inhibits telomerase activity and results in progressive telomere shortening and increased $p14^{ARF}$ expression. A functional relationship between *p53* and sensitivity to AZT has also been suggested (Datta et al., 2006). Ritonavir, developed as a protease against HIV-1, also has an anti-ATL effect. Ritonavir decreases NF-κB activity linked to the inhibition of IκBα phosphorylation and induces the apoptosis of ATL cells. In addition, it very efficiently prevents tumor growth and leukemic infiltration in various organs of NOG mice when administered at the same dose as that used in the treatment of patients with AIDS (Dewan et al., 2006). Together, several signaling networks are deregulated in leukemic cells, and these are a specific molecular feature of ATL (**Figure 2**). At present, signal interception is the most effective strategy to treat ATL. At the same time, the occurrence of incidental side effects should be carefully considered because these signaling pathways are essential for normal cell function and survival.

CYTOKINES PRODUCTION AND ATL

CD4+ T cells play a central role in the immune response by controlling cells such as B cells, dendritic cells, and cytotoxic cells and their responses through various cytokines. Deregulation of the associated signaling pathways leads to abnormal gene expressions, including that of several cytokines. ATL has been implicated in the production of various cytokines, including IL-1 (Wano et al., 1987), TGF-β (Niitsu et al., 1988), TNF-α, IFN-γ, GM-CSF (Yamada et al., 1996), and PTHrP (Watanabe et al., 1990). Especially, an elevated serum C-terminal PTHrP level is a characteristic marker of the HTLV-1 carrier status, and the determination of this level in ATL patients could be useful for assessing the prognosis (Yamaguchi et al., 1994). SCID mice model of ATL also showed clearly elevated serum levels of calcium and C-terminal PTHrP, resulting in the development of hypercalcemia (Takaori-Kondo et al., 1998). The high frequency of hypercalcemia is one of the notable clinical characteristics of ATL, in particular, the aggressive types of ATL (Kiyokawa et al., 1987). Besides PTHrP, which plays an important role in bone resorption by stimulating osteoclasts, abnormal expression of the RANK ligand (RANKL) has also been demonstrated in ATL with hypercalcemia (Nosaka et al., 2002). Recent studies have revealed that a central region of HTLV-1 gp46 acts as an antagonist for osteoprotegerin and leads to hypercalcemia (Sagara et al., 2007, 2009).

SCID mice engrafted with cells from Tax-transgenic mice that develop lymphoma produced TNF-α, PDGF-BB, sICAM-1, and sVCAM-1 as factors that may contribute to high levels of organ infiltration (Watters et al., 2010). PDGF, in particular, the BB isoform, is a well-known potent osteotropic factor that stimulates the osteoclasts and osteoblasts functions (Yi et al., 2002). High IL-2 production was not observed in previous ATL studies or in our microarray data (Yamagishi et al., 2012). Nevertheless, IL-2 is an HTLV-1-induced cytokine associated with the NF-κB pathway (Hoyos et al., 1989). In contrast, receptor subsets for IL-2 (IL2Rs) are generally overexpressed in ATL cells.

Interestingly, smoldering/chronic ATL PBMCs spontaneously proliferate *ex vivo* in an IL-12-, IL-9-, and IL-15-dependent manner, whereas acute type ATL PBMCs do not proliferate or proliferate independent of cytokines. Furthermore, purified leukemic cells from indolent ATL cases produce IL-2/IL-9 and the downstream JAK–STAT pathway is activated. Thus, autocrine/paracrine cytokine stimulation of leukemic cell proliferation may occur in patients with smoldering/chronic ATL (Chen et al., 2010).

CELL SURFACE MARKERS AND THEIR FUNCTIONS

IL-2 receptor α (CD25) was the first marker of ATL and HTLV-1-infected cells. CD25 expression is dependent on NF-κB activity (Ruben et al., 1988). Global gene expression analysis has also validated the high CD25 mRNA level in ATL patient samples (Yamagishi et al., 2012).

Chemokines and their receptors mainly function in the migration and tissue localization of lymphocytes. The expression of the following ATL-specific chemokine receptors has been identified: CCR4 (Yoshie et al., 2002), CCR7 (Hasegawa et al., 2000), CCR8 (Ruckes et al., 2001), and CXCR4 (Twizere et al., 2007). In addition, other cell surface proteins such as OX40 (Imura et al., 1997) and TSLC1 (Sasaki et al., 2005) are highly expressed in ATL cells and are thus the molecular hallmarks of ATL; they may also participate in leukemogenesis. For example, TSLC1, a well-known tumor suppressor in various carcinomas, is overexpressed in ATL. The cytoplasmic domain of TSLC1 directly interacts with the PDZ domain of TIAM1 and induces the formation of lamellipodia through Rac activation in HTLV-1-transformed and ATL cell lines. TIAM1 may integrate signals from TSLC1 to regulate the actin cytoskeleton through Rac activation (Masuda et al., 2010).

Some ligands are also expressed by ATL cells; therefore, autocrine/paracrine stimulation is implicated. Tax develops a strategy based on the activation of the SDF-1α/CXCR4 axis in infected cells (Twizere et al., 2007). In Tax-transgenic mice and their transplantation model, AMD3100, a CXCR4 antagonist, inhibits the infiltration of lymphomatous cells into tissues *in vivo*, indicating the involvement of the SDF-1α/CXCR4 interaction in leukemic cell migration (Kawaguchi et al., 2009). CCR4 expression is clinically considerable. The defucosylated anti-CCR4 monoclonal antibody KW-0761 induces CCR4-specific antibody-dependent cellular cytotoxicity (ADCC) against CCR4-positive ATL cells. In view of its molecular functions, CCR4 expression may also account for the frequent infiltration of ATL cells into the skin and lymph nodes (Yoshie et al., 2002). Specific surface markers are therefore worthy of attention to identify concentrations of leukemic cells as well as minor infected cells in asymptomatic carriers. A recent study reported the development of a new method for concentrating leukemic cells by multi-color flow cytometry. The majority of leukemic cells are included in the CD4+, CD3-dim, and CD7-low subpopulations (Tian et al., 2011). Consequently, characteristic expression of cytokines and their receptors is clearly required for leukemic cell behavior, which in turn may be used as landmarks and/or therapeutic motifs.

NEW PARADIGM FROM miRNA

According to the summary of previous ATL studies described above, we can fight ATL to a certain extent. However, we cannot cure ATL because of relapse with multidrug resistance, immunodeficiency, and strong invasiveness. In addition to the previously proposed molecular hallmarks (**Figure 2**), we urgently need a conceptual advance that can promote understanding of the source of

disrupted gene expression. Indeed, in the course of our remarkable progress in researching ATL and other malignancies, new observations have helped in clarifying and modifying the original formulations of the hallmark capabilities.

One of the most significant recent advances in biomedical research has been the discovery of the 22-nt-long class of noncoding RNA designated miRNA that posttranscriptionally regulates gene expression by binding to the target mRNAs. miRNA is expressed by all metazoans and plants, as well as by several DNA viruses; it regulates cellular processes such as development, differentiation, growth, homeostasis, stress responses, apoptosis, and immune activation (Esquela-Kerscher and Slack, 2006). In ATL filed, some studies have been reported, and several miRNA aberrations have been identified in HTLV-1-infected cells and ATL samples.

Pichler et al. (2008) first identified abnormal miRNA expression in HTLV-1-infected cells. They explored the interconnections between HTLV-1 and cellular miRNAs by using several HTLV-1-transformed cell lines. miR-21, miR-24, miR-146a, and miR-155 were found to be upregulated and miR-223 was found to be deregulated in HTLV-1-infected cells. In particular, miR-146a expression was directly stimulated by Tax through the NF-κB pathway. *In silico* analysis predicts that many candidate genes may be deregulated by miRNA changes (Pichler et al., 2008).

Yeung et al. (2008) performed miRNA microarray analysis of 327 well-characterized human miRNAs in 7 HTLV-1-related cell lines and four acute ATL patient samples. They found that miR-18a, miR-93, and miR-130b were overexpressed in ATL samples. Of note, these miRNAs were also upregulated by PHA-mediated T cell activation. Tumor protein p53 inducible nuclear protein 1 (TP53INP1) is a gene targeted by one of miR-93 and miR-130b, and reduced TP53INP1 expression mediated by miRNA upregulation contributes to cell proliferation and survival (Yeung et al., 2008).

Bellon et al. (2009) also reported the result of miRNA array analysis of 7 ATL samples and normal PBMC and CD4+ T cells and revealed that miR-150, miR-155, miR-223, miR-142-3p, and miR-142-5p are upregulated, whereas miR-181a, miR-132, miR-125a, and miR-146b are downregulated in ATL. They discussed that miRNAs involved in normal hematopoiesis and immune responses are profoundly deregulated in ATL tumor cells *ex vivo* (Bellon et al., 2009).

Each of these studies has identified interesting miRNAs that are deregulated in ATL-related cells; however, no identical miRNA patterns have been observed. The amount of cellular miRNAs may be susceptible to various environmental conditions such as transcriptional activity, maturation processing, and epigenetic regulation. The end results appear to be affected by the methodology employed and the conditions and types of samples used. Very recently, we established global gene expression analyses of a large cohort ATL study that included analyses of mRNA expressions, miRNA levels, and genomic copy number (Yamagishi et al., 2012). A strict threshold ($p < 1 \times 10^{-5}$) and two-dimensional hierarchical clustering analysis revealed 61 miRNAs with significantly altered expression levels in ATL cells ($n = 40$) compared with control CD4+ T cells ($n = 22$). It is most important that primary ATL samples show global miRNA downregulation, similar to observations in other cancer researches (Lu et al., 2005; Gaur et al., 2007). Fifty-nine of the 61 miRNA (96.7%) showed decreased expression in ATL. The amount of cellular miRNA may be susceptible to various environments such as transcriptional activity, maturation processing, and also epigenetic regulation. Among them, miR-31 is the most profoundly repressed miRNA in all ATL individuals (fold change, 0.00403). It is a known tumor suppressor that may also be associated with metastatic breast cancer (Valastyan et al., 2009). Other downregulated miRNAs found in ATL patients may also be involved in the hallmark capabilities of ATL, since they are uniformly decreased in tested ATL samples and each miRNA may regulate a large number of genes.

Several predictions and experimental approaches have defined a novel miR-31 target gene, MAP3K14 (also called NIK), which is a persistent NF-κB activator in various malignancies, including B cell lymphoma (Pham et al., 2011), multiple myeloma (Annunziata et al., 2007), breast cancer (Yamamoto et al., 2010), pancreatic cancer (Nishina et al., 2009), and ATL (Saitoh et al., 2008). Interestingly, all these malignancies have low miR-31 levels. Manipulation of the miR-31 level clearly indicated that the miR-31 level was negatively correlated with cellular NF-κB activity. Importantly, enforced miR-31 expression in B cells attenuated both BAFF and CD40L-mediated NIK accumulation and subsequent canonical and non-canonical NF-κB signaling. As discussed above, NF-κB activity dominates the regulation of apoptosis and subsequent cell survival. Induced miR-31 expression or NIK knockdown reduces apoptotic resistant proteins such as BCL-XL and XIAP, resulting in strong apoptosis in ATL cell lines as well as in primary leukemic cells from ATL patients (Yamagishi et al., 2012). Several lines of evidence definitively support two notions: (1) miR-31 acts as a tumor suppressor in T cells and (2) NIK-regulated NF-κB is of pivotal importance to cancer cell survival (Uribesalgo et al., 2012).

The fact that deregulated miRNA expression predominates NF-κB activity is a conceptual advance. Regulation of global miRNA downregulation and each regulatory network may shed light on our understanding of the next-generation molecular hallmarks of ATL and of molecules suitable for therapeutic targeting (**Figure 4**). Since a single miRNA can regulate the expression of multiple genes, pleiotropic miRNA may have potential as molecular therapy. Profound miR-31 loss is a characteristic of ATL; however, decreased miR-31 expression seems to be commonly observed in various malignancies. The regulatory mechanism of miR-31 had not been identified until our discovery. In general, down modulation of gene expression is coordinated by some contents of transcriptional factors and an epigenetic regulatory mechanism.

EPIGENETIC DEREGULATION OBSERVED IN ATL

Technological advances in genomics and epigenomics have supplied new methods to distinguish one cell type from another. The epigenetic code consists of the combined on–off states of hundreds of genes, which coordinately dictate cellular identity and function. Increasing attention is being paid to global regulatory factors and molecular mechanisms by which control gene transcription. This genome programming operates fundamentally through DNA methylation, histone chemical modification, and protein complex binding in these environments.

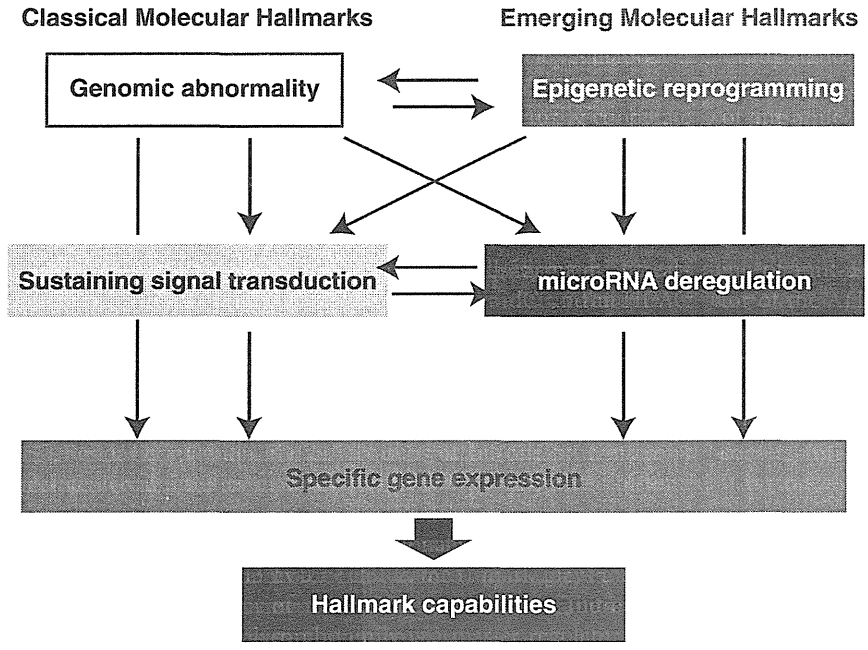

FIGURE 4 | Emerging molecular hallmarks, microRNA deregulation and epigenetic reprogramming. An increasing body of research suggests that two additional molecular hallmarks are involved in the pathogenesis of ATL. In addition to the genomic abnormality, epigenetic imbalance widely governs the downstream molecular capabilities. Deregulation of the cellular miRNA levels directly influence hundreds of genes expression. Importantly, cross talking among each category can attain more complex gene regulatory network that is indispensable for exercise of various functions at appropriate timing.

Cancer-associated epigenetic reprogramming has been suggested because DNA methylation is a transcriptional regulator. In ATL, no attempt has been made to determine global epigenetic statements that can explain deregulated gene expression. Analysis of epigenetic factors such as DNA methylation and related gene silencing has been reported, particularly in some tumor suppressor genes such as the CDK inhibitor family.

The CpG island of *CDKN2A* gene is more frequently methylated in fresh tumor cells isolated from patients with acute ATL (47%) or lymphoma type ATL (73%) than in fresh tumor cells isolated from patients with chronic (17%) and smoldering (17%) ATL, which are relatively less malignant (Nosaka et al., 2000). No *CDKN2A* gene is methylated in asymptomatic carriers or uninfected individuals. A possible inverse correlation between *CDKN2A* mRNA expression and gene methylation status is suggested. Methylation-specific polymerase chain reaction (MSP) also suggested the presence of an additional DNA methylation in *CDKN2B* gene (20%; Hofmann et al., 2001). In addition to the cell cycle regulators, multifunctional factors involved in cell proliferation, differentiation, and apoptosis, e.g., bone morphogenetic protein (BMP) is deregulated by aberrant DNA methylation in malignant lymphomas (Daibata et al., 2007) and also ATL (Taniguchi et al., 2008). Above all, the BMP6 promoter is hypermethylated in ATL: acute (96%), lymphoma (94%), chronic (44%), and smoldering (20%). BMP6 promoter methylation seems to be a common epigenetic event at later stages of ATL. The adenomatous polyposis coli (APC) gene is also a tumor suppressor, and its mRNA level is at least partially regulated by DNA methylation (Tsuchiya et al., 2000). In ATL, 48% of primary samples have methylated promoter DNA in the APC region (Yang et al., 2005).

The methylated CpG island amplification/representational difference analysis method revealed 53 aberrantly hypermethylated DNA sequences in ATL (Yasunaga et al., 2004). Among them, kruppel-like factor 4 (KLF4) and early growth response 3 (EGR3) were found to be responsible for apoptotic resistance in ATL cell lines, implicating that DNA methylation is involved in leukemogenesis. Abnormal DNA demethylation may also be involved. MEL1S, an alternatively spliced form of MEL1, is frequently expressed in ATL cells because of DNA hypomethylation at an alternative transcriptional start site. Aberrant MEL1S expression is associated with dysregulation of TGF-β-mediated signaling (Yoshida et al., 2004). Thus, altered DNA methylation pattern, including DNA demethylation, is one of the molecular hallmarks of ATL linking leukemogenesis to gene transcription control.

Histone modifications such as histone acetylation and specific methylations confer dynamic exchanges of transcription. Although a global survey of histone modification in ATL (such as by ChIP-on-chip analysis) has not been reported, experimental evidence with epigenetic drugs strongly suggests that epigenetic reprogramming is the background of the molecular hallmarks of ATL. Histone deacetylase (HDAC) inhibitors effectively inhibit the proliferation of several cancers (Spiegel et al., 2012) as well as that of HTLV-1-infected cell lines and primary ATL samples (Nishioka et al., 2008). Analysis of signaling cascades suggested that HDAC inhibition can block nuclear translocation of NF-κB components. Paradoxically, another study implicated that the HDAC inhibitors

can actively modulate the NF-κB pathway through RelA acetylation (Chiechio et al., 2009). Anyway, abnormal histone deacetylation may be involved in cell survival and cell cycle regulation in ATL cells.

Histone acetylation and DNA methylation actually cooperate in regulating a cohort of genes during multiple processes of leukemogenesis. For example, thioredoxin-binding protein-2 (TBP-2) expression is lost during the transformation step in HTLV-1-infected T cells (Nishinaka et al., 2004). TBP-2 seems to play a crucial role in the growth regulation of T cells. Sequential treatment with a DNA methylation inhibitor, 5-Aza-dC, and an HDAC inhibitor can restore the TBP-2 expression, suggesting that loss of TBP-2 expression is caused by both DNA methylation and histone deacetylation in transformed infected cell lines (Ahsan et al., 2006).

Besides acetylation, the N-terminus of histone proteins contains several residues that can be methylated. Integrated histone modification consequently decides the degrees of chromatin condensation and subsequent transcriptional sensitivity. Trimethylation of the histone H3 Lys27 (H3K27me3) mark plays a central role in the repression of transcription, mainly in the euchromatin region. The Polycomb family is a master regulator of the H3K27me3 level by inducing and maintaining the histone mark. Progress over the past decade has defined two main protein complexes: Polycomb repressive complex 1 (PRC1) and PRC2, with fundamental roles in Polycomb-mediated gene silencing (Schuettengruber et al., 2007). PRC2 methylates the histone histone 3 lysine 27 (H3K27). PRC1 is commonly viewed as an important, direct executor of silencing at target genes. Although H3K27 methylation is a key chromatin mark, there is ongoing debate about its molecular consequences. In the context of cancer research, deregulation by the Polycomb family confers a specific gene expression pattern responsible for chronic proliferation, survival, peculiar development, and cancer-associated stemness in various cancer types, including ATL (Sparmann and van Lohuizen, 2006).

The involvement of the Polycomb family in ATL was first revealed by global gene expression analysis. Significantly higher levels of enhancer of zeste homolog 2 (EZH2) as well as RING1 and YY1 binding protein (RYBP) transcripts with enhanced H3K27me3 levels were found in ATL cells compared with normal CD4+ T cells (Sasaki et al., 2011). EZH2 serves as the catalytic subunit in the PRC2 and mediates gene silencing by catalyzing the trimethylation of H3K27 at the promoters of target genes. EZH2 is highly expressed in many cancer types, including breast and prostate cancer and lymphomas, and it is often correlated with advanced stages of tumor progression and a poor prognosis. Importantly, EZH2 inhibition by 3-deazaneplanocin A and the HDAC inhibitor panobinostat showed a synergistic effect in killing the ATL cell lines. Because the Polycomb family generally contributes to silencing of tumor suppressor genes, e.g., the *CDKN2* family, the genes silenced in ATL should be addressed to elucidate the functional significance of the Polycomb family in the leukemogenic process.

We recently identified a notable gene silenced by Polycomb. A human gene that encodes miR-31, *hsa-miR-31*, is located at 9p21.3, which is adjacent to clusters of the *CDKN2* and *IFNA* families. In addition to the genetic loss (12.5% of ATL cases), transcription of the miR-31 precursor is completely lost in ATL cells. Computational predictions and experimental evidence clearly demonstrated that an assembly of YY1 binding motifs upstream of the miR-31 region is responsible for the occupancy of the Polycomb family at the target region, which leads to H3K27me3-dependent transcriptional repression. Overexpression of EZH2 and suppressor of zeste 12 (SUZ12) homolog, components of PRC2, in ATL cells can induce and maintain the epigenetic silencing of miR-31. Of note, given that miR-31 is a master regulator of the ATL-specific gene expression pattern described above, Polycomb-mediated loss can influence gene expression downstream of miR-31 (**Figure 4**). Indeed, the amount of EZH2 and SUZ12 directly strengthens cellular miR-31 depletion, which in turn activates the NF-κB pathway through NIK induction and confers anti-apoptotic features to T cell (Yamagishi et al., 2012). It is noteworthy that the molecular and biological interconnections between Polycomb–miR-31–NF-κB are conserved in breast cancer cells and B lymphocytes. By organizing the new principle, various cell types may realize the more complex gene regulatory network required for maintenance and execution of cellular functions. Imbalance of this network probably switches the cell fate from one to another.

The origin of epigenetic reprogramming observed in ATL cells remains elusive. In addition to self-dysfunction of the epigenetic machinery, a possible mechanism is viral hijacking; HTLV-1 Tax can physically associate with the key histone modifiers HDAC1 (Ego et al., 2002), SUV39H1 (Kamoi et al., 2006), and SMYD3 (Yamamoto et al., 2011). However, at present, the possible influence of the Tax-epigenetic association on gene regulation is unknown. Governing the epigenetic system by Tax may disrupt gene expression, leading to chronic proliferation and abnormal survival of HTLV-1-infected cells. In the context of viral gene regulation, epigenetic changes, mainly DNA methylation, in the HTLV-1 provirus may facilitate ATL cell evasion of the host immune system by suppressing viral gene transcription (Koiwa et al., 2002; Taniguchi et al., 2005). Recent studies using *in vivo* models strongly suggested that Tax and also other viral proteins are directly linked to leukemogenesis, despite viral gene expression being rare in circulating leukemic cells in patients (Hasegawa et al., 2006; Ohsugi et al., 2007b; Banerjee et al., 2010; Satou et al., 2011). Furthermore, not only histone methylation but also other histone modifications such as phosphorylation and ubiquitination are intriguing for understanding the molecular and physiological hallmarks of ATL.

THERAPEUTIC TARGETING OF ATL

To establish more effective molecular-targeted therapies for ATL, we need to understand the exact molecular underpinnings of ATL. In addition to classical molecular characteristics, the emerging hallmarks of miRNA deregulation and epigenetic reprogramming broaden the scope of conceptualization of the responsible molecular mechanism (**Figure 4**). As highlighted in this review, ATL possesses six molecular hallmarks: genomic abnormality, specific changes in gene expression, sustaining activated signaling, producing cytokines, miRNA deregulation, and epigenetic reprogramming. These molecular hallmarks confer robustness to leukemic

cell hallmark capabilities: resisting cell death, promoting cell cycle, invasiveness, chronic proliferation, replicating immortality, and drug resistance (**Figure 5**). Consideration of hallmark principles should aid in developing future therapeutics. Several studies with inhibitory agents have clearly indicated that blockade of signaling drivers appears to be both practical and feasible for inducing leukemic cell apoptosis. However, common clinical traits of ATL include relapse and drug resistance. Importantly, each of the core hallmark capabilities is regulated by a partially redundant signaling pathways. Consequently, a targeted therapeutic agent that inhibits only one key pathway in ATL may not completely shut off another hallmark capability, allowing some ATL cells to survive with residual function until they or their progeny eventually adapt to the selective pressure imposed by the therapy. In this case, given that the number of parallel signaling pathways supporting a given hallmark is limited, it may become possible to target all of these supporting pathways therapeutically, thereby preventing the development of adaptive resistance. However, it is possible that this could involve critical side effects. Alternatively, most upstream elements that can act pleiotropically in leukemic cells, e.g., miRNA and epigenetics, may be heralded as one of the fruits of remarkable progress into understanding the ATL mechanism. Moreover, selective co-targeting of multiple core and emerging molecular hallmarks in mechanism-guided combinations therapies will result in more effective and durable therapies for aggressive ATL.

CONCLUSION AND FUTURE DIRECTION

We have explored our present understanding of the molecular aspects of ATL to refine and extend the six specific traits, the molecular hallmarks of ATL, which have provided a useful conceptual framework for understanding the complex biology of ATL (**Figure 5**).

Other areas are currently in rapid flux. In recent years, the biological importance of several elaborate ATL models, including the Tax-transgenic model (Hasegawa et al., 2006), HBZ transgenic model (Satou et al., 2011), and HTLV-1-infected humanized SCID mice (Banerjee et al., 2010), has been proposed. ATL-initiating

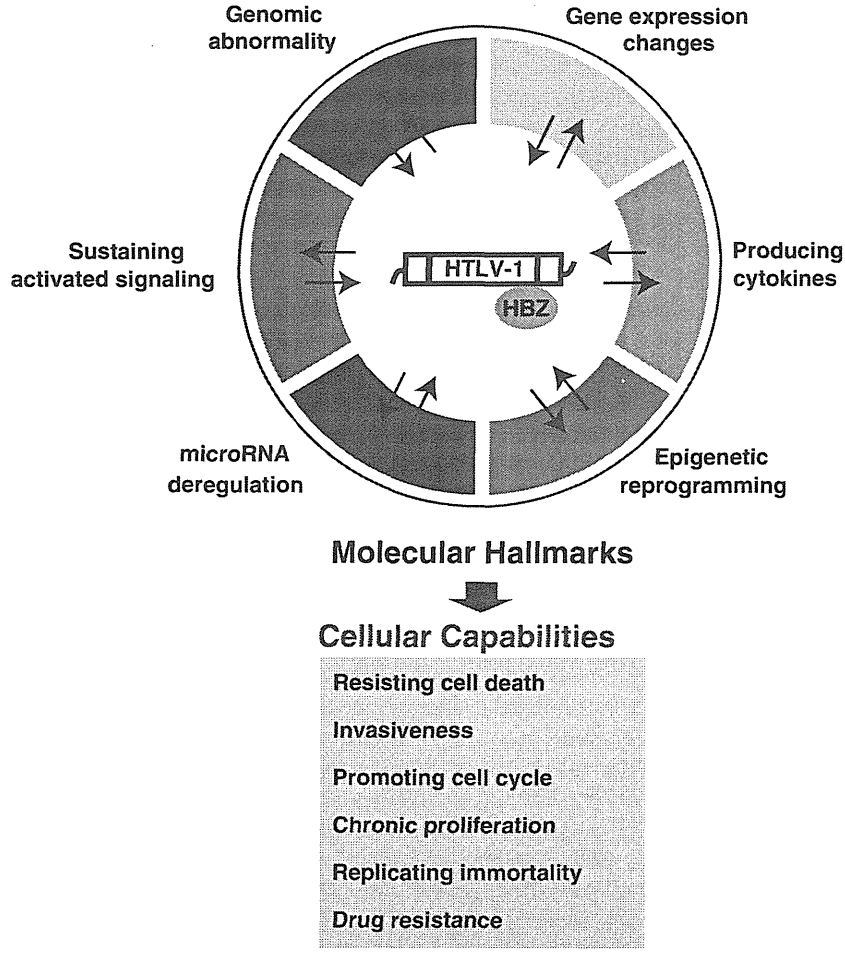

FIGURE 5 | Conceptual illustration of the molecular hallmarks of ATL. This illustration encompasses the six molecular hallmarks of ATL. These organized principles provide characteristics of ATL itself. Because they may be directly associated with the clinical traits of ATL, targeting the one outstanding hallmark or co-targeting of multiple molecular hallmarks in mechanism-guided combinations will result in more effective and durable therapies for aggressive ATL.

stem cell theory has also been developed (Yamazaki et al., 2009; El Hajj et al., 2010). Similar to other lymphomas and solid cancers, leukemic cells in tissues may be encompassed by a tumor microenvironment that contributes to leukemogenesis. The organized principles of the molecular basis of ATL may be helpful in the coming decade of ATL study.

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Clinical pathophysiology of human T-lymphotropic virus-type 1-associated myelopathy/tropical spastic paraparesis

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Human T-lymphotropic virus type 1 (HTLV-1), a human retrovirus, is the causative agent of a progressive neurological disease termed HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HAM/TSP is a chronic inflammatory disease of the central nervous system and is characterized by unremitting myelopathic symptoms such as spastic paraparesis, lower limb sensory disturbance, and bladder/bowel dysfunction. Approximately 0.25–3.8% of HTLV-1-infected individuals develop HAM/TSP, which is more common in women than in men. Since the discovery of HAM/TSP, significant advances have been made with respect to elucidating the virological, molecular, and immunopathological mechanisms underlying this disease. These findings suggest that spinal cord invasion by HTLV-1-infected T cells triggers a strong virus-specific immune response and increases proinflammatory cytokine and chemokine production, leading to chronic lymphocytic inflammation and tissue damage in spinal cord lesions. However, little progress has been made in the development of an optimal treatment for HAM/TSP, more specifically in the identification of biomarkers for predicting disease progression and of molecular targets for novel therapeutic strategies targeting the underlying pathological mechanisms. This review summarizes current clinical and pathophysiological knowledge on HAM/TSP and discusses future focus areas for research on this disease.

Keywords: epidemiology, diagnosis, HAM/TSP, HTLV-1, pathogenesis, prognosis, retrovirus, treatment

EPIDEMIOLOGY

Human T-lymphotropic virus type 1 (HTLV-1), the first human retrovirus discovered (Poiesz et al., 1980), infects approximately 10–20 million people worldwide (de Thé and Bomford, 1993). Endemic areas of HTLV-1 infection include the Caribbean, southern Japan, Central and South America, the Middle East, Melanesia, and equatorial Africa (Blattner and Gallo, 1985; Gessain and de Thé, 1996). Although majority of the infected individuals remain lifelong asymptomatic carriers, approximately 0.25–3.8% develop a progressive neurological disease termed HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP; de Thé et al., 1985; Osame et al., 1986a) and 2–5% develop an aggressive mature T cell malignancy termed adult T cell leukemia/lymphoma (ATLL; Uchiyama et al., 1977; Hinuma et al., 1981). HAM/TSP is two to three times more common in women than men. In a prospective cohort analysis, the onset period after infection ranged from 4 months to 30 years (median, 3.3 years; Maloney et al., 1998). HTLV-1 is primarily transmitted by breast feeding, but also spread via sexual intercourse, blood transfusion, and sharing of needles. While ATLL is mainly associated with breast feeding, HAM/TSP can be occurred in infected individuals of any route of transmission (Sugiyama et al., 1986; Tajima et al., 1987; Osame et al., 1990a; Krämer et al., 1995; Maloney et al., 1998). In Japan, nationwide routine screening of the anti-HTLV-1 antibody for blood donations is conducted after the high incidence of HAM/TSP in recipients of blood transfusion reported in 1986 (Osame et al., 1986b) and such screening has proven to be an effective way of curbing transfusion-related infection (Kamihira et al., 1987). In Japan, the lifetime risk of developing HAM/TSP among approximately one million HTLV-1-infected individuals is 0.25% (Kaplan et al., 1990). The lifetime risk of HAM/TSP in the estimated 22,000 HTLV-1-infected individuals in England is 3% (Tosswill et al., 2000). Seroprevalence of HTLV-I in blood donors in the United States is 1 per 10,000 individuals. A recent study estimates that approximately 266,000 individuals are infected with HTLV-1 or 2, and that there are likely more than 3600 people in the United States with unrecognized HAM/TSP (Orland et al., 2003).

CLINICAL FEATURES

HTLV-1-associated myelopathy/tropical spastic paraparesis mainly presents as a slowly progressive spastic paraparesis with neurogenic bladder disturbance (Nakagawa et al., 1995; Araújo et al., 1998). The first major symptoms are typically gait disturbance, tendency to fall, stumbling, leg weakness, back pain, bladder/bowel, and sexual dysfunction, which are usually insidious but occasionally occur abruptly over weeks. Symptoms in the lower limbs are mostly symmetrical. Neurogenic bladder symptoms such as urinary frequency, urgency, incontinence, and/or retention are very common and seen very early in the course of the disease; sometimes, these symptoms precede the development of paraparesis by many years. The patients have a spastic gait with weakness of the lower limbs, which is most evident proximally. Hyperreflexia of the lower limbs

is commonly seen, often accompanied by clonus and Babinski's sign, and hyperreflexia of upper limbs is occasionally observed in some patients. Upper limb power is usually retained throughout the course of the disease. Sensory disturbance – typically paresthesia of the feet and occasionally of the hands – is observed in some HAM/TSP patients and is generally mild. Sensory level is occasionally observed at the lower thoracic spinal cord, although a clear-cut sensory level is unusual. Loss of light touch sensation and pain in the lower limbs were reported in 27–53% of patients in three clinical series, with impairment of vibration sense recorded in 3–48% of the patients (Vernant et al., 1987; Bhigjee et al., 1990; Araújo et al., 1993). Pain and numbness, usually at the lumbar level and lower limbs, is present in approximately 5–50% of the patients (Gotuzzo et al., 2004). In some cases, pain is severe and more distressing than gait disturbance. Back pain, constipation, and sexual dysfunction are also very common (Verdonck et al., 2007). The less common signs and symptoms include cerebellar signs, optic neuritis and atrophy, and nystagmus (**Table 1**).

Table 1 | Clinical features of HAM/TSP.

Motor Disturbance
Symptoms: gait disturbance, tendency to fall, stumbling, and leg weakness
Signs: spastic paraparesis, weakness and hyperreflexia of the lower limbs, clonus, and Babinski's sign
Sensory Disturbance
Symptoms: pain and numbness at the lumbar level and lower limbs and back pain
Signs: paresthesia of the feet and occasionally of the hands, sensory level at the lower thoracic spinal cord, loss of light touch sensation
Autonomic Dysfunction
Symptoms: urinary frequency, urgency, incontinence, retention, constipation, and sexual dysfunction
Signs: neurogenic bladder, overactive bladder, diminished peristalsis, and erectile dysfunction

Human T-lymphotropic virus type 1 is also associated with non-neoplastic inflammatory conditions such as HTLV-1-associated uveitis (Mochizuki, 1992), Sjögren syndrome (Eguchi et al., 1992), bronchoalveolitis (Nakagawa et al., 1995), arthritis (Nishioka et al., 1989), and polymyositis (Morgan et al., 1989), in which high tissue concentrations of HTLV-1-infected T lymphocytes have been observed. Importantly, some HAM/TSP patients have more than one of these HTLV-1-associated inflammatory conditions (Nakagawa et al., 1995).

DIAGNOSIS

The diagnosis of HAM/TSP is based upon a combination of characteristic clinical features and confirmation of HTLV-1 infection, along with exclusion of other disorders presenting spastic paraparesis (**Figure 1**). For confirmation of HTLV-1 infection, serological screening for HTLV-1 antibodies can be performed by using a commercially available enzyme immunoassay or particle agglutination test. Confirmatory testing for screening-positive individuals is necessary to eliminate false positives and discriminate between HTLV-1 and HTLV-2. Serological confirmation can be performed by using a commercially available western blot test. Polymerase chain reaction analysis on a blood sample may also be required if the western blot test provides some indeterminate results.

Diagnostic criteria for HAM/TSP were agreed upon by a World Health Organization (WHO) (1989; **Table 2**). However, a recent recommendation proposes a redefinition of the WHO diagnostic guidelines by formulating levels of ascertainment (definite, probable, and possible), where a patient with definite HAM/TSP manifests non-remitting progressive spastic paraparesis and positive serology and/or detection of proviral DNA, with other disorders being excluded (De Castro-Costa et al., 2006).

Detection of anti-HTLV-1 antibodies in cerebrospinal fluid (CSF) is necessary for the diagnosis of HAM/TSP, based on the WHO diagnostic guidelines. CSF examination revealed mild lymphocyte pleocytosis in approximately one-third of cases as well as mildly elevated protein concentration and increased

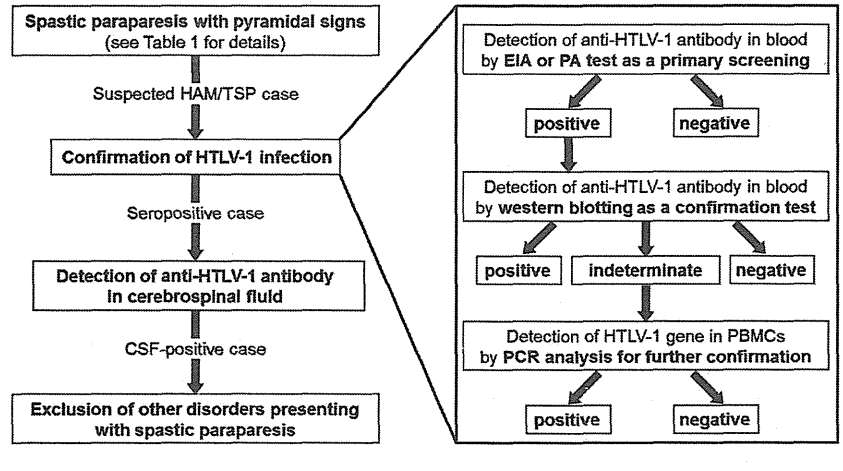

FIGURE 1 | Flow chart for clinical diagnosis of HAM/TSP. EIA, enzyme immunoassay; PA, particle agglutination; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction.

Table 2 | World Health Organization diagnostic criteria for HAM/TSP.

Age and sex incidence	Mostly sporadic and adult, but sometimes familial; occasionally seen in childhood; females predominant
Onset	Usually insidious but may be sudden
Main neurological manifestations	Chronic spastic paraparesis, which usually progresses slowly, sometimes remaining static after initial progression Weakness of the lower limbs, more marked proximally Bladder disturbance usually an early feature; constipation usually occurs later; impotence or decreased libido is common Sensory symptoms such as tingling, pins and needles, and burning are more prominent than objective physical signs Low lumbar pain with radiation to the legs is common Vibration sense is frequently impaired; proprioception is less often affected Hyperreflexia of the lower limbs, often with clonus and Babinski's sign Hyperreflexia of the upper limbs, positive Hoffman's and Tromner signs frequent; weakness may be absent Exaggerated jaw jerk in some patients
Less frequent neurological findings	Cerebellar signs, optic atrophy, deafness, nystagmus, other cranial nerve deficits, hand tremor, absent, or decreased ankle jerk. Convulsions, cognitive impairment, dementia, or impaired consciousness are rare Muscular atrophy, fasciculations (rare), polymyositis, peripheral neuropathy, polyradiculopathy, cranial neuropathy, meningitis, encephalopathy
Systemic non-neurological manifestations	Pulmonary alveolitis, uveitis, Sjogren's syndrome, arthropathy, vasculitis, ichthyosis, cryoglobulinemia, monoclonal gammopathy, adult T cell leukemia/lymphoma
Laboratory diagnosis	Presence of HTLV-1 antibodies or antigens in blood and CSF CFS may show mild lymphocyte pleiocytosis Lobulated lymphocytes may be present in blood and/or CSF Mild to moderate increase of protein may present in CSF

CSF, cerebrospinal fluid.

concentrations of inflammatory markers such as neopterin (Nakagawa et al., 1995; Milagres et al., 2002). These abnormalities can persist for as long as 10 years or more after symptom onset (Moreno-Carvalho et al., 1995).

Spinal cord magnetic resonance imaging (MRI) was abnormal in 3/21 (14%) patients with HAM/TSP in a small series where spinal cord atrophy was reported mainly in the thoracic region (Bagnato et al., 2005). High signal intensity and contrast enhancement with or without associated spinal cord swelling located at cervical or thoracic levels are occasionally observed (Umehara et al., 2007). It has been suggested that patients with more rapidly progressive disease who are scanned earlier in the disease course are more likely to show high signal intensity and contrast enhancement in the spinal cord on MRI, possibly because this reflects highly active spinal cord inflammation.

The differential diagnosis for HAM/TSP includes multiple sclerosis (MS), neuromyelitis optica (NMO), spinal cord compression (e.g., cervical spondylosis and spinal tumors), transverse myelitis, collagen vascular disease, Sjögren syndrome, hereditary spastic paraparesis, primary lateral sclerosis, subacute combined degeneration secondary to vitamin B12 and folate deficiency, human immunodeficiency virus-associated vacuolar myelopathy, neurosyphilis, and Lyme disease, among others. Differentiating, rapidly progressing HAM/TSP from NMO is important. NMO shows more rapid progression than HAM/TSP, and HAM/TSP usually does not present with optic neuritis. Importantly, from our clinical experience, HAM/TSP patients are negative for a specific diagnostic antibody for NMO termed NMO-IgG or anti-aquaporin-4 antibodies (data not published). Furthermore, differentiating HAM/TSP from primary progressive MS is occasionally a diagnostic challenge, since the two conditions are clinically indistinguishable and the mere presence of positive HTLV-1 serology does not necessarily lead to neurological disease. This diagnostic difficulty is compounded by the fact that sometimes, white matter abnormalities are found on brain MRI of HAM/TSP patients (Kira et al., 1991; Alcindor et al., 1992; Kuroda et al., 1995). CSF pleocytosis, when present, typically falls within a similar range, and oligoclonal bands may be present in both. A recent study suggests that a high ratio of proviral DNA load in CSF to peripheral blood mononuclear cells (PBMCs) may distinguish HAM/TSP from HTLV-1-infected patients with MS (Puccioni-Sohler et al., 2007). In general, HTLV-1 proviral loads measured in the CSF of HAM/TSP patients are typically greater than twice the proviral load in PBMCs (Nagai et al., 2001; Takenouchi et al., 2003), whereas the ratio of CSF to peripheral blood HTLV-1 proviral loads are typically lower in asymptomatic carriers (Lezin et al., 2005; Puccioni-Sohler et al., 2007), reflecting either recruitment or expansion of HTLV-1-infected cells in the central nervous system (CNS).

PATHOPHYSIOLOGY

The primary neuropathological feature of HAM/TSP is chronic meningomyelitis of the white and gray matter, followed by axonal degeneration preferentially affecting the middle to lower thoracic cord. Histopathological studies have shown loss of myelin and axons in the lateral columns, with variable damage to anterior and posterior columns in patients with HAM/TSP. The lesions are associated with perivascular and mild parenchymal lymphocytic infiltration with the presence of foamy macrophages, proliferation of astrocytes, and fibrillary gliosis. Later in the course of the

disease, the process becomes less cellular and more atrophic. Interestingly, patients who underwent prior steroid treatment show a lesser degree of inflammation (Iwasaki, 1990; Yoshioka et al., 1993; Izumo et al., 2000). Proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-1β were detected in perivascular infiltrating cells (Umehara et al., 1994). There is no direct evidence that HTLV-1 infects neurons, astrocytes, or microglia, but infected $CD4^+$ T cells have been observed within spinal cord lesions (Matsuoka et al., 1998), and $CD8^+$ T cells directed against HTLV-1 antigens accumulate in the CSF of patients with HAM/TSP (Nagai et al., 2001; Kubota et al., 2002). Immunohistochemical analysis of affected spinal cord lesions in early-stage HAM/TSP patients revealed the presence of infiltrating $CD4^+$ and $CD8^+$ lymphocytes, among which $CD8^+$ cells become increasingly dominant over the duration of the illness (Umehara et al., 1993). The expression of HLA class I antigens has also been found in such lesions (Moore et al., 1989). In addition, infiltrating $CD8^+$ CTLs were positive for TIA-1, a CTL marker (Umehara et al., 1994). The number of TIA-1^+ cells was clearly related to the amount of proviral DNA *in situ*, and the number of infiltrating $CD8^+$ cells appeared to correlate with the presence of apoptotic cells.

Human T-lymphotropic virus type 1-1-infected CD4+ T cells may primarily contribute to development of HAM/TSP, since the number of HTLV-1-infected T cells circulating in the peripheral blood is higher in patients with HAM/TSP than in asymptomatic HTLV-1-infected individuals (Nagai et al., 1998; Yamano et al., 2002); this number is even higher in the CSF of patients with HAM/TSP (Nagai et al., 2001). Recently, $CD4^+CD25^+CCR4^+$ T cells, which mainly include suppressive T cell subsets such as regulatory T (T_{reg}) cells under healthy conditions, are the predominant viral reservoir of HTLV-1 in both ATLL and HAM/TSP (Yoshie et al., 2002; Yamano et al., 2009). Interestingly, cells of this T cell subset become Th1-like cells with overproduction of IFN-γ in HAM/TSP, while in ATLL patients, leukemogenesis develops, and maintains the T_{reg} phenotype. These results indicate that HTLV-1-infected T cells are increased and abnormally modified, favoring the development of HAM/TSP.

Human T-lymphotropic virus type 1-associated myelopathy/tropical spastic paraparesis patients show extremely high cellular and humoral acquired immune responses, such as high frequencies of Tax-specific $CD8^+$ T cells in peripheral blood and CSF (Jacobson et al., 1990; Nagai et al., 2001); high antibody titer to HTLV-1 (Ishihara et al., 1994; Akahata et al., 2012); and increased production of proinflammatory cytokines such as IL-6, IL-12, and IFN-γ (Furuya et al., 1999). Recently, overexpression of a subset of IFN-stimulated genes in HAM/TSP patients was demonstrated using systems biology approaches (Tattermusch et al., 2012).

While the acquired immune response is accelerated, HAM/TSP patients demonstrate reductions in the amount and efficacy of cellular components of innate immunity; this is vital for regulating the immune response against general viral infections and cancers. The numbers and functions of $CD56^+CD16^+$ natural killer (NK) cells in HAM/TSP patients are significantly lower than those observed in healthy controls (Yu et al., 1991; Azakami et al., 2009). In addition, HAM/TSP patients also have a decreased frequency of invariant natural killer T (iNKT) cells in peripheral blood (Azakami et al., 2009; Ndhlovu et al., 2009).

Although the exact cellular and molecular events underlying the induction of chronic inflammation in the spinal cord by HTLV-1 are still unclear, the most widely accepted hypothesis is that HAM/TSP is the result of "bystander damage" (Ijichi et al., 1993; Nagai et al., 2001; Osame, 2002). The sequence of events leading to bystander damage may be as follows. Activation of HTLV-1-infected $CD4^+$ T cells induce high-migration activity (Furuya et al., 1997; Kambara et al., 2002) and allows the migration of infected $CD4^+$ T cells across the blood–brain barrier from the peripheral blood to the CNS. Migrated HTLV-1-infected $CD4^+$ T cells start to express viral antigens, including Tax, and secrete proinflammatory cytokines such as IFN-γ (Hanon et al., 2001; Kambara et al., 2002), which stimulate the resident cells to produce multiple chemokines. These chemokines recruit more proinflammatory cells including HTLV-1-infected $CD4^+$ T cells and HTLV-1-specific $CD8^+$ T cells that are preferentially recruited and/or expanded in the CNS. Thus, HTLV-1-specific immune responses and secondary inflammations inflated in the CNS may lead to the subsequent CNS damage (**Figure 2**).

PROGNOSIS

The symptoms usually begin during adulthood, most frequently after the age of 40 years (range, 6–75 years). The disease usually progresses slowly without remission. However, there is a subgroup of patients with rapid progression who are unable to walk within 2 years, and another subgroup of patients with very mild progression (Nakagawa et al., 1995; Gotuzzo et al., 2004; Olindo et al., 2006; Lima et al., 2007; Martin et al., 2010). Indeed, in HAM/TSP, the clinical course and rate of progression may vary greatly among patients (**Figure 3**). In a study of 123 patients with a 14-year follow-up, the median time from symptom onset to need for unilateral walking aid was 6 years; bilateral walking, 13 years; and wheelchair dependence, 21 years. Nineteen of those 123 patients died

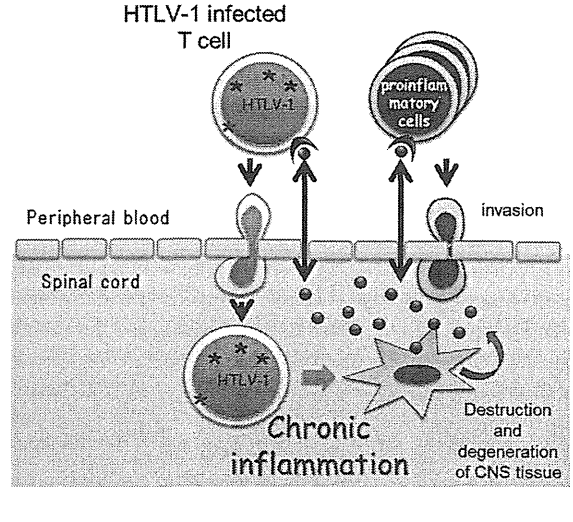

FIGURE 2 | Cellular mechanisms underlying pathogenesis of human T-lymphotropic virus type 1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). CNS, central nervous system. *HTLV-1.

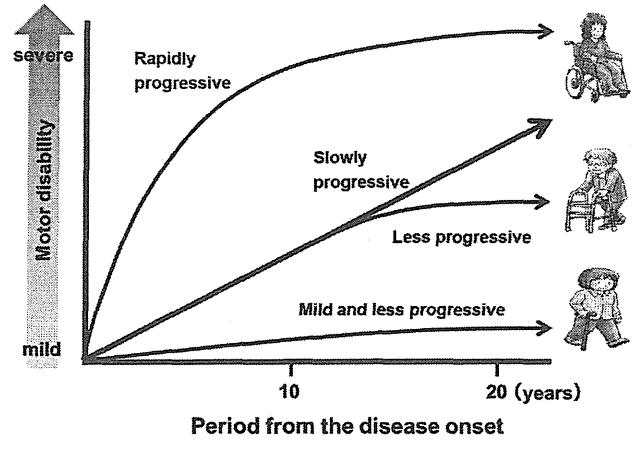

FIGURE 3 | A schematic representation of the clinical course of human T-lymphotropic virus type 1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).

due to complications of HAM/TSP, and the mean age at death was approximately 15 years shorter than the life expectancy in the cohort area (Olindo et al., 2006). In a study of 48 patients with a 15-year follow-up, the median time from symptom onset to the need for unilateral walking aid was 11 years; bilateral walking, 11.2 years; and wheelchair dependence, 18 years. The conditions of 3 of these 48 patients worsened rapidly, and they were unable to walk within 2 years, while in six patients, the progression was slow or the condition did not worsen; 5 of the 48 patients died, and the median age at death was 57 years (range, 36–78 years). Importantly, a timed 10-m walk was found to be a more sensitive scale to identify motion deterioration and recognize patients in need of therapeutic intervention (Martin et al., 2010). In terms of vital prognosis, it is also important to recognize that HAM/TSP patients have a risk to develop ATLL (Tamiya et al., 1995).

Since HAM/TSP is a chronic progressive neurological disease, the progression of clinical disease is usually subtle; this hampers the evaluation of disease progression even over the course of a year. Therefore, information about quantitative biomarkers associated with disease prognosis and disease activity is important for assessing the effect of therapy as well as conducting clinical trials of novel therapeutics with statistically significant endpoints. Although few well-designed studies have evaluated the usefulness of potential biomarkers as surrogate markers, accumulating evidence supports the relationship between HTLV-1 proviral load and long-term disease prognosis. Indeed, in a study with 100 untreated HAM/TSP patients, a significant association was demonstrated between higher HTLV-1 proviral load and poor long-term prognosis (Olindo et al., 2005); later, the authors confirmed this result in a bigger cohort (Olindo et al., 2006). Analysis of observational studies also showed a relationship between HTLV-1 proviral load and disease prognosis (Matsuzaki et al., 2001; Takenouchi et al., 2003). Older age at onset has also been demonstrated to be associated with poor long-term prognosis (Nakagawa et al., 1995; Matsuzaki et al., 2001; Olindo et al., 2006). In terms of biomarkers of disease activity, recent work by our research group showed that CSF cell count, neopterin concentration, and CSF levels of C-X-C motif chemokine 10 are well correlated with disease progression over 4 years, better even than HTLV-1 proviral load in PBMCs (manuscript in preparation). A prospective study to determine whether these indicators are useful as prognostic biomarkers will be necessary.

TREATMENT

Since the discovery of HAM/TSP, various therapeutic approaches have been used for HAM/TSP patients. However, no effective therapeutic strategy has been established thus far. Because induction of chronic inflammation by HTLV-1-infected T cells in the spinal cord is considered the major pathogenic mechanism underlying HAM/TSP, anti-inflammatory, or antiviral therapies have been tested. Clinical improvements in open-label studies have been reported for a number of agents including corticosteroids (Nakagawa et al., 1996), danazol (Harrington Jr. et al., 1991), pentoxifylline (Shirabe et al., 1997), and IFN-β1 (Oh et al., 2005). With the exception of IFN-α (Izumo et al., 1996), however, these drugs lack evidence required to merit strong recommendation for their use in HAM/TSP. The role of IFN-α in HAM/TSP is also not clear, as no study has conclusively shown its long-term benefit. Here, I summarize the results of recent trials and discuss the need for the identification of novel drug targets (**Table 3**).

Soon after the definition of HAM/TSP, corticosteroids were reported to decelerate the progression of this disease (Osame et al., 1990b). In a large-scale case series study (Nakagawa et al., 1996), oral prednisolone was effective in 81.7% of 131 patients, with 69.5% of the 131 patients showing more than one grade of improvement, as determined by Osame's motor disability scale. Furthermore, oral prednisolone therapy decreased the concentration of neopterin, which is an inflammatory marker of HAM/TSP, in CSF (Nakagawa et al., 1996). A recent open-cohort study of 39 patients with HAM/TSP with a mean follow-up of 2.2 years showed an improvement in overall disability following pulsed intravenous methylprednisolone (Croda et al., 2008). However, a few studies reported no such benefit (Kira et al., 1991; Araújo et al., 1993), and there has been no randomized clinical trial. Although steroidal therapy is not recognized as a radical therapy since it does not eliminate the HTLV-1-infected cells, in practice, steroids are the most commonly prescribed drug, despite the poor evidence for their efficacy. This is probably because some patients experience highly active inflammation or there is a significant inflammatory phase relatively early in disease. Since the clinical course and disease activity of HAM/TSP vary among patients (**Figure 3**), the treatment plan should be designed based on the patient's background such as activity or phase of the disease.

It is also notable that some patients' condition worsened after the dose of prednisolone was reduced, and hence, these patients remain dependent on drug administration (Nakagawa et al., 1996). In my research group, we had similar experiences; we found that such patients usually have high inflammatory levels in CSF, which increase even more as the clinical situation worsens after the dose of prednisolone is decreased. Since long-term use of prednisolone therapy is not desirable due to its variety of side effects, the development of steroid-sparing agents is urgently required for these patients. Candidate steroid-sparing agents could be

Table 3 | Summary of reports on treatment for HAM/TSP.

Authors	Country	Study design	Reagents	Treatment regimen	Study period	No. of patients	Rate of Efficacy	Note
Osame et al. (1990b)	Japan	Open-label	Prednisolone	60–80 mg qod for 2 month → 10 mg off/month for 6 month → 5 mg qod for 3 month	11 Month	65	90.8% (59/65) 56.9% (>1)	Incidence of side effects: 20% (13/65)
Croda et al. (2008)	Brazil	Case series	Methylprednisolone	1 g × 3 days/month for 3–4 month	2.2 Years	39	24.5%	Transient effect
Nakagawa et al. (1996)	Japan	Open-label	Prednisolone	1–2 mg/kg qd or qod for 1–2 month → tapering	6–12 Month	131	81.7% 69.5% (>1)	Decrease of CSF neopterin
			Methylprednisolone	500 mg–1g × 3 days		10	30.0%	For rapid progression
			Interferon-α	3 MU/day × 30 days	1–3 Month	32	62.5% 21.9% (>1)	Transient effect Incidence of side effects: 65.6% (21/32)
Martin et al. (2012)	UK	Open-label	Cyclosporine A	2.5–5 mg/kg/day bd for 48 week	72 Week	7	71.4% (5/7) after 3 Month	Clinical failure: two patients
Izumo et al. (1996)	Japan	Multicenter double-blind RCT	Interferon-α	0.3 MU/day × 28 days 1 MU/day × 28 days 3 MU/day × 28 days	8 Week	15 17 16	7.1% 23.5% 66.7%	Incidence of side effects: 26.7% (4/15) 29.4% (5/17) 50.0% (8/16)
Yamasaki et al. (1997)	Japan	Case series	Interferon-α	6 MU/day × 14 days → 6 MU/3 times/week × 22 week	6 Month	7	71.4% (5/7)	Clinical failure: two patients
Arimura et al. (2007)	Japan	Phase IV	Interferon-α	3 MU/day × 4–793 days (median 30 days)	6 Month	167	66.2% 29.2% (>1)	Side effects: 87.4% Serious side effects: 7.0%
Taylor et al. (2006)	UK and Japan	Double-blind RCT	Zidovudine + lamivudine	AZT 300 mg + 3TC 150 mg bd	48 Week	16	No clinical improvement	No change in proviral load
Macchi et al. (2011)	UK	Case series	Tenofovir	245 mg/day	2–16 Month	6	No clinical improvement	No change in proviral load

> 1, improvement of more than one grade in the Osame's motor disability score.

No., number; qod, every other day; mo: month(s); yr, year(s); qd, every day; MU, million unit; wk, week(s)' bd, twice daily; RCT, randomized controlled trial; AZT, zidovudine; 3TC, lamivudine.

anti-inflammatory and/or antiviral in nature. In fact, there is a recent report on the high efficacy of cyclosporine A therapy targeted at early phase or progressive HAM/TSP patients. In this study, clinical improvement was observed in five of seven patients, with reduction of provirus DNA load observed in the CSF (Martin et al., 2012).

Type I IFNs (α and β), which have immunomodulatory and antiviral properties (Borden et al., 2007), have been tested as anti-HAM/TSP drugs. IFN-α demonstrated clinical benefits in a multicenter, randomized, double-blind, controlled trial of HAM/TSP patients in Japan (Izumo et al., 1996). In this study, 3 million units (MU) of human lymphoblastoid natural IFN-α given daily by intramuscular injection for 28 days showed better clinical benefit than 0.3 or 1 MU of IFN-α. The reduction of proviral DNA load and memory $CD8^+$ cells in PBMCs (Saito et al., 2004) and the reduction of CD4/CD8 ratio and $CD4^+CCR5^+$ cells in CSF (Kambara et al., 2002) after short-term IFN-α therapy was demonstrated. However, the benefit of long-term IFN-α therapy has not been well demonstrated. A small study extending IFN-α treatment for 24 weeks reported sustained clinical response (Yamasaki et al., 1997). In a post-marketing surveillance of IFN-α in Japan, sustained improvements in motor disability for 5 months after cessation of IFN-α administration were observed in 11 of 30 patients, and a high adverse event rate (536 events reported in 146 patients; 46 classified as serious) was indicated (Arimura et al., 2007). In this surveillance study, it is notable that IFN-α treatment was more effective in patients with lower motor disability and shorter duration of illness and progression phase, suggesting the existence of therapeutic windows of opportunity in the treatment of HAM/TSP. It is also notable that rapidly progressing HAM/TSP patients showed no response and dropped out from the IFN-α therapy (Yamasaki et al., 1997; Arimura et al., 2007). Therefore, well-designed controlled clinical trials to guide the clinician with regard to the appropriate target, time of initiation, and the dose or duration of IFN-α therapy in HAM/TSP will be important for future studies.

Thus, corticosteroids and IFN-α may have therapeutic efficacy for HAM/TSP to some extent; however, the effect may not be sufficient for avoiding long-term disability. Moreover, in some cases, it might be difficult to continue therapy because of the side effects of these drugs and their insufficient benefit. Therefore, it is essential that revolutionary drugs that can lead to a paradigm shift in the therapeutic strategies for HAM/TSP be developed. Considering the pathogenesis of HAM/TSP, therapies to eliminate HTLV-1-infected cells from the peripheral blood and CNS should be developed. However, antiviral therapy has not been successful in the clinical trial for HAM/TSP. A randomized, double-blind, placebo-controlled, 6-month study of zidovudine and lamivudine combination therapy, which demonstrated activity against HTLV-1 reverse transcriptase *in vitro*, was conducted in 16 patients, and no significant changes were observed in the clinical symptoms and HTLV-1 proviral load (Taylor et al., 2006). A pilot trial of tenofovir, which also demonstrated activity against HTLV-1 reverse transcriptase *in vitro*, was conducted in six patients, and no significant change in HTLV-1 proviral load was observed (Macchi et al., 2011). Thus, the impact of therapy with viral reverse transcriptase inhibitors with the aim of reducing the HTLV-1 proviral load *in vivo* has been minimal. These results support the hypothesis that HTLV-1 proviral load in HTLV-1-infected patients is mainly maintained through cell division of infected cells and not by viral replication and new infection (Wattel et al., 1995; Cavrois et al., 1998). Therefore, development of therapies directly targeting HTLV-1-infected cells could be more promising to reduce the viral load. Recently, we have demonstrated that CC chemokine receptor 4 (CCR4), expressed on the surface of ATLL cells (Ishida et al., 2004), is also expressed on HTLV-1-infected cells in HAM/TSP patients (Yamano et al., 2009). More recently, a humanized anti-CCR4 monoclonal antibody has been developed; the safety and efficacy of this antibody has been proven in phase I and II studies (Yamamoto et al., 2010; Ishida et al., 2012) and subsequently approved by the Ministry of Health, Labour and Welfare as a therapeutic agent for relapsed patients with ATLL in Japan. Further clinical trials on the safety and efficacy of anti-CCR4 therapy for HAM/TSP patients should be conducted in future studies.

CONCLUSION

Advances in study of the epidemiology and pathogenesis of HAM/TSP have led to the identification of several biomarkers and therapeutic targets. However, these findings have not yet translated into an optimal therapeutic strategy for this hitherto intractable neurological disease. Well-designed clinical trials in HAM/TSP will provide opportunities for further quantification of biomarkers and refinement of therapeutic drugs. The development of an effective therapy to improve long-term prognosis in HAM/TSP is of paramount importance, and clinical trials for the validation of HAM/TSP relevant biomarkers and new therapeutic targets will be key challenges in this therapy.

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