brooklyn, NY and that ATLL is endemic in this community. [5-7] Here we review the clinical and pathologic features of Caribbean ATLL and present our findings in a cohort of ATLL from central Brooklyn, NY. Our data suggest that Caribbean ATLL is not only clinically but also molecularly distinct from Japanese ATLL. Epigenetically targeted therapy may be a potential effective adjunct therapy in treatment of ATLL.

**Clinical and pathologic features**

ATLL exhibits diverse clinical features, and can involve almost all the organ systems, including skin, gastrointestinal tract, lung, liver, spleen, bone, and CNS. It is classified into four major clinical subtypes: acute, lymphomatous, chronic, and smoldering, based on the percentage of abnormal T-lymphocytes in peripheral blood, lactate dehydrogenase (LDH) level, calcium values, and organ involvement. [8] The acute and lymphomatous types are considered aggressive ATLLs, while the smoldering and chronic types are indolent ATLLs. Rarely ATLL presents as a localized mass lesion.[9] The clinical presentations of ATLL vary among geographic locations. Western/Caribbean ATLL patients are found to present at a much younger age (5-10 years younger) compared to the Japanese ATLL patients, and manifest as more aggressive (acute and lymphomatous) subtypes.[7,10,11]

42 Caribbean ATLL patients admitted to Kings County Hospital Center from 2004-2017 were studied:21 males and 21females.43% were lymphomatous subtype and 45% acute subtype. Male patients were slightly younger (median age 55, range from 35 to 76) than female patients (median age 59, range from 40 to 85). Female patients appeared to have more indolent subtypes than male patients (3 chronic & 1 smoldering vs 1 chronic). Younger patients (</= 50) presented predominantly with acute subtype (64%), while chronic and lymphomatous subtypes were more common among older patients. The overall survival was 9.5 months. Hypercalcemia was observed in 35% of our patients at initial presentation, and up to 77% patients during the clinical course. Lymphopenia has been shown to be an independent prognostic factor for poor prognosis in many hematologic malignancies, including Hodgkin's Lymphoma, diffuse large B-cell lymphoma, follicular lymphoma and peripheral T-cell lymphoma, [12] however its role in ATLL has not been investigated. We found that lymphopenia (ALC< $1\times 10^9$/L) is present in more than half (53%) of the lymphomatous subtype at disease onset and that was inversely associated with survival. 21% of the lymphomatous patients developed peripheral blood involvement at a later stage of the disease.

The diagnosis of ATLL requires a histologically proven malignant T-cell infiltrate with typical immunophenotype (most commonly CD3+, CD4+, CD8-, CD7-, CD25, CD30-/+) and positive HTLV-1 serology. In the absence of organ involvement, more than 5% abnormal T lymphocytes is required to be present in the peripheral blood for diagnosis of ATLL. [8] The morphology of the ATLL cells are quite variable, including small cell, pleomorphic, large cell, anaplastic, and angioimmunoblastic variants, and can mimic different types of lymphomas. In the peripheral blood, the ATLL cells are characterized by so-called ‘flower cells’, which show polylobated nuclei with hyperchromatic and condensed chromatin, small or absent nucleoli, and agranular and basophilic cytoplasm; however, ‘flower cells’ are present only in a subset of cases. [13] ATLL cells may also be found occasionally in other fluids such as sputum.[14] It is not uncommon to see ATLL cases misdiagnosed as other lymphomas, i.e., mycosis fungoides, anaplastic large cell lymphoma, angioimmunoblastic T cell lymphoma, or Hodgkin lymphoma, due to its rarity in non-endemic regions. If a mature CD4+ T-cell leukemia/lymphoma is diagnosed in patients from endemic regions, performing a CD25 immunohistochemistry and serology testing for HTLV-1 antibody can be very helpful in reaching an expeditious diagnosis. Forkhead box protein P3 (FOXP3) is a crucial regulator

 

of regulatory T (Treg) cell gene expression. It was observed in 62% of the ATLL cases and correlated with pleomorphic small and medium cell types. Fox3p may be lost upon large cell transformation associated with de novo CD30 expression. [15]

**Cytogenetics**

Chromosome analysis plays an important role in identifying the genes responsible for the development of hematologic malignancies and remains the fundamental tool to delineate and understand the relationship between clonal evolution and disease progression at single cell level. Cytogenetic analysis of Caribbean ATLL showed highly complex karyotype with frequent numerical and structural chromosomal abnormalities involving nearly every chromosome pair.[16] Complex karyotype with ≥3 aberrations is detected in all acute and lymphomatous subtypes of ATLL, of which 61% exhibited high complex karyotype with > 10 aberrations associated with significant shorter survival. Karyotype heterogeneity (presence of subclones) is observed in majority of ATLL (65%). [16] High frequency of copy number loss is seen in chromosome 14, followed by 13, 19, 5 and 17. Among deletions, 6q and 3q were most frequent. The most common recurrent chromosomal rearrangement breakpoints identified are 6q21, 1q21, 3p21, and 14q32. Chromosome band 6q21 is reported to be one of the most frequent target regions in T-cell lymphoma for both translocations and deletions. [17] PRDM1 is considered a candidate gene associated with 6q21 deletion in extra-nodal NK/T cell lymphoma, nasal type.[18] 3p21 band is the location for RhoA GTPase gene, which has been shown to play an important role in the pathogenesis of angioimmunoblastic T-cell lymphoma (AITL) and other subtypes of PTCL.[19] The *TCL1* oncogene on human chromosome 14q32 is involved in the development of T cell leukemia in humans. Its expression is activated by chromosomal translocations and inversions at 14q32.1. [20] Aberrations of 17p are seen in a significant number (36%) of cases. Although no distinct/recurrent cytogenetic abnormalities are identified, the findings show chromosomal instability and clonal diversity of ATLL, providing evidence of clinical aggressiveness and chemo-resistance seen in ATLL patients.[16] It also sheds lights on the genomic basis for future studies. These findings show both similarity and differences compared to Japanese ATLLs.

**Molecular genetic findings**

The molecular pathway driving from HTLV-1 infection to ATLL development is not well understood. Single, random viral integration site is identified in host genome of most cases of ATLL, associated with aberrant transcripts of HTLV-1, e.g., the tax expression was almost totally lost.[21] Tax is considered to be a main viral transcription activator protein involved in T cell proliferation and immortalization of the infected cells. [22] Large-scale whole-exome sequencing revealed frequent driver mutations in components of the T-cell receptor/NF-κB signaling and T cell trafficking pathways, including activating mutations in the PLCG1, PRKCB, IRF4, CARD11, VAV1, CCR4, CCR7, gene fusions involving co-stimulatory/inhibitory pathway (*CD28*, *CTLA4*, and *ICOS*).[21] Molecules associated with immune surveillance, such as HLA-A/B, CD58 and FAS, are affected recurrently. A substantial number of ATLL cases also show accumulation of repressive epigenetic changes, including DNA hypermethylation frequently affects the CpG islands of MHC class 1 genes.[21] Mutations in epigenetic and histone modifying genes, such as *TET2* and *EP300*, are also observed in

 

ATLL. These mutations were reported to be more prevalent in North American patients than in Japanese ATLL patients, suggesting a possible difference in mutational profile among different ethnic groups.[23]

Our preliminary sequencing data from Caribbean ATLL patients provides further support of such differences. We performed whole exome sequencing from paraffin sections dissecting tumors and their paired non-tumor control tissue. We identified average 109±51 somatic mutations per patient. For samples without control tissue we identified 1863±461 mutations per patient, demonstrating the importance of using paired control tissue to exclude false positive results when interpreting next generation sequencing data. Next, to validate our data, we performed a targeted sequencing including 32 candidate genes (Table 1). Using 5 paired tumor samples and 11 un-paired samples, we validated mutations in: CD5, FOXP1, MLLT1, PHF21A, PLCG1, SMARCB1 and PKHD1(unpublished data). Of the top 32 recurrently mutated genes identified from our study, only five (16.7%) of the genes overlap with those detected in Japanese ATLL. Similar to a previous report that north American ATLL has a high frequency of epigenetic mutations, [23] 27% of the frequently mutated genes in our study are epigenetic regulators (MLLT1, PHF21A, BRD1, ARID1A, ARID1B, ATXN3, SMARCC2, SMARCB1). 27% of the genes are involved in signaling pathways (e.g., PLCG1, NFAT, PRKCB, TYK2); 10% are tumor suppressors (e.g., P53, FBXW7), and 7% are involved in ubiquitination (SKP1, HUWE1).

Although further studies are needed to consolidate our sequencing data, the findings provide additional support for differences between Caribbean ATLL and Japanese ATLL at the molecular level, particularly at epigenetic regulation. Epigenetic modifications influence the transcription and function of a gene without affecting the DNA sequence itself, and play an important role in tumorigenesis. [24] Our findings, together with others, suggest that epigenome-targeted therapy maybe a promising strategy for treatment of this extremely chemo-refractory disease.

 

**Table 1.** Top 32 recurrently mutated genes identified by whole exome sequencing of our study

| **Candidate list TOP 32** | |
|---|---|
| **1** | CACNA1D |
| **2** | BRD1 |
| **3** | CDKN1B |
| **4** | GUSB |
| **5** | NFAT |
| **6** | PHF21A |
| **7** | PKHD1 |
| **8** | PLCG1 |
| **9** | PRKCB |
| **10** | SMARCB1 |
| **11** | ARID1A |
| **12** | ARID2 |
| **13** | ATXN3 |
| **14** | FOXP1 |
| **15** | MAP3K1 |
| **16** | PCDHB2 |
| **17** | RHOA |
| **18** | SMARCC2 |
| **19** | TARP |
| **20** | TCEB3B |
| **21** | TYK2 |
| **22** | ARID1B |
| **23** | FBXW7 |
| **24** | JARID2 |
| **25** | NOTCH1 |
| **26** | RANBP10 |
| **27** | SKP1 |
| **28** | TRIM5 |
| **29** | MLLT1 |
| **30** | CD5 |
| **31** | TP53 |
| **32** | HUWE1 |

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