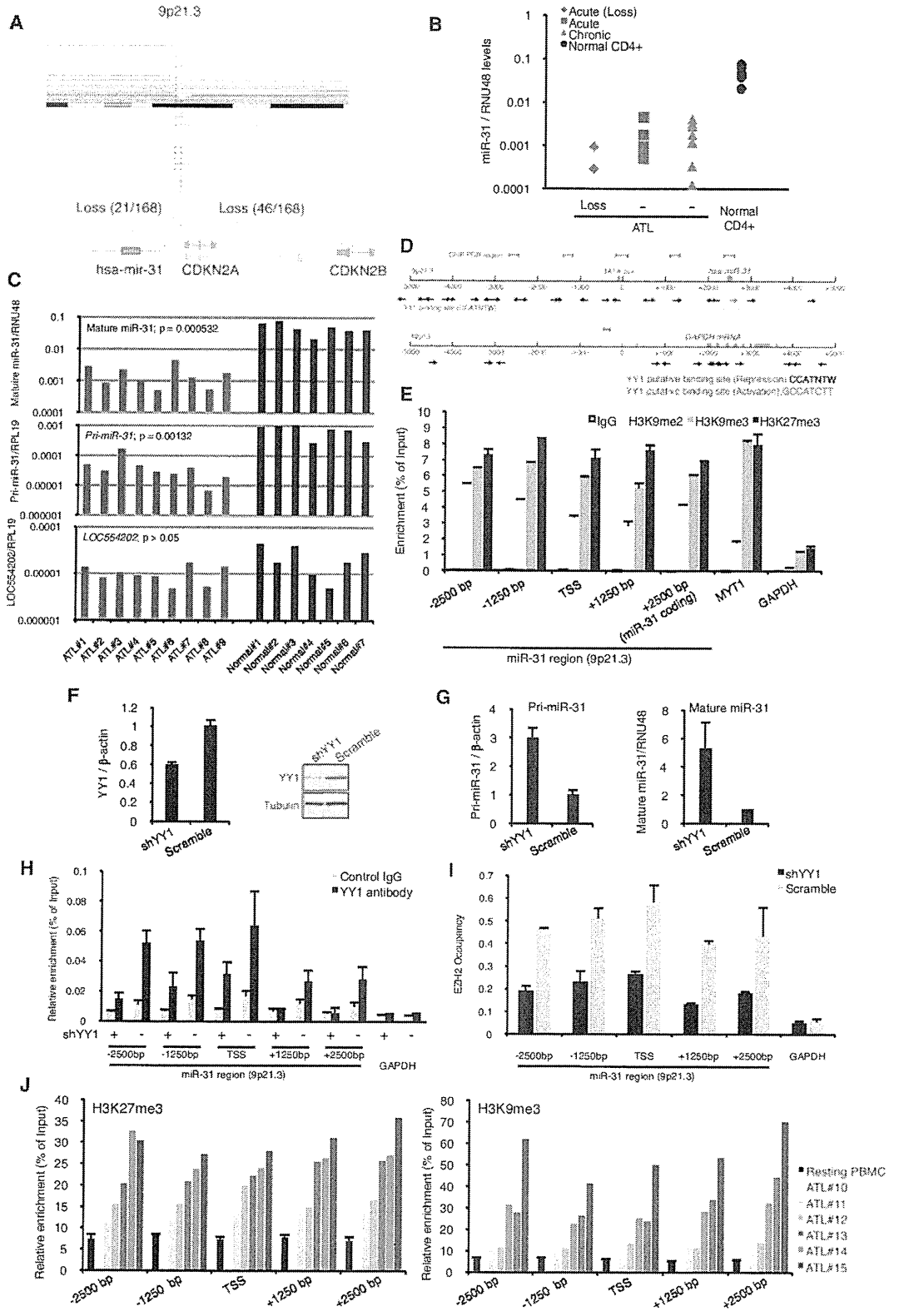

A
9p21.3
Loss (21/168)
Loss (46/168)
hsa-mir-31
CDKN2A
CDKN2B
B
Acute (Loss)
Acute
Chronic
Normal CD4+
miR-31 / RNU48 levels
Loss
ATL
Normal CD4+
C
Mature miR-31; p = 0.000532
Pri-miR-31; p = 0.00132
LOC554202; p > 0.05
Mature miR-31/RNU48
Pri-miR-31/RPL19
LOC554202/RPL19
D
E
IgG H3K9me2 H3K9me3 H3K27me3
Enrichment (% of Input)
-2500 bp
-1250 bp
TSS
+1250 bp
+2500 bp (miR-31 coding)
MYT1
GAPDH
miR-31 region (9p21.3)
F
YY1 / β-actin
shYY1
Scramble
YY1
Tubulin
G
Pri-miR-31
Pri-miR-31 / β-actin
Mature miR-31
Mature miR-31/RNU48
H
Control IgG
YY1 antibody
Relative enrichment (% of Input)
shYY1
GAPDH
miR-31 region (9p21.3)
I
shYY1
Scramble
EZH2 Occupancy
J
H3K27me3
H3K9me3
Relative enrichment (% of Input)
Resting PBMC
ATL#10
ATL#11
ATL#12
ATL#13
ATL#14
ATL#15

ChIP assays detected higher levels of trimethylated H3K27 and EZH2 occupancy in cells showing lower expression levels of miR-31 (Figure S4H). Furthermore, knockdown of EZH2 or SUZ12 restored miR-31 transcription in MDA-MB-453 and MCF7 cells (Figures 5F and 5G; Figure S4K, respectively), which are consistent with the results obtained with ATL cells. These results indicate a link between Polycomb-mediated epigenetic regulation and miR-31 transcription in ATL and breast cancer cell lines.

Polycomb Group Regulates NF-κB Pathway by Controlling miR-31 Expression

Based on our findings, we considered an aspect of the biological communication between epigenetic silencing and the NF-κB pathway through miR-31 regulation. The microarray data sets showed positive correlations between PRC2 components and miR-31 target gene, *NIK* expression (Figure 6A). The results also suggested that these factors tend to show higher levels in the aggressive subtype (acute type) than in the indolent subtypes (chronic and smoldering types), implying that these genes may play important roles in the clinical phenotype and prognosis of ATL. To examine this notion, we performed PRC2 knockdown in ATL cell lines. Western blots of these cells demonstrated decreased levels of NIK, p52, and phospho-IκBα (Figure 6B; Figure S5A), suggesting suppression of both canonical and noncanonical NF-κB cascade and activity (Figure 6C; Figures S5B and S5C). These results are consistent with those of miR-31 overexpression (Figures 3C–3F). Then, we tested whether exogenous manipulation of miR-31 could restore the effect of PRC2 loss. Anti-miR-31 treatment rescued impaired NF-κB activity in PRC2-disrupted cells (Figure 6D). On the other hand, overexpression of EZH2 induced NF-κB activation, which was partially canceled by the introduction of miR-31 precursor (Figure 6E; Figure S5D). These results suggest that Polycomb-mediated miR-31 suppression leads to NF-κB activation. Indeed, knockdown of the PRC2 complex led to reduced levels of cell proliferation and greater sensitivity to serum deprivation in ATL cells (Figure 6F; Figure S5E). In addition, PRC2 disruption showed a reduction in cell migration (Figure S5F).

To gain further insight into this general network, we studied the functions of miR-31 and the PRC2 complex in breast cancer cell lines. NF-κB activity was downregulated by knockdown of PRC2 components in MDA-MB-453 cells (Figure 6G; Figures S5G and S5H), although no significant differences were observed in cell proliferation (data not shown). Repression of NF-κB activity induced by knockdown of PRC2 components was partially restored by treatment with a miR-31 inhibitor, suggesting that PRC2 knockdown-mediated relief of NF-κB repression is at least a part of the result of the miR-31 induction. In addition, knockdown of PRC2 components resulted in a reduced level of receptor-initiated accumulation of NIK in B cells (Figure 6H). Our findings indicate a common molecular mechanism comprising Polycomb-mediated epigenetic regulation, miR-31 expression and the NF-κB signaling pathway.

Regulation of NF-κB by Polycomb family may in turn control the cellular apoptosis responses. We found that lentivirus-mediated EZH2 knockdown led to increased apoptotic sensitivity in TL-Om1 cells (Figure 6I). Additional expression of NIK inhibited the cell death induced by EZH2 knockdown, suggesting the reciprocal relationship between Polycomb and NF-κB cascades. By using primary tumor cells from patient, we tested the killing effect induced by miR-31, NIK knockdown, and EZH2 knockdown (Figure 6J; Figures S5I and S5J). All tested samples showed strong death response, demonstrating that survival of ATL cells was closely associated with miR-31, NIK, and EZH2, all of which show deregulated expression in ATL cells.

By qRT-PCR we finally examined the expression levels of some genes involved in the noncanonical NF-κB pathway. As shown in Figure 6K, the results clearly demonstrated higher expression levels of positive regulators such as *NIK*, *CD40*, and *LTBR*, and lower expression levels of the negative regulators such as *BIRC2/3* (cIAP1/2), which are involved in proteasomal degradation of NIK (Zarnegar et al., 2008a). These observations are in line with a previous report on Multiple Myeloma cells (Annunziata et al., 2007). In addition to these data, we obtained convincing evidence for a molecular aspect of NIK accumulation in ATL cells. Polycomb-dependent epigenetic gene silencing may be associated with miR-31 loss, followed by NF-κB activation and other signaling pathways (Figure 7).

DISCUSSION

Constitutive activation of NF-κB contributes to abnormal proliferation and inhibition of apoptotic cell death in cancer cells,

Figure 4. Genetic and Epigenetic Abnormalities Cause miR-31 Loss in ATL Cells

(A) Genomic loss of chromosome 9p21.3 in primary ATL cells. Copy number analyses revealed tumor-associated deletion of miR-31 region (21/168) and *CDKN2* region (46/168). Recurrent genetic changes are depicted by horizontal lines based on CNAG output of the SNP array analysis.

(B) miR-31 expression in various sample sets. Expression levels were evaluated by real-time PCR.

Loss, samples with genomic loss of the miR-31 region; (–) samples without genomic loss of the miR-31 region.

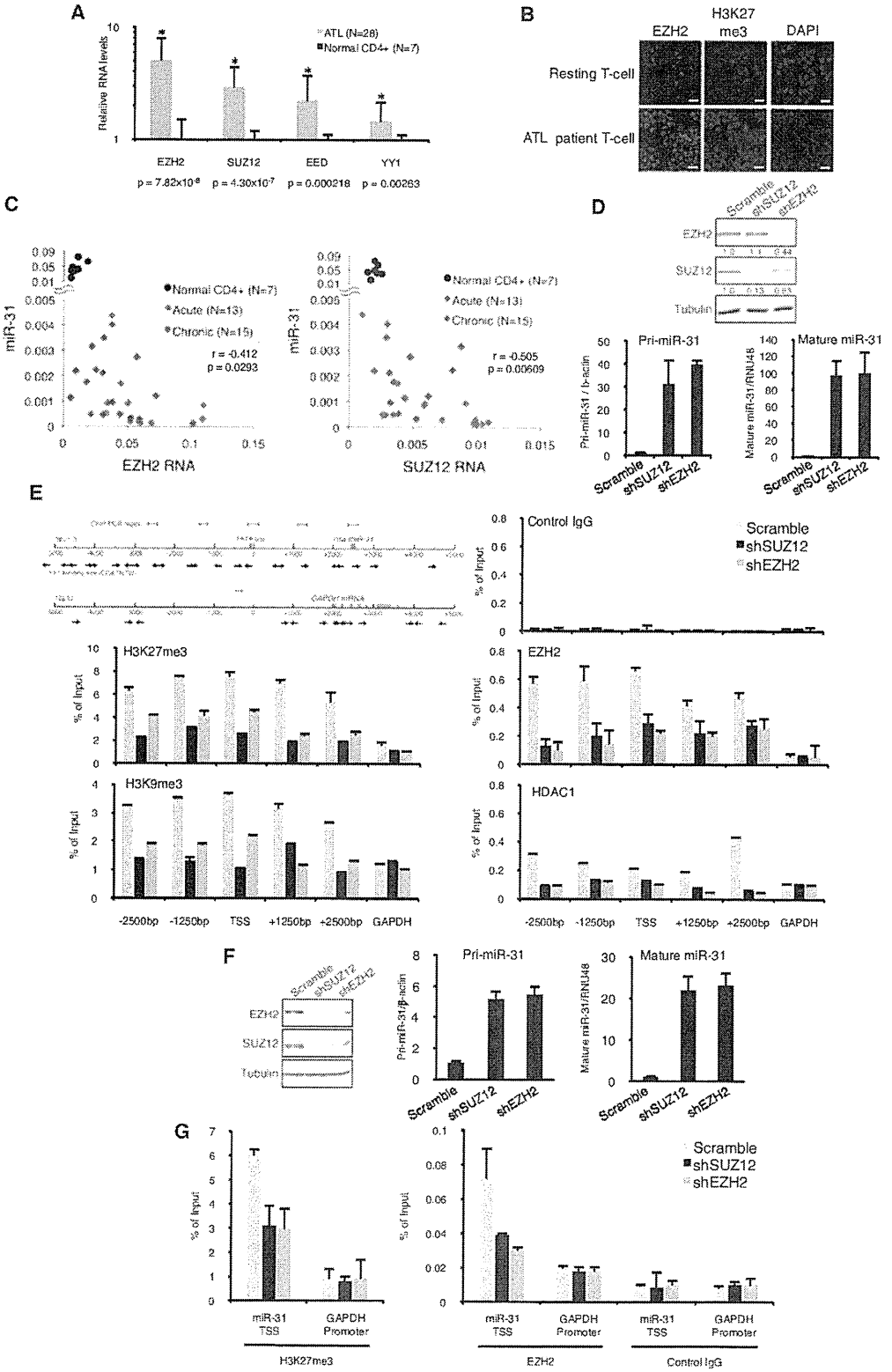
(C) PCR-based miR-31 quantifications in primary ATL samples. ATL samples without genetic loss in miR-31 region (n = 9, Figure S3B), and normal CD4+ T cells (n = 7) were tested. p values (ATL versus normal) are shown.

(D) YY1 binding motif cluster around transcriptional start site (TSS) of miR-31 region. Arrows represent positions of the motifs. Regions of PCR amplification for ChIP assay are shown.

(E) Repression-associated histone methylation in miR-31 region determined by ChIP assay (n = 3, mean ± SD). The results of relative enrichment against input control are presented and distance from miR-31 TSS is described. *MYT1* and *GAPDH* promoters are as positive or negative controls, respectively.

(F–I) YY1-dependent EZH2 occupancy in miR-31 locus. (F) YY1 knockdown in TL-Om1 cells. qRT-PCR (left, n = 3, mean ± SD) and western blotting (right) showed decreased YY1 level. (G) YY1 knockdown led to both primary and mature miR-31 restoration in TL-Om1 cells (n = 3, mean ± SD). (H) YY1 occupancy in miR-31 region analyzed by ChIP (n = 3, mean ± SD). YY1 occupancy in miR-31 locus was reduced by YY1 knockdown. (I) EZH2 occupancy in miR-31 region analyzed by ChIP (n = 3, mean ± SD). YY1 knockdown inhibited EZH2 recruitment in miR-31 region.

(J) Aberrant accumulation of repression-associated histone methylations widely in miR-31 region of primary ATL cells. PBMCs freshly isolated from ATL patients (n = 6) were analyzed by ChIP assay. PBMC from healthy adults were used for normal controls. See also Figure S3.


A
Relative RNA levels
ATL (N=28)
Normal CD4+ (N=7)
EZH2
SUZ12
EED
YY1
p = 7.82x10-8
p = 4.30x10-7
p = 0.000218
p = 0.00263
B
H3K27 me3
EZH2
DAPI
Resting T-cell
ATL patient T-cell
C
miR-31
Normal CD4+ (N=7)
Acute (N=13)
Chronic (N=15)
r = -0.412
p = 0.0293
EZH2 RNA
r = -0.505
p = 0.00609
SUZ12 RNA
D
Scramble
shSUZ12
shEZH2
EZH2
SUZ12
Tubulin
Pri-miR-31
Pri-miR-31 / b-actin
Mature miR-31
Mature miR-31/RNU48
E
Control IgG
Scramble
shSUZ12
shEZH2
% of Input
H3K27me3
EZH2
H3K9me3
HDAC1
-2500bp
-1250bp
TSS
+1250bp
+2500bp
GAPDH
F
Pri-miR-31
Mature miR-31
G
miR-31 TSS
GAPDH Promoter
H3K27me3
EZH2
Control IgG

including ATL, diffuse large B cell lymphoma (DLBCL), Hodgkin lymphoma, breast cancer, prostate cancer and others (Prasad et al., 2010). NF-κB is also essential for various cell functions, including inflammation, innate immunity, and lymphocytic development (Hayden and Ghosh, 2008). Identification of NF-κB determinants will lead to marked progress in understanding molecular pathology.

Our global analyses demonstrated an interesting miRNA expression signature as well as an aberrant mRNA expression profile, which may be associated with leukemogenesis in the primary ATL cells (Figures 1 and 6A). We revealed downregulation of tumor-suppressive miRNA including Let-7 family, miR-125b, and miR-146b, which can contribute to aberrant tumor cell signaling. Recent studies have suggested unique expression profiles of miRNAs in ATL (Yeung et al., 2008; Bellon et al., 2009), but loss of miR-31 has not been focused. Cellular amount of miRNAs may be susceptible to various environments such as transcriptional activity, maturation processing, and also epigenetic regulation. The end results appear to be affected by methodology employed and conditions and types of samples used. Our integrated expression profiling of primary ATL cells are based on a significantly larger number of samples and fruitfully provides intriguing information that may be useful in improving the understanding of T cell biology as well as in the identification of biomarkers for diagnosis.

Pleiotropy of miR-31 was first reported by Valastyan et al. (2009). The authors elegantly demonstrated the function of miR-31 in vivo and also identified several target genes that contribute to cell migration and invasiveness. In the present study, we focused on the functional significance of miR-31 in the regulation of NF-κB signaling that contributes to tumor cell survival.

Overexpression of NIK acts as an oncogenic driver in various cancers. In the present study, NIK was identified as a miR-31 target based on several lines of evidence. First, luciferase-3′ UTR reporter assay showed that *NIK* 3′ UTR sequence has a role for negative regulation (Figure S1B). By combining a specific inhibitor and mutations in miR-31-binding site, we demonstrated that miR-31 recognizes and negatively regulates the *NIK* 3′ UTR (Figures 2A and 2D). Second, by introducing a miR-31 precursor or inhibitor, we showed that amount of miR-31 inversely correlates with levels of NIK expression and downstream signaling (Figures 2E–2K). Third, genetic evidence indicated strong base pairing and biological conservation (Bartel, 2009) (Figures S1L–S1O). Our experimental approach illustrated that mmu-miR-31 regulates mouse *Map3k14* gene. Fourth, individual assessments using gene expression data clearly revealed an inverse correlation between the expression levels of miR-31 and *NIK* (Figure 3A). Collectively, we provide definitive evidence for the notion that miR-31 negatively regulates NIK expression and activity.

It is well known that the NIK level directly regulates NF-κB activity in various cell types (Thu and Richmond, 2010). We experimentally showed that miR-31 regulates noncanonical NF-κB activation stimulated by BAFF and CD40L, both of which are major B cell activating cytokines. Since signals from receptors are essential for the development and activity of B cells, the negative role of miR-31 in cytokines-induced NIK accumulation appears to be widely important in the noncanonical regulation of NF-κB in B cells and other cell types (Figures 2H–2K). Again, our findings revealed the role of NIK in the regulation of canonical NF-κB pathway. Strict regulation of NIK appears to be closely associated with the fate of lymphocytes.

The level of miR-31 was drastically suppressed in all tested primary ATL cells, and its magnitude is greater than that which has been reported in other cancers. Our results demonstrated a profound downregulation of miR-31 (fold change, 0.00403; Figure 1B) in all ATL cases, suggesting that miR-31 loss is a prerequisite for ATL development. Restoration of miR-31-repressed NF-κB activity in ATL cells, resulting in impairment of the proliferative index and apoptosis resistance (Figure 3). Furthermore, our results demonstrate that inhibition of NF-κB promotes tumor cell death in cell lines and also primary tumor cells from ATL patients (Figures 3 and 6), which are consistent with our previous observation (Watanabe et al., 2005). Since it is highly possible that miR-31 and relevant factors are pivotal in cancers, their expressions would have a great importance in view of biomarkers for the aberrant signaling and clinical outcomes.

By studying clinical samples and in vitro and ex vivo models, we obtained several biologically interesting results. First, we identified the Polycomb protein complex as a strong suppressor of miR-31. Generally, the Polycomb group constitutes a multimeric complex that negatively controls a large number of genes involved in cellular development, reproduction, and stemness (Sparmann and van Lohuizen et al., 2006). However, the key molecules involved in cancer development, progression, and prognosis are not yet fully understood. In breast and prostate cancers, oncogenic functions of EZH2 and NF-κB activation were reported independently (Kleer et al., 2003; Varambally et al., 2002; Suh and Rabson, 2004). Interestingly, these tumors show low miR-31 levels (Valastyan et al., 2009; Schaefer et al., 2010). Recently, Min et al. (2010) reported that EZH2 activates NF-κB by silencing the DAB2IP gene in prostate cancer cells.

Figure 5. Amount of PRC2 Components Epigenetically Links to miR-31 Expression in T Cells and Epithelial Cells

(A) Overexpression of PRC2 components in primary ATL cells measured by qRT-PCR (ATL, n = 28; normal, n = 7; mean ± SD). These results were supported by the data of gene expression microarray (Table S3).

(B) Escalation of EZH2 protein and trimethylated H3K27 levels in primary ATL cells illustrated by immunocytostaining (n = 4, a representative result is shown). Resting T cells were as normal control. Scale bars = 20 μm.

(C) Statistical correlation among the levels of miR-31, *EZH2*, and *SUZ12* in individual ATL samples. Correlation coefficients within ATL samples are shown in the graphs.

(D and E) Loss of PCR2 function causes chromatin rearrangement and miR-31 upregulation. (D) TL-Om1 cells expressing shSUZ12, shEZH2, and scrambled RNA were established by retroviral vector. The levels of EZH2, SUZ12, *Pri-miR-31*, and mature miR-31 were measured by western blotting and qRT-PCR (n = 3, mean ± SD). (E) Results of ChIP assays with indicated antibodies (n = 3, mean ± SD). Amounts of immunoprecipitated DNA were analyzed by region-specific PCR. *GAPDH* promoter served as a region control.

(F and G) Knockdown of Polycomb family proteins in MDA-MB-453 cells. (F) EZH2 and SUZ12 are shown by western blot. miR-31 level was examined by qRT-PCR (n = 3, mean ± SD). (G) Histone methylation and EZH2 occupancy evaluated by ChIP assay (n = 3, mean ± SD). See also Table S3 and Figure S4.

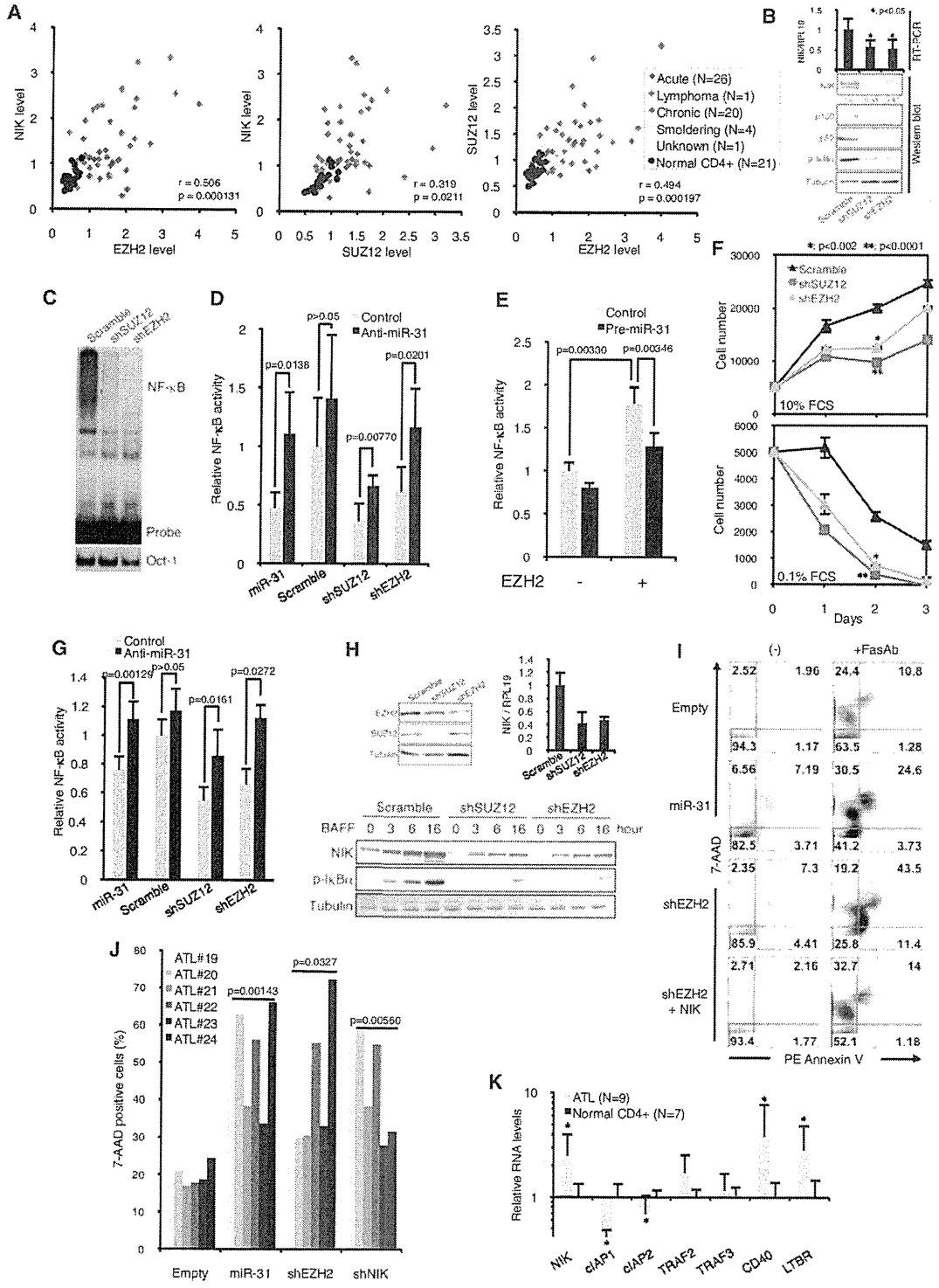


Figure 6. Epigenetic Change Driven by Polycomb Group Mediates NF-κB Signaling through miR-31 Regulation

(A) Reciprocal relationship of mRNA expression between *NIK* and Polycomb group in primary samples. Pearson's correlation coefficients among ATL samples are shown.

(B) PRC2 knockdown negatively affects NF-κB signaling in TL-Om1 cells. After establishment of PRC2 knockdown, the levels of *NIK* RNA (n = 4, mean ± SD) and proteins of NIK, p52/p100, and phospho-IκBα were examined.

(C) Downregulation of NF-κB activity in PRC2-disrupted cells detected by EMSA.

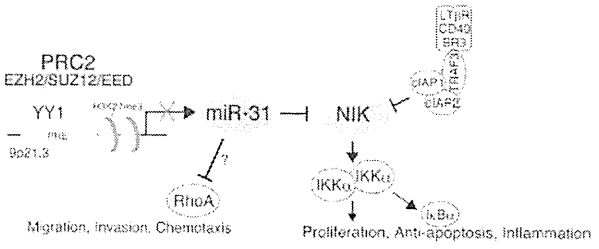


Figure 7. Proposed Model for ATL and Other Tumor Cells
Polycomb repressive factors are linked to NIK-dependent NF-κB activation via miR-31 regulation.

In the present study, we found that the Polycomb group regulates miR-31 expression and that elevated expression of EZH2 leads to NF-κB activation via NIK-miR-31 regulation in ATL and breast cancer cells (Figure 6). We also showed that restoration of miR-31 partially impaired Polycomb-mediated NF-κB operation (Figures 6D, 6E, and 6G), suggesting that miR-31 is involved in this relationship. Furthermore, a connection between NIK and PRC2 was observed in B cells (Figure 6H). Polycomb group proteins are essential in lymphocyte development and activation (Su et al., 2003, 2005). Further, given the NF-κB is a pivotal transcription regulator in normal and oncogenic functions, practical participations of epigenetic regulators and miR-31 in NF-κB signaling will increase our understanding of the molecular mechanisms of T cell functions. For generalization of the molecular axis in other cancers and normal cells, further study will be needed.

Second, YY1 is a recruiter of PRC2 to the miR-31 region. In humans, the Polycomb response element (PRE) has not been precisely identified. A good candidate for a mammalian recruiter of PRC2 is YY1, the homolog of *D. melanogaster* PHO (Simon and Kingston, 2009). We found an assembly of the YY1 binding motif in the miR-31 locus and demonstrated that YY1 knockdown dislodged EZH2 in this region (Figure 4I), which supports previous findings (Caretti et al., 2004). The detailed mechanism by which YY1 mediates recruitment of the Polycomb family may be important in the context of epigenetic regulation of orchestrated gene expression and T cell functions.

Third, Polycomb family proteins can control miRNA expression in an epigenetic fashion. The amount of PRC2 factors strongly influenced the degree of suppression of miR-31 expression (Figure 5). We speculate that, in addition to controlling the transcription, the Polycomb group can modulate translation via miRNA regulation. Furthermore, miR-101 and miR-26a are known to regulate EZH2 expression (Sander et al., 2008; Varambally et al., 2008), which is supported by our observation (Figure S4C). This signaling circuit will permit multiple gene regulation. Whereas genetic loss at the miR-31 locus is observed in some cases of ATL (Figure 4A), no genetic deletion in the miR-101-1 or miR-101-2 region was detected in ATL, which is not consistent with a previous finding in prostate cancer. Our results also suggested putative association between Let-7 family and EZH2 (Figure S4). Aberrant downregulations of these miRNAs in the primary ATL cells will be the next important questions to be addressed in efforts to improve understanding of the oncogenic signaling network.

By collaborative profiling of miRNA and mRNA expression, we identified a notable relationship between ATL subtypes and a gene cluster that contains miR-31, NIK, EZH2, and SUZ12. This finding suggests that an aberrant gene expression pattern correlates with the malignant phenotype, and this provides important clues about clinical manifestations and may help identify therapeutic targets against ATL cells (Figure 6A). Although HDAC inhibitors did not show effective responses (Figures S4I and S4J), emerging epigenetic drug such an EZH2 inhibitor (Fiskus et al., 2009) may pave a pathway leading to cures for various malignancies that involve constitutive activation of NF-κB.

In summary, we show that genetic and epigenetic loss of miR-31 is responsible for oncogenic NF-κB activation and malignant phenotypes in ATL. This provides evidence for the idea that miR-31 is an important tumor suppressor. An emerging pathway involving an epigenetic process, miR-31, and NF-κB will provide a conceptual advance in epigenetic reprogramming, inflammatory signaling, and oncogenic addiction.

EXPERIMENTAL PROCEDURES

Cell Lines and Primary ATL Cells

The primary peripheral blood mononuclear cells (PBMCs) from ATL patients and healthy volunteers used in the present work were a part of those collected with an informed consent as a collaborative project of the Joint Study on Prognostic Factors of ATL Development (JSPFAD). The project was approved by the Institute of Medical Sciences, the University of Tokyo (IMSUT) Human Genome Research Ethics Committee. Additional ATL clinical samples for copy number analysis were provided by Drs. Y. Yamada, Nagasaki University,

(D) NF-κB activity evaluated by reporter assays in the presence or absence of miR-31 inhibitor (n = 5, mean ± SD). Anti-miR-31 treatment partially rescued the NF-κB activity in PRC2 knockdowned TL-Om1 cells.
(E) Overexpressed EZH2 activates NF-κB via miR-31. Jurkat cells were transfected with an EZH2 plasmid together with miR-31 precursor or control RNA (n = 5, mean ± SD).
(F) PRC2 dysfunction changes TL-Om1 cell proliferation and response to serum starvation. Under conditions of 10% or 0.1% of FCS, cell growth curves were examined (n = 3, mean ± SD). PRC2 downregulation decreased cell growth with statistical significance.
(G) NF-κB activity in PRC2-knockdowned MDA-MB-453 cells in the presence or absence of miR-31 inhibitor were examined (n = 5, mean ± SD).
(H) PRC2 disruption inhibits BAFF-dependent NIK accumulation and IκBα phosphorylation in BJAB cells.
(I) Apoptotic cell death induced by lentivirus-mediated EZH2 knockdown in TL-Om1. Venus-positive populations were analyzed by Annexin V/7-AAD stainings (n = 3) and representative of FACS data are shown.
(J) Summary of primary tumor cell death. Lentivirus-based miR-31 expression, NIK knockdown, and EZH2 knockdown showed killing effects in six primary ATL samples. Statistical significances are shown in the graph. Results of FACS and qRT-PCR are shown in Figures S5I and S5J.
(K) Expression levels of genes involved in noncanonical NF-κB pathway in primary ATL cells (ATL, n = 9; normal, n = 7; mean ± SD). Relative expression levels were tested by qRT-PCR (*$p < 0.05$). See also Figure S5.

and K. Ohshima, Kurume University, where the projects were approved by the Research Ethics Committees of Nagasaki University and Kurume University, respectively. PBMC were isolated by Ficoll separation. ATL cells, primary lymphocytes, and all T cell lines were maintained in RPMI1640 supplemented with 10% of FCS and antibiotics. Clinical information of ATL samples is provided in Table S1.

Expression Analyses

Clinical samples for microarrays were collected by a collaborative study group, JSPFAD (Iwanaga et al., 2010). Gene expression microarray was used 4x44K Whole Human Genome Oligo Microarray (Agilent Technologies) and miRNA microarray was used Human miRNA microarray kit v2 (Agilent Technologies), respectively. Quantitative RT-PCR was performed with SYBRGreen (TAKARA). Mature miRNA assays were purchased from Applied Biosystems.

Copy Number Analyses

Genomic DNA from ATL patients was provided from the material bank of JSPFAD, Nagasaki University, and Kurume University, and was analyzed by Affymetrix GeneChip Human Mapping 250K Nsp Array (Affymetrix). Obtained data were analyzed by CNAG/AsCNAR program (Chen et al., 2008).

Oligonucleotides, Plasmids, and Retrovirus Vectors

All RT-PCR primers and oligonucleotides are described in Supplemental Experimental Procedures. miRNA precursor and inhibitor were from Applied Biosystems. Transfection of small RNA and other plasmid DNA were performed by Lipofectamine2000 (Invitrogen). For miRNA or shRNA expression, retroviral vectors (pSINsi-U6, TAKARA) were used.

3′ UTR-Conjugated miR-31 Reporter Assay

HeLa cells were cotransfected with 3′ UTR-inserted pMIR-REPORT firefly plasmid (Ambion), RSV-Renilla luciferase plasmid, and miRNA inhibitor. The cells were collected at 24 hr posttransfection, and Dual-luciferase reporter assay was performed (Promega).

Analysis of NF-κB Pathway

NF-κB activity was evaluated by EMSA and reporter assays as previously described (Horie et al., 2004). Antibodies for western blots are described in supplemental information. Cell proliferative assay was performed by Cell Counting Kit-8 (Dojindo).

Lentivirus Vectors and Apoptosis Analysis

A lentivirus vector (CS-H1-EVBsd) was provided from RIKEN, BRC, Japan. Lentivirus solution was produced by cotransfection with packaging plasmid (pCAG-HIVgp) and VSV-G- and Rev-expressing plasmid (pCMV-VSV-G-RSV-Rev) into 293FT cells. After infection of lentivirus, the apoptotic cell was evaluated by PE Annexin V / 7-AAD staining (BD PharMingen) and analyzed by FACS Calibur (Becton, Dickinson). Collected data were analyzed by FlowJo software (Tree Star).

ChIP Assay

ChIP assay was previously described (Yamagishi et al., 2009). Briefly, cells were crosslinked with 1% of formaldehyde, sonicated, and subjected to chromatin-conjugated IP using specific antibodies. Precipitated DNA was purified and analyzed by real-time PCR with specific primers (see Supplemental Experimental Procedures).

Computational Prediction

To identify miR-31 target genes, we integrated the output results of multiple prediction programs; TargetScan, PicTar, miRanda, and PITA. RNAhybrid was for secondary structure of miRNA-3′ UTR hybrid. TSSG program was for TATA box and TSS predictions. DNA methylation site was predicted by CpG island Searcher.

Statistical Analyses

Data were analyzed as follows: (1) Welch's t test for Gene Expression Microarray (p value cutoff at 10^{-6}) and miRNA Microarray (p value cutoff at 10^{-5}); (2) Pearson's correlation for two-dimensional hierarchical clustering analysis and individual assessment of microarray data sets; (3) two-tailed pared Student's t test with $p < 0.05$ considered statistically significant for in vitro cell lines and primary cells experiments, including luciferase assay, RT-PCR, ChIP assay, cell growth assay, and migration assay. Data are presented as mean ± SD.

ACCESSION NUMBERS

Coordinates have been deposited in Gene Expression Omnibus database with accession numbers, GSE31629 (miRNA microarray), GSE33615 (gene expression microarray), and GSE33602 (copy number analyses).

SUPPLEMENTAL INFORMATION

Supplemental Information includes three tables, five figures, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.ccr.2011.12.015.

ACKNOWLEDGMENTS

We thank Dr. M. Iwanaga, Mr. M. Nakashima, and Ms. T. Akashi for support and maintenance of JSPFAD. We thank Drs. H. Miyoshi and A. Miyawaki for providing the Venus-encoding lentivirus vectors. We also thank Dr. R. Horie for experimental advices, and Drs. T. Kanno and T. Ishida for providing the MDA-MB-453. Grant support: Grants-in-Aid for Scientific Research from Ministry of Education, Culture, Sports, Science, and Technology of Japan to T.W. (No. 23390250) and by Grants-in-Aid from the Ministry of Health, Labour and Welfare to T.W. (H21-G-002 and H22-AIDS-I-002).

Received: November 3, 2010
Revised: August 12, 2011
Accepted: December 19, 2011
Published: January 17, 2012

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Meeting Report

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EXPERT REVIEWS

Third annual forum on T-cell lymphoma

Expert Rev. Anticancer Ther. 11(5), 693–695 (2011)

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Third Annual T-cell Lymphoma Forum

San Francisco, CA, USA, 27–29 January 2011

The Third Annual T-cell Lymphoma Forum, held on 27–29 January 2011 in San Francisco (CA, USA), continued in the spirit of the two earlier conferences and provided a collegial venue for clinicians and scientists to discuss advances in the science and treatment of T-cell lymphomas. More than 40 experts from around the world presented updates on classification, epidemiology and prognosis; rare and T-cell lymphomas of unspecified origin, CD30+ T-cell lymphomas; new treatment strategies; new agents and rational combinations; and transplantation. Of particular interest this year was a discussion on the link between breast implants and anaplastic large-cell lymphoma, which coincided with the US FDA announcement of this rare but noteworthy relationship. Submitted abstracts and poster presentations rounded off each of the sessions.

Conference history

The T-cell Lymphoma Forum was first held in Washington (DC, USA) in 2008 to share the latest research, exchange ideas and increase exposure for these rare types of lymphomas. The second forum, held in Hawaii (USA) in 2010, continued the momentum begun by the first conference and focused on epidemiologic and clinicopathologic characteristics of and treatment strategies for T-cell and natural killer (NK)-cell lymphomas. The third forum, held on 27–29 January 2011 in San Francisco (CA, USA) under the chairmanship of Francine Foss of the Yale Cancer Center in New Haven (CT, USA) and Kensei Tobinai, once again provided a platform for discussion about the classification, epidemiology, prognosis and pathogenesis of several T-cell lymphoma subtypes, and novel agents and treatment approaches. The conferences are designed for hematologists, oncologists, and other clinicians and scientists interested in T-cell lymphoma research and treatment. All three conferences have encouraged participation in T-cell registries and collaborations. The fourth T-cell lymphoma forum is scheduled for January 2012.

Classification, epidemiology & prognosis

James O Armitage (University of Nebraska, NE, USA) delivered the keynote address on classifying T-cell lymphomas. The difficulty in characterizing peripheral T-cell lymphomas (PTCLs) is that clonality is difficult to establish, variable immunological patterns exist, cytogenetics are only occasionally characteristic, and there are few characteristic oncogenes. In addition, as new treatments emerge, the optimal system established today for classification will most likely become obsolete. Lymphoma classification remains a moving target.

Dan Jones (Quest Diagnostics Nichols Institute, VA, USA) stressed the importance of T-cell function in the classification of T-cell lymphomas. Both clinicopathologic elements and molecular genetic entities must be considered in classifying post-thymic T-cell malignancies. For example, tumor types that overexpress the T-cell oncogenes *ALK*, *TCL1* and *SYK* bypass the upstream regulatory function of the T-cell pathway, and will most likely require different kinds of immunomodulation and different therapy targets.

Gene-expression profiling studies with large datasets and linkage to clinical outcome data are beginning to emerge that will help determine gene signatures that impact survival. Randy D Gascoyne (British Columbia Cancer Agency, BC, Canada) discussed recent work from the International Peripheral T-cell Lymphoma Project and gene signatures that impact survival in the angioimmunoblastic T-cell lymphoma (AITL) subtype [1]. He also discussed novel translocations in PTCL, such as those that target the IRF4/MUM1 locus on chromosome 6p25 [2], the identification of which may be important to T-cell biology and survival predictions.

 10.1586/ERA.11.33 ISSN 1473-7140

Rare & T-cell lymphomas of unspecified origin

T-cell lymphomas represent approximately 40% of all non-Hodgkin's lymphoma in children and adolescents, and more than 90% occur in the three subtypes of T-lymphoblastic lymphoma, systemic T-anaplastic large-cell lymphoma (ALCL) and PTCLs not otherwise specified [3]. Mitchell S Cairo (Columbia University, NY, USA) discussed the characteristics of each of these subtypes, recent investigations, and the novel therapies for each, such as nelarabine, vinblastine and brentuximab vedotin.

Christian Gisselbrecht (Institut d'Hématologie, Hôpital Saint Louis, Paris, France) presented data on rare T-cell lymphomas collected through a comprehensive translational program launched in 2008 by pathologists with data from the Groupe d'Etude des Lymphomes de l'Adulte (GELA), Groupe Ouest-Est des Leucémies et Autres Maladies du Sang (GOELAMS) and Société Française des Cancers de L'Enfant (SFCE) centers. The program collected over 605 frozen PTCL samples with corresponding clinical data. Preliminary results describe some major pathways important for rational use and design of new drugs. Prospective collection of *de novo*, clinical and histological material is ongoing.

Gene-expression profiling revealed a distinct molecular signature for one of the rare types of T-cell lymphoma, namely, hepatosplenic T-cell lymphomas, as described by Marion Travert (Hôpital Henri Mondor, Créteil, France) in a poster presentation. A comparison of hepatosplenic T-cell lymphoma cells with normal γδ T cells found overexpression of genes encoding NK cell-associated molecules (*NCAM1*), oncogenes (*MAFB*, *FOS* and *JUN*), multidrug-resistance molecules (*ABCB1*), tyrosine kinases (*SYK*) and others. Among the dowregulated genes were those associated with activated cytotoxic molecules, tumor-suppressor genes (*AIM1*) and CD5.

CD30+ T-cell lymphomas

Lauren C Pinter-Brown (Geffen School of Medicine at UCLA, CA, USA) discussed CD30+ diseases of the skin, including the newly recognized entity of ALCL surrounding breast implants. Primary breast lymphomas are extremely rare, representing 0.04–0.05% of all breast malignancies. Of these, less than 5% are T-cell lymphomas. Of the 36 reported cases of NHLs associated with breast implants, 81% were ALCLs, and 100% of the cases with anaplastic lymphoma receptor tyrosine kinase (ALK) data were ALK negative. The implants had been in place for an average of 6.9 years. One reason for the association may be the textured surface of the implants, both saline and gel. Study of implants removed for various reasons shows that most cases are localized to the capsule and/or a seroma within the fibrous capsular space.

Max Robinowitz (Division of Immunology and Hematology Devices, US FDA) expanded on the FDA announcement that coincided with the Forum. He said that the FDA is working with the American Society of Plastic Surgeons and many of the clinical experts at the forum to establish a patient registry of ALCL associated with breast implants. ALCL should be suspected if a patient develops a late seroma, swelling, asymmetry or contracture adjacent to her breast implant years after the breast implant incision is fully healed.

Barbara Pro (Fox Chase Cancer Center, PA, USA) reported the interim results of a Phase II study of brentuximab vedotin (SGN-35) in 58 relapsed or refractory systemic ALCL patients. The overall response rate (ORR) by an independent review panel was 86%, with 53% complete remissions. Median duration of response and survival has not yet been reached. Brentuximab vedotin resulted in complete remissions (CRs) in 50% of both ALK-negative and -positive patients. Adverse events were manageable, including peripheral neuropathy, and the investigators concluded that brentuximab vedotin is a promising new agent for CD30+ malignancies.

New treatment strategies

Adult T-cell leukemia-lymphoma (ATL) is associated with human T-cell lymphotropic virus type 1 and has the worst prognosis among various PTCLs according to the International T-Cell Lymphoma Project [4]. Southwestern Japan has the highest prevalence of human T-cell lymphotropic virus type 1 infection and the highest incidence of ATL in the world. Among the new agents targeting ATL is KW-0761, a humanized anti-CCR4 antibody, which produced an overall response rate of 31% in a Phase I study [5]. A Phase II study is underway. KW-0761 is also being studied in combination with VCAP–AMP–VECP. The latter chemotherapy regimen followed by allogeneic stem cell transplant is also being evaluated for untreated aggressive ATL. In addition, a Phase III study is being planned to compare zidovudine plus IFN-α with watchful waiting for indolent ATL.

In a poster presentation, Madeleine Duvic (University of Texas MD Anderson Cancer Center, TX, USA) presented the preliminary results of a Phase II study of KW-0761 in cutaneous T-cell lymphoma and PTCL, excluding ATL. Of 32 evaluable patients, the ORR was 38%, with three patients achieving CR.

New agents & rational combinations

A plethora of new drugs and combinations have been or are being developed, representing a crucial step forward in the treatment of PTCL. Among them are the histone deacetylase inhibitors (HDIs), particularly panobinostat, belinostat, romidepsin and vorinostat. Susan E Bates (National Cancer Institute, MD, USA) pointed out that while the HDIs are similar, they are not the same, having different affinities for the histone deacetylases. She discussed the remarkable sensitivity of HDIs in T-cell lymphoma, and described the potential relationship with the increased expression of pro-apoptotic proteins with inhibitors of cell cycle progression due to direct effects on chromatin.

Purine nucleoside analogs are another class of novel agent for T-cell lymphomas, and Duvic presented information on these promising agents, in particular, gemcitabine, pentostatin, fludarabine, nelarabine and the newly synthesized forodesine. A dose-escalating Phase I/II trial of oral forodesine achieved an ORR of 39%, with acceptable safety profiles in cutaneous T-cell lymphoma patients. A Phase II trial in 120 patients has been completed.

Steven M Horwitz (Memorial Sloan–Kettering Cancer Center, NY, USA), in addition to a discussion of folate analogs, presented a poster on the results of a Phase II study of romidepsin in progressive

or relapsed PTCL following prior systemic therapy. The ORR was 26% as confirmed by an independent review committee, with 13% of the 130 patients achieving CR. The median duration of response was 12 months for the entire responding population.

Transplantation

Stem cell transplantation is potentially a curative treatment option for patients with relapsed/refractory PTCL and for those with poor prognosis subtypes. Foss reviewed the role of reduced-intensity (RI) regimens in allogeneic transplant, which has not been fully explored in PTCL. In a registry review of data reported to the Center for International Blood and Marrow Transplant Research on RI transplants performed between 1996 and 2005, there were no significant differences in the transplant-related outcomes when comparing reduced conditioning with myeloablative [6]. A retrospective study by the Société Française de Greffe de Moëlle et de Thérapie Cellulaire (France) found no impact of conditioning regimen on overall or event-free survival [7]. In addition, in a series reported by the European Group for Blood and Marrow Transplantation in patients with relapsed and refractory AITL, there was again no difference between ablative and RI conditioning in terms of overall survival and progression-free survival [8]. Other trials are being planned using RI regimens.

Andrei R Shustov (Fred Hutchinson Cancer Research Center, WA, USA) discussed who should receive autologous transplantation and when in T/NK-cell lymphomas. After reviewing the evidence from a number of retrospective and prospective clinical trials, Shustov concluded that high-dose therapy (HDT) with autologous hematopoietic cell transplant (aHCT) is feasible in primary therapy of T/NK-cell lymphoma, but current evidence does not support the routine use of HDT–aHCT in all patients, although relapses eventually occur in the majority of them. Shustov said that patients with AITL might benefit from upfront HDT–aHCT, but patients with high-risk PTCL might not benefit and should be treated in clinical trials. HDT–aHCT might be appropriate for patients who achieve a partial remission after initial therapy. Prospective randomized trials are needed to define the role of HDT–aHCT in T/NK-cell lymphoma.

Financial & competing interests disclosure

Kensei Tobinai has received grant/research funding from Celgene, Kyowa Hakko Kirin, Merck, Mundipharma, Novartis and Pfizer. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Kensei Tobinai acknowledges the support of Erilyn B Riley, Hemedicus, Inc., for writing assistance, funded by Jonathan Wood & Associates.

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Review article

Peripheral T-cell lymphoma

Francine M. Foss,[1] Pier Luigi Zinzani,[2] Julie M. Vose,[3] Randy D. Gascoyne,[4] Steven T. Rosen,[5] and Kensei Tobinai[6]

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Peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of clinically aggressive diseases associated with poor outcome. Studies that focus specifically on PTCL are emerging, with the ultimate goal of improved understanding of disease biology and the development of more effective therapies. However, one of the difficulties in classifying and studying treatment options in clinical trials is the rarity of these subtypes. Various groups have developed lymphoma classifications over the years, including the World Health Organization, which updated its classification in 2008. This article briefly reviews the major lymphoma classification schema, highlights contributions made by the collaborative International PTCL Project, discusses prognostic issues and gene expression profiling, and outlines therapeutic approaches to PTCL. These include the standard chemotherapeutic regimens and other modalities incorporating antifolates, conjugates, histone deacetylase inhibitors, monoclonal antibodies, nucleoside analogs, proteasome inhibitors, and signaling inhibitors. As this review emphasizes, the problem has now evolved into an abundance of drugs and too few patients available to test them. Collaborative groups will aid in future efforts to find the best treatment strategies to improve the outcome for patients with PTCL. (*Blood.* 2011;117(25): 6756-6767)

Introduction

Peripheral T-cell lymphomas (PTCL), a subdivision of T-cell non-Hodgkin lymphomas (NHLs) and distinct from the more common cutaneous T-cell lymphomas, are a diverse group of disorders that, for the most part, carry a poor prognosis. Classification of PTCL is complex, has resulted in many classification schemes, and has been further hampered by a paucity of molecular markers. Older lymphoma classification systems include the Rappaport system, which was used until the mid 1970s,[1] the Kiel system, introduced in 1974,[2] and the National Cancer Institute's Working Formulation, introduced in the 1980s.[3] The work of Lukes, Collins, and Lennert in the 1970s first suggested that the T-cell lymphomas should be identified as distinct from B-cell lymphoma, but this was initially met with skepticism. In 1994, the International Lymphoma Study Group proposed the REAL classification (revised European-American Classification of Lymphoid Neoplasms), which featured the major histologic, immunologic, and genetic characteristics of B- and T-cell neoplasms and Hodgkin lymphoma.[4] A clinical evaluation of the International Lymphoma Study Group classification of NHL published in 1997 concluded that the classification could be readily applied and identified clinically distinctive types of NHL.[5]

The International Lymphoma Epidemiology Consortium (InterLymph) proposed in 2007 a nested classification of lymphoid neoplasm subtypes.[6] They based their classification on the World Health Organization (WHO) classification of lymphoid neoplasms[7] and the *International Classification of Diseases for Oncology* (3rd ed).[8] The fourth edition of the *WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues* was published in 2008.[9] The new WHO classification includes many additional subtypes of PTCL (Table 1); nevertheless, it does not necessarily help clinicians determine the best therapeutic strategy for each specific subtype. The International PTCL Project is a collaborative effort that was designed to gain better understanding of the distribution and outcomes of aggressive T-cell lymphomas. A total of 22 institutions and more than 1300 eligible patients in North America, Europe, and Asia participated in and submitted clinical and pathologic information on PTCLs diagnosed and treated at their respective centers.[10]

Treatment advances in PTCLs have been slow compared with other lymphomas. Very often, PTCL patients are treated with the same therapy used in B-cell lymphomas, but this approach generally has a very poor outcome. Relapse is common after the administration of most currently available agents, and there are few effective options for salvage therapy. In addition, only small numbers of patients have each type of PTCL, which further complicates studying new treatments for these diseases in clinical trials.

Classification

The WHO classification system classifies the mature T-cell lymphomas into 4 groups based on their clinical features. Most of the aggressive T-cell lymphomas are included within the nodal, extranodal, and leukemic groups.

The nodal lymphoma group includes PTCL, not otherwise specified (NOS), accounting for 25.9% of cases,[10] anaplastic large cell lymphoma (ALCL), and angioimmunoblastic T-cell lymphoma (AITL). ALCL is further separated into the ALK^+ and ALK^- entities. The ALK^- ALCLs are morphologically and immunophenotypically similar to ALK^+ ALCL but lack ALK expression and have distinctive molecular features.[11] According to the International PTCL study, ALK^+ ALCL accounts for 6.6% and ALK^- ALCL for 5.5% of PTCL cases.[10] The cutaneous ALCLs are

Submitted May 3, 2010; accepted March 16, 2011. Prepublished online as *Blood* First Edition paper, April 14, 2011; DOI 10.1182/blood-2010-05-231548.

Table 1. Old and new WHO classifications of PTCLs

Old WHO classification[7]	New WHO classification[9]
Precursor T-cell lymphoma	
T-lymphoblastic lymphoma/leukemia	
Mature T-cell lymphomas	
T-cell prolymphocytic leukemia	T-cell prolymphocytic leukemia
T-cell granular lymphocytic leukemia	T-cell large granular lymphocytic leukemia
Aggressive NK-cell leukemia	Aggressive NK-cell leukemia
	Indolent large granular NK-cell lymphoproliferative disorder (provisional)
ATL/adult T-cell leukemia (HTLV1+)	ATL/adult T-cell leukemia
Extranodal NK-/T-cell lymphoma, nasal type	Extranodal NK-/T-cell lymphoma, nasal type
Enteropathy-type T-cell lymphoma	EATL
Hepatosplenanic T-cell lymphoma	Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma	Subcutaneous panniculitis-like T-cell lymphoma (αβ only)
	Primary cutaneous γδ T-cell lymphoma
Mycosis fungoides/Sézary syndrome	Mycosis fungoides/Sézary syndrome
Anaplastic large-cell lymphoma, systemic or cutaneous	ALCL: ALK+
	ALCL: ALK− (provisional)
PTCL, unspecified	PTCL, NOS
AITL	AITL
	Primary cutaneous CD30+ T-cell LPD
	LYP and primary cutaneous ALCL
	Primary cutaneous CD4+ small/medium T-cell lymphoma (provisional)
	Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma (provisional)
	Systemic EBV+ T-cell LPD of childhood
	Hydroa vacciniforme-like lymphoma

The WHO classification for PTCLs was updated in 2008. The new classification expanded some existing disease types and added several new provisional diseases.

WHO indicates World Health Organization; PTCL, peripheral T-cell lymphoma; NK, natural killer; HTLV1, human T-lymphotropic virus type 1; EATL, enteropathy-associated T-cell lymphoma; and ALCL, anaplastic large cell lymphoma; AITL, angioimmunoblastic T-cell lymphoma; and NOS, not otherwise specified.

included as a separate disease entity because their treatment and prognosis are distinct from the systemic ALCLs. AITL is the second largest category, accounting for 18.5% of cases in the International PTCL Project.[10] The lymphoepithelioid cell variant appears to be distinct from the others and is usually characterized by CD8+ T cells and a predominance of epithelioid cells in the background.[12]

The extranodal group includes a number of less common entities described primarily by their tissue tropism. Hepatosplenic γδ T-cell lymphoma is a disease of children and young adults,[13,14] accounting for 1.4% of PTCL cases.[10] This tumor is derived from immature or nonactivated γδ T cells, which infiltrate the liver, spleen, and bone marrow sinusoids. A characteristic chromosomal abnormality is isochromosome 7q, and patients with hepatosplenic T-cell lymphoma often have a poor outcome with a median survival of less than 2 years. The disease is more common in young males who often present with B-symptoms and cytopenias. The cells are typically CD2+, CD3+, CD4−, CD5−, CD7+, CD8+, or CD8− and may express natural killer (NK) markers as well.

Enteropathy-associated T-cell lymphoma (EATL) accounts for 4.7% of cases of T-cell lymphoma.[10] EATL is more common in geographic areas with a higher incidence of celiac disease, as evidenced by the fact that it represents 5.8% and 9.1% of the PTCLs in North America and Europe, respectively, but only 1.9% of those in Asia. The disease often presents with pain, weight loss, and bowel perforation. There are 2 morphologic variants that are the pleomorphic type, associated with celiac disease and usually CD3+, CD7+, and CD56−, and the monomorphic type, which is CD56+ and often not associated with celiac disease.[15] Chromosomal abnormalities found in EATL include gains at chromosome 9q33-q34 in up to 70% of cases.[16] The intestinal T- and NK-cell lymphomas diagnosed in Asian countries are associated with Epstein-Barr virus (EBV) infection and are part of the spectrum of nasal-type NK-/T-cell lymphoma.[17] The mucocutaneous γδ T-cell lymphomas,[18] the nasal type NK-/T-cell lymphomas, and even ALCLs can present as an intestinal lymphoma.[19,20]

Panniculitis-like T-cell lymphomas constitute only 0.9% of PTCLs, and in the International PTCL Project were more common in men (75% of cases) than in women. Patients usually present with subcutaneous nodules that may become necrotic. The neoplastic cells are typically CD3+, CD4−, and CD8+, with either T-cell receptor (TCR)–α/β+ or TCR-γ/δ+ and infiltrate in a rim-like fashion around the fat cells. The γδ panniculitis-like T-cell lymphomas have now been reclassified as cutaneous γδ T-cell lymphoma because their outcome is significantly inferior to that of patients with the αβ type.[21]

The leukemia group consists of adult T-cell lymphoma (ATL) associated with human T-lymphotropic virus type I (HTLV-1), T-cell chronic large granular lymphocytic (LGL) leukemia, aggressive NK-cell leukemia, and T-cell prolymphocytic leukemia. Most patients with LGL leukemia have an indolent disorder that is often associated with neutropenia and can be treated with immunosuppressive agents, whereas those with aggressive NK-cell leukemia and ATL often have a poor outcome.

Several other types are delineated in the WHO classification: hydroa vacciniforme-like lymphoma, which is usually of a T-cell origin, NK-cell lymphoma caused by mosquito bite allergy, and systemic EBV-positive T-cell lymphoproliferative disease of childhood, which is part of the spectrum of chronic, active EBV infection.

Incidence of T-cell lymphomas

Based on the United States Surveillance, Epidemiology, and End Results registry, the incidence of PTCL is < 1 case per 100 000 people in the United States.[22] Results from the International PTCL Project showed that, around the world, the most common subtypes are the nodal T-cell lymphomas, with PTCL-NOS (25.9%) being the most frequent, along with AITLs (18.5%) and the ALCLs (12%). NK-/T-cell lymphomas composed 10.4%, whereas enteropathy-associated T-cell lymphomas (4.7%), hepatosplenic T-cell lymphomas (1.4%), and panniculitis-like T-cell lymphomas (0.9%) were rare, even in this large study. Among the common subtypes, there are regional differences in frequency. PTCL-NOS is more common in North America and less common in the European and Asian countries; AITL is more common in Europe than in Asia or North America; ALK+ ALCL is more common in North America; ALK− ALCL is slightly more common in Europe; and the NK-/T-cell lymphomas and ATL are more common in Asia.[23] The epidemiology of EATL is closely associated with the human

leukocyte antigen DQ, which results in either overt or silent celiac disease, a European disease.[24] The EBV-associated lymphoproliferative, T-cell, and NK-cell neoplasms are seen mainly in Japan, Korea, and Northern China, but also in Native American populations from Central and South America. NK-cell nasal and nasal-type lymphomas, occurring more frequently in Asia and Latin America, have a greater incidence in males than in females.

Viral associations with T-cell lymphomas

The increased incidence of T-NK-cell lymphomas in East Asia related to the frequency of endemic HTLV-1 and EBV infections. The HTLV-1 virus was first identified by Gallo's group at the National Cancer Institute from a cell line established from a patient with cutaneous T-cell lymphoma.[25] At the same time, Yoshida et al in Japan identified a retrovirus that was immunoreactive to sera from patients with T-cell leukemia.[26] The unique genome structure of HTLV-1 identified it as distinct from any other animal retroviruses, thus founding a new retroviral group. Other members of this viral group include HTLV-2, STLV, and BLV. Viral transmission is thought to require the transfer of live infected T lymphocytes, that is, T cells in breast milk, T cells in semen, and fresh T cells in blood carriers of HTLV-1 proviruses.

HTLV-1-associated lymphoma

HTLV-1-associated lymphomas include ATL, smoldering ATL, which is characterized by small numbers of circulating leukemia cells without nodal involvement, lymphomatous ATL, which presents with lymphadenopathy without leukemic involvement, and chronic ATL, which is characterized by skin lesions, leukemic, nodal, and visceral disease without hypercalcemia, gastrointestinal involvement, bone, or central nervous system disease. The geographic distribution is reflective of the areas where HTLV-1 infections are prevalent, specifically Japan and the Caribbean basin. ATL is characterized by a clonal expansion of $CD4^+$ T lymphocytes frequently associated with skin rash, lymph node and visceral involvement, and hypercalcemia. It is suggested that the transformation of T cells in the early stages of ATL is mediated by HTLV-1 Tax protein and persistent nuclear factor-κB activation.

Angioimmunoblastic T-cell lymphoma

AITL shows distinctive features, with a polymorphous infiltrate containing medium-sized neoplastic cells, prominent arborizing blood vessels, proliferation of follicular dendritic cells, and scattered EBV^+ B-cell blasts.[27] Recent studies indicate that AITL is derived from the unique T-cell subset located in the germinal center, which are called the follicular helper T cells (T_{FH}).[28-31] Viruses have been implicated in the transformation of these T_{FH} cells. Besides EBV, which is found in B-cell blasts and is likely to play a role in the development of an EBV-associated B-cell lymphoma in some AITL patients, HHV6B, another human herpes virus, has been reported in approximately half of AITL cases. Although viral infection most probably reflects the underlying immune dysfunction, it could also suggest that EBV and/or HHV6B may modulate cytokines, chemokines, and membrane receptors.

NK-/T-cell lymphoma

NK-cell lymphomas include extranodal NK-/T-cell lymphoma, nasal type, blastic NK-cell lymphoma, and aggressive NK-cell leukemia account for 10.4% of PTCL cases. Most NK-cell lymphomas have been recognized and are included in the classification of lymphoid neoplasms, but some other NK-cell lineage neoplasms are not yet categorized by the WHO. These include myeloid/NK-cell precursor acute leukemia, precursor NK-cell acute lymphoblastic leukemia, and chronic reactive NK-cell lymphocytosis.[32] EBV has been implicated and found in the tumor cells of nasal/NK-cell lymphomas and aggressive NK-cell leukemia.[33] However, there may be other causative factors. In one recent study from China, the occurrence of epithelial carcinomas in 10 of 23 cases of NK-/T-cell lymphoma of the oral cavity suggests that other oncogenic factors may be implicated as well as EBV.[34]

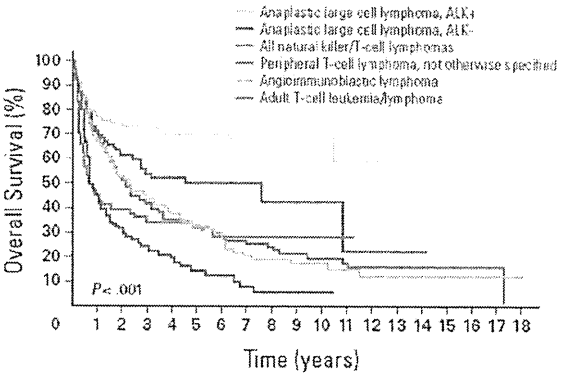

Figure 1. OS of patients with the common subtypes of PTCL. Data from Vose et al[10] with permission.

Prognostic features of T-cell lymphomas

Overall, patients with PTCL had a very poor outcome compared with patients with aggressive B-cell lymphomas.[10] The International T-cell Lymphoma Study demonstrated that the overall survival (OS) and failure-free survival (FFS) with PTCL-NOS at 10 to 15 years was 10% (Figure 1). The International Prognostic Index (IPI), when applied to patients with aggressive T-cell lymphomas, identified a group with high IPI score who had an adverse outcome compared with patients with low IPI, similar to diffuse large B-cell lymphoma.[35] However, even patients in the best prognostic category did not have a favorable outcome, and patients in the highest risk category had very short survival. When examined by histopathologic subset, the 5-year OS for patients with PTCL-NOS and AITL with IPI of 0 to 1 were 56% and 50%, respectively, whereas those with IPI of 4 or 5 were 11% and 25%, respectively. For patients with ALCL, the 5-year survivals for IPI 0 to 1 patients were excellent at 90% and 74% for ALK^+ and ALK^- patients, respectively; however, patients with IPI of 4 to 5 had a poor outcome with 5-year survivals of 33% and 13%, suggesting that IPI is an important predictor, even in ALK^+ patients.[23] The IPI has not been predictive of outcome in patients with ATLL, enteropathy-associated and hepatosplenic T-cell lymphoma, or extranasal NK-/T-cell lymphoma.

A new prognostic index specifically designed for PTCL,[36] the prognostic index for PTCL (PIT), is similar to the IPI, including age, lactate dehydrogenase, performance status, and then bone marrow involvement. When applied to patients with PTCL-NOS, the index separated patients into more specific prognostic groups than the IPI. Of 322 patients studied, 20% had no adverse features, 34% had one feature, 26% had 2 features, and 20% had 3 or more.

Table 2. Biologic factors and outcome in PTCL

Variable	Prognostic significance
p53	Worse
Ki-67	Worse
BCL-2, BCL-XL	Worse
CD26	Worse
EBV	Worse
MDR	Worse
CCND2	Worse
CCR4	Worse
NF-κB	Favorable
CCR3	Favorable
CXCR3	variable
PRDM1	Worse
ALK-1	Favorable
TCR BF1	Favorable
TCR gamma1	Worse

TCR indicates T-cell receptor.

The 5-year OS for the most favorable group with no adverse prognostic features was 62% compared with 18% for patients with 3 or 4 adverse prognostic factors.

Aside from conventional histopathology, chemokine expression and proliferative signature have been shown to have prognostic significance, including p53 and Ki-67 (Table 2). In one study, p53 was the most important prognostic factor and was correlated with expression of P-glycoprotein, which confers resistance to chemotherapy.[37] In addition, 40% to 60% of patients with PTCL express BCL2- and $BCLX_L$-associated proteins.[38] ALK^+ ALCL is usually BCL2 negative, whereas other subtypes with worse outcomes may be $BCL2^+$.

Two chemokine receptors, CXCR3 and CCR4, were found to be expressed in 63% and 34% of PTCL-NOS cancers, respectively.[8] The dominant chemokine expression found in this study was CXCR3-positive/CCR4-negative; this phenotype was shown by multivariate analysis to be an independent prognostic factor and significantly prognostic of a poor prognosis in both PTCL-NOS and ALK-negative ALCL.

In a study reported by Rudiger et al of PTCL-NOS category, which excludes AITL, the presence of > 70% transformed blasts, > 25% Ki67 proliferation, CD56 and CD30 expression, EBV infection, and a background of > 10% $CD8^+$ cells were all adverse prognostic factors.[39] The results indicated that immunophenotyping is absolutely necessary to determine prognosis. In another study, multivariate analysis of all prognostic factors indicated that, on controlling for IPI, if the number of transformed cells is > 70%, the hazard ratio for OS is 2.2 and 1.6 for FFS.[40] In patients with ALCL, the presence of the small cell variant can be associated with a worse prognosis. Finally, the expression of cytotoxic molecules also has an adverse impact on survival.[41]

Gene expression, cytogenetic and comparative genomic hybridization data

Several studies of gene expression profiling of PTCLs have been published. Importantly, expression profiling of purified normal T-cell subpopulations has provided the framework for understanding cell-of-origin and functional properties of T-cell malignancies.[42-47] In aggregate, these studies have helped define the normal counterpart of several specific PTCLs, identified pathways frequently altered in PTCL, including nuclear factor-κB signaling and cell cycle deregulation, and highlighted the importance of proliferation as a potential biomarker for prognosis.[48-57] However, most of these studies are largely underpowered to allow definitive statements regarding survival prediction. Large sample sizes will be needed to build molecular outcome predictors in PTCL and will probably require international collaboration.

More recently, gene expression profiling studies of larger numbers of patients have been undertaken and do provide novel insights into several PTCL entities.[58,59] Iqbal et al studied 144 cases and were able to confirm several previous observations.[58] In addition, they were able to show a major role for the microenvironment in AITL and developed a 15-gene outcome predictor highly correlated with survival and independent of the IPI. Similarly, they discovered a subgroup of PTCL-NOS with cytotoxic characteristics and inferior survival. Huang et al studied a much smaller number of extranodal NK-/T-cell lymphomas, nasal type by combining microarray analysis with array comparative genomic hybridization.[59] These authors established marked differences in gene expression in these tumors compared with normal NK cells, particularly the significant overexpression of granzyme H; provided evidence for overexpression of PDGFRA similar to PTCL-NOS; and suggested a possible role for the novel tumor suppressor gene, HACE1, mapping to the 6q21 region, in NK-/T-cell lymphomas.

Classic cytogenetic studies have also provided insight into copy number alterations in PTCL (Table 3).[60] A recent study of PTCL-NOS, AITL, and ALK^- ALCL revealed recurrent alterations associated with specific entities, including gains of 5q, 21, and 3q in AITL, commonly associated with trisomies of chromosome 5 and 21. Loss of genetic material involving chromosome 6q was also frequent. ALK^- ALCL was characterized by gains of 1q and 3p and losses of 16pter, 6q13-21, 15, 16qter, and 17p13. PTCL-NOS showed frequent gains of 7q22-31, 1q, 3p, 5p, and 8q24qter and losses of 6q22-24 and 10p13pter. Cases with a complex karyotype had shorter OS.

Although many subtypes of PTCL are characterized by clonal expansion of TCR rearranged lymphocytes, this may not be a characteristic of all cases of NK neoplasms. Two studies recently examined the lack of TCR rearrangements in these patients and reported rearrangements at the TCR locus in 0.8% to 1.2% of cases.[61,62]

Comparative genomic hybridization was used to examine genomic alterations in patients with ALK^+ and ALK^- ALCL. A study of 74 patients showed that ALK^+ ALCL samples had gains of 17p and losses of 4q13-q21 and 11q14, whereas gains of 1q and 6p21 were more frequent in ALK^- samples.[11] Another study, which used genomic profiling in patients with PTCL-NOS (n = 42) and ALK^- ALCL (n = 37),[63] showed that genetics of PTCL-NOS and ALK^- ALCL differ substantially from other T-cell lymphomas, including EATL, T-cell prolymphocytic leukemia, and ATL. Those with PTCL-NOS had recurrent chromosomal gains, high-level amplifications, and recurrent chromosomal losses on 13q, 6q, 9p, 10q, 12q, and 5q. Cutaneous ALCL and ALK^+ ALCL did not show imbalances, whereas ALK^- ALCL showed chromosomal gains of 1q and losses of 6q and 13q.

Lastly, it is probable that significant progress in our understanding of the genetic complexity of PTCL will require an international effort to study a number of well-annotated cases using next-generation sequencing strategies.[64] It can safely be hypothesized that this approach will yield important information about recurrent mutations and novel fusions that underlie the critical events in the biology of these tumors. Matched constitutional DNA will be required to properly annotate all of the somatic changes.

Table 3. Cytogenetics of PTCL subtypes

	TCR	Chromosomal/histopathologic features	Distinguishing features	5-year survival, %
PTCL-NOS	αβ	Loss 13q22.3 adverse prognosis; gains 8q,9p,19q,loss 3q,9p	Heterogeneous, variable morphology	20-30
AITL	αβ	Follicular dendritic cell signature CXCL13$^+$, PD-1$^+$; gains 2,5,13q22.3 adverse prognosis	Immunodeficiency and immune dysregulation Helper B cells may respond to cyclosporine	32
ALCL ALK$^+$	αβ	(2,5) translocation; gain 1q, loss6q, 13q	Bone marrow involvement in only 29%, median age 34 y	70
ALK ALK$^-$	αβ	Pax 5$^-$, CD15$^-$	Median age 58 y	49
NK-/T-cell		CD56$^+$, often EBV$^+$	Stage I/II patients respond to radiotherapy or radiochemotherapy Serum EBV copy number predictive of outcome	Nasal type 64 Extranasal type < 20
SPTCL- αβ SPTCL- γδ	Aβγδ	CD3$^+$CD8$^+$CD56$^-$ 5q,13q gains CD3$^+$CD8$^-$ CD56$^{+/-}$	Nodules and plaques nodules, often ulceration Hemophagocytic syndrome	82; 11
Hepatosplenic T-cell lymphoma	γδ	Isochromosme 7q, CD3$^+$, CD4$^-$CD8$^-$, may be CD56$^+$	Infiltration of sinusoids in liver, spleen, bone marrow Erythrophagocytosis	2-year OS 20
EATL	αβ	Gains at chromosome 9q33-q34 CD3$^+$ CD7$^+$, may be CD8$^+$ CD56$^+$ with monomorphic type	Associated with celiac sprue, malabsorption	4

PTCL indicates peripheral T-cell lymphoma; TCR, T-cell receptor; NOS, not otherwise specified; AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; NK, natural killer; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; and EATL, enteropathy-associated T-cell lymphoma.

Therapeutic approaches

Standard first-line therapy

T-cell lymphomas have traditionally been treated much like the B-cell lymphomas, with a combination chemotherapy regimen. CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) was the most widely used, although there were no randomized studies that proved that it was the best therapy. A retrospective meta-analysis of 2912 patients treated with CHOP or CHOP-like regimens reported a 5-year OS of 37%.[65] Several groups have tried more intensive chemotherapeutic treatments for PTCL patients. The French Groupe d'Etude des Lymphomes de l'Adulte (GELA) used more intensive chemotherapy: ACVBP (dose-intensified doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone) plus consolidation with autologous stem cell transplantation (ASCT) for younger, fitter patients.[66] The German High Grade Non-Hodgkin Lymphoma Study Group used several variations of CHOP,[67,68] and an Italian group used high-dose sequential chemotherapy with ASCT.[69] The M. D. Anderson Cancer Center used an alternating triple therapy regimen and hyper-CVAD (fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone).[70] The GELA demonstrated that ACVBP had higher toxicity but superior event-free survival and OS compared with CHOP.[71] CHOP-14 proved superior to CHOP-21 or CHOP plus etoposide in elderly patients,[68] and CHOP plus etoposide should be used in younger patients.[67]

Anthracycline-containing regimens for PTCL have been associated with median 5-year OS rates < 40%.[65] ALCL patients have better outcomes than non-ALCL patients, with an overall response rate (ORR) > 75% and 5-year OS rates > 60%.[23] ALK$^+$ ALCL patients have slightly higher OS and FFS rates compared with ALK$^-$ ALCL patients, but this may be the result of a difference in age, as ALK$^+$ patients tend to be younger than ALK$^-$ patients. However, even in ALK$^+$ ALCL patients, 5-year FFS rates were only 49%, suggesting a definite need for new therapies or different subtype-specific treatments. It is unclear why patients with aggressive T-cell lymphomas are less responsive to conventional B-cell lymphoma regimens, but data are emerging regarding the expression of P-glycoprotein and other drug resistance pathways in subsets of patients, particularly patients with NK-/T-cell lymphomas.

Novel approaches

New therapeutic approaches and the incorporation of novel agents into these therapeutic regimens are necessary to improve the outcome for PTCL patients. Table 4 lists some of the new agents that are currently being used or are in clinical trials.

Monoclonal antibodies and immunoconjugates

The addition of the anti-CD20 monoclonal antibody rituximab to chemotherapy regimens, such as CHOP, has significantly improved treatment outcomes in B-cell lymphoma. As such, several monoclonal antibodies and targeted immunoconjugates and fusion proteins are currently being tested in PTCL, including alemtuzumab, iratumumab, siplizumab, zanolimumab, denileukin diftitox, and brentuximab vedotin.

Alemtuzumab, an anti-CD52 monoclonal antibody, has been shown to have activity in heavily treated patients with PTCL with an ORR of 36% in one early study.[72] Alemtuzumab has been used in combination with CHOP or EPOCH (etoposide, prednisone, vincristine, cyclophophamide, and doxorubicin) with some success in PTCL, with half of patients achieving a complete response (CR).[73,74] Another study compared combinations of alemtuzumab with CHOP, EPOCH, and IMVP16-PL (ifosfamide plus mesna, methotrexate, etoposide, and prednisone),[75] and a third study used alemtuzmab with CHOP and ESHAP (etoposide, methylprednisolone, high-dose cytarabine, and cisplatin) in newly diagnosed PTCL patients.[76] Although overall response rates have been high, there has been an increased risk of opportunistic infections associated with the immunosuppressive effects of alemtuzumab. Patients on these trials have required routine surveillance for reactivation of cytomegalovirus. A randomized trial comparing CHOP-14 to alemtuzumab-CHOP 14 is currently underway as a European intergroup study.

Two anti-CD30 monoclonal antibodies, iratumumab and SGN-30, have shown efficacy in CD30$^+$ ALCL.[77,78] In vitro studies have shown that both drugs are synergistic or additive with conventional chemotherapy.[79-81] An immunoconjugate of SGN-30 and monomethyl auristatin, brentuximab vetodin (SGN-35), has demonstrated an ORR of 41% with a median response duration of 7.3 months in patients with relapsed and refractory CD30$^+$ lymphomas.[82] A study is currently underway to evaluate the combination

Table 4. Novel agents in use or trials in PTCL

Type of agent	Name	Description	Disease(s)	Status
Antifolates	Pralatrexate	10-Deazaminopoterin	PTCL, CTCL	Approved for PTCL
Conjugates	LMB-2	Anti-Tac) anti-CD25 fused to Pseudomonas toxin	CTCL, PTCL (especially ATLL)	Phase 2
	Denileukin diftitox	IL-2 targeting domain fused with diphtheria toxin	CTCL, PTCL	Approved for CTCL
	Brentuximab vedotin	CD30 antibody conjugated to monomethylauristan-E	CD30+ T-cell lymphoma	Phase 2
HDAC inhibitors	Belinostat	PXD101	CTCL, PTCL	Phase 2
	Panobinostat	LBH589	CTCL, ATL	Phase 2
	Romidepsin	Depsipeptide	CTCL, PTCL	Approved for CTCL
	Vorinostat	Suberoylanilide hydroxamic acid	CTCL	Approved for CTCL
Immunomodulatory agents	Lenalidomide	Derivative of thalidomide	PTCL, CTCL	Phase 2
Immunosuppressive agents	Cyclosporine	Inhibitor of the NF-AT transcription complex	AITL	Phase 2
Monoclonal antibodies	Alemtuzumab	Anti-CD52	PTCL	Phase 3
	Bevacizumab	Anti-VEGF	PTCL (especially AITL), NK-cell	Phase 2
	Iratumumab	Anti-CD30	CD30+ ALCL	Phase 1/2
	KW-0761	Anti-CCR4	ATLL, PTCL	Phase 2
	SGN-30	Anti-CD30	CD30+ ALCL	Phase 2
	Siplizumab	Anti-CD2	PTCL, NK-cell, ATLL	Phase 1
	Zanolimumab	Anti-CD4	CTCL, PTCL	Phase 2
Nucleoside analogs	Cladribine	Purine nucleoside analog	PTCL	Phase 4
	Clofarabine	Purine nucleoside analog	PTCL, NK-cell	Phase 1/2
	Fludarabine	Purine nucleoside analog	PTCL, CTCL	Phase 2
	Forodesine	Metabolic enzyme inhibitor	PTCL, CTCL	Phase 2
	Gemcitabine	Pyrimidine nucleoside analog	PTCL	Phase 2
	Nelarabine	Purine nucleoside analog	T-ALL, T-NHL	Phase 2
	Pentostatin	Metabolic enzyme inhibitor	PTCL	Phase 2
Proteasome inhibitors	Bortezomib	Proteasome inhibitor	CTCL	Phase 2
Signaling inhibitors	Enzastaurin	Selective inhibitor of protein kinase C	PTCL, CTCL	Phase 2
	R788	Syk inhibitor	PTCL	Phase 2

There are several other experimental agents in various stage clinical trials for T-cell lymphoma.

PTCL indicates peripheral T-cell lymphoma; CTCL, cutaneous T-cell lymphoma; ATLL, adult T-cell lymphoma; HDAC, histone deacetylase; NF-AT, nuclear factor of activated T cell; AITL, angioimmunoblastic T-cell lymphoma; NK, natural killer; ALCL, anaplastic large cell lymphoma; T-ALL, T-cell acute lymphoblastic leukemia; and T-NHL, T-cell non-Hodgkin lymphoma.

of CHOP with brentiximab vetodin in newly diagnosed patients with ALCL.

Siplizumab is an anti-CD2 monoclonal antibody. CD2 is an adhesion molecule highly expressed on activated T cells and NK cells and on the majority of cells from patients with T-cell lymphoma and leukemia. Siplizumab eliminated both $CD4^+$ and $CD8^+$ T cells and NK cells without affecting B cells. In a phase 1 trial in patients with $CD2^+$ lymphoproliferative disease, siplizumab showed clinical activity, inducing CRs in 2 patients with LGL leukemia, 3 partial responses (PRs) in patients with ATL, and 1 PR in a patient with cutaneous T-cell lymphoma (CTCL).[83] A subsequent dose escalation study produced a PR in a patient with NK-cell LGL and a CR in a PTCL patient.[84] However, siplizumab also predisposes patients to the development of lymphoproliferative syndrome,[85] although it may be possible to prevent that with prophylactic rituximab.

CD4 is expressed in half of all T cells and by most CTCL and nodal PTCL cells. Zanolimumab, an anti-CD4 monoclonal antibody, is being used in both disease types, although clinical development for CTCL is farther along. Zanolimumab was shown to be active and well tolerated in a study of 21 PTCL patients, with an ORR in 24% of patients.[86] Clinical studies of zanolimumab in combination with CHOP are ongoing and include a phase 1/2 dose escalation trial in patients with noncutaneous $CD4^+$ PTCL.

Bevacizumab, an antivascular endothelial growth factor (VEGF) monoclonal antibody, will most probably have the largest impact in AITL, as AITL is characterized by the overexpression of angiogenic factors, such as VEGF. At least one relapsed AITL patient has achieved a CR after treatment with bevacizumab[87] and was being studied along with CHOP in a clinical trial for patients with PTCL or NK-cell neoplasms by the Eastern Cooperative Oncology Group. However, preliminary results of this trial reported a high incidence of cardiac events related to the therapy.[88]

Denileukin diftitox, a fusion protein that combines IL-2 receptor-binding domain with diphtheria toxin, has demonstrated activity in both cutaneous and aggressive T-cell lymphomas. In a single-center phase 2 study at M. D. Anderson Cancer Center, denileukin diftitox at a dose of 18 μg/kg per day for 5 days on a 21-day cycle demonstrated a response rate of 48% in heavily pretreated patients with relapsed PTCL.[89] Responses were seen in 4 of 10 patients with PTCL-NOS, 2 of 3 with AITL, and 2 of 2 with ALCL. In this trial, the expression of CD25 by immunohistochemistry was not predictive of response to denileukin diftitox.

Based on these data, a combination of denileukin diftitox and CHOP was studied in untreated patients with PTCL. This study enrolled 49 patients. Denileukin diftitox was administered at 18 μg/kg per day on days 1 and 2 of each cycle, followed by CHOP chemotherapy on day 3, and granulocyte colony-stimulating factor support starting day 4 of each 21-day cycle.[90] In 37 efficacy-evaluable patients (> 2 cycles), the ORR was 86% (CR 75%). Median progression-free survival (PFS) was 15 months, and 2-year estimated OS was 60%. A large randomized study comparing CHOP to denileukin diftitox with CHOP is being initiated.[90]

LMB-2 is an anti-Tac (anti-CD25) single-chain monoclonal antibody conjugated to Pseudomonas toxin. LMB-2 has shown clinical activity in phase 2 trials in CLL, CTCL, and hairy cell leukemia. ATL is the PTCL subtype that is most sensitive to LMB-2, but clinical responses have been limited to do rapid

disease progression after > 95% tumor reduction and immunogenic reactions.[91] A phase 2 clinical trial for this population is being planned in which LMB-2 will be given after chemotherapy with fludarabine and cyclophosphamide.

HDAC inhibitors

Histone deacetylase (HDAC) inhibitors are potent inducers of histone acetylation, which results in the expression of tumor suppressor genes that had been previously silenced by deacetylation. This gene expression leads to cell cycle arrest and apoptosis. There are a number of HDAC inhibitors being used or studied in T-cell lymphoma, including vorinostat, romidepsin (also known as depsipeptide), panobinostat, and belinostat. Vorinostat and romidepsin have shown single-agent activity in CTCL,[92,93] and vorinostat was approved by the Food and Drug Administration (FDA) in 2006 for the treatment of advanced and refractory CTCL. Romidepsin was also recently FDA-approved in 2009 for advanced and refractory CTCL based on a demonstrated ORR in 2 clinical trials of 34%.[94] The median response duration was 15 months (range 1-20+), and median time to progression was 8.3 months in early and 6.4 months in more advanced disease. A phase 2 study of romidepsin was completed in patients with relapsed and refractory PTCL.[95] This phase 2, open-label, multiarm, multicenter study enrolled 43 PTCL patients from the National Cancer Institute and 9 extramural sites. Of 43 patients, 31 received ≥ 2 cycles of therapy. Mean number of prior therapies was 3.9 (range, 1-12). Objective response rate was 39% overall or 55% for patients who received at least 2 cycles of therapy. The overall median duration of response was 8.3 months (range, 1.6 months to 4.8+ years) for all patients. A multicenter, multinational phase 2B registration study of romidepsin at the same dose and schedule in relapsed and refractory PTCL has completed accrual and results have been recently reported. Of 130 patients with a median of 2 prior therapies, the ORR was 26% with 15% CR by radiographic documentation. The median response duration was 12 months, and toxicities included gastrointestinal and constitutional events and thrombocytopenia.

Belinostat, a hydroxamic acid-derived HDAC inhibitor, has been studied in both intravenous and oral formulations. Belinostat was administered intravenously at 1000 mg/m^2 daily for 5 days every 3 weeks in 53 patients, including 19 with refractory PTCL and 29 with refractory CTCL.[96] The objective response rate in PTCL was 32% with 2 CR and a median response duration of 8.9+ months, and 14% in CTCL, with a response duration of 9.1 months. A multicenter phase 2 registration trial of belinostat in relapsed PTCL patients is underway, and a cohort dose escalation study of oral belinostat is ongoing in patients with relapsed lymphoma.

Additive and synergistic activity has been demonstrated in vitro for combinations of HDAC inhibitor with a number of agents, including topoisomerase inhibitors, bortezomib, and cytotoxic chemotherapy drugs, and clinical trials are underway to explore the activity of these combinations in T-cell lymphomas.

Antifolates

Pralatrexate is a novel folate antagonist whose activity is associated with binding to the reduced folate carrier.[97] In a phase 1/2 dose escalation trial of pralatrexate in refractory lymphoma patients, the ORR was 31%, with response durations ranging from 3 to 26 months.[98] The response rate in that trial was 54% for patients with T-cell lymphomas. Based on these encouraging data, the PROPEL trial was initiated. In this trial, 111 patients with relapsed or refractory PTCL were treated with pralatrexate weekly for 6 weeks on a 7-week cycle. The median prior therapies was 3, and 63% of patients had no response to their last line of therapy. The ORR was 29% and the median response duration was 10.1 months. Five patients with relapsed/refractory PTCL who responded to single-agent pralatrexate went onto stem cell transplantation.[99] Toxicities included mucositis in70% of patients and thrombocytopenia in 40%. Pralatrexate was approved in September 2009 by the United States FDA as a single agent to treat relapsed or refractory patients with PTCL. A number of recent studies have explored the potential synergy between pralatrexate and other active agents in T-cell lymphoma. A phase 1 study combining pralatrexate with gemcitabine is underway.

Immunomodulators and immunosuppressants

Cyclosporine is an immunosuppressive agent that inhibits the nuclear factor of activated T-cell transcription complex, which activates the genes encoding cytokines and cell surface molecules involved in cell-to-cell communication and death.[100] Because AITL is characterized by immune dysregulation, cyclosporine was administered to 12 patients in a phase 2 trial.[101] Two-thirds (3 CRs, 5 PRs) of the patients responded, but there were 4 deaths. A phase 2 trial of cyclosporine in AITL was conducted by Eastern Cooperative Oncology Group but closed early because of slow accrual.

Other immune-modulating and antiangiogenic agents, including bevacizumab,[87] rituximab,[102] lenalidomide, and thalidomide, are also being explored as single agents and in combination with chemotherapy. A phase 2 study of lenalidomide at a dose of 25 mg/m^2 daily for 21 days of a 28-day cycle was conducted in 24 relapsed PTCL patients.[103] The overall response rate was 30% with a PFS of 95 days. Toxicities included neutropenia and thrombocytopenia in 20% and 33% of patients, respectively.

Nucleoside analogs

Nucleoside analogs are chemotherapeutic agents that primarily inhibit DNA replication and repair. Gemcitabine is the most effective pyrimidine nucleoside analog in PTCL. It has been active both as a single agent[104,105] and in combination with alemtuzumab[106] and bortezomib.[107,108] The purine nucleoside analogs include cladribine, fludarabine, clofarabine, and nelarabine. Both cladribine and fludarabine have shown efficacy in PTCL, and clofarabine and nelarabine are currently in several clinical trials in T-cell lymphoma.

The metabolic enzyme inhibitors, which include deoxycoformycin (pentostatin) and forodesine, do not incorporate into DNA, unlike the other nucleoside analogs. Pentostatin inhibits adenosine deaminase, increasing the deoxyadenosine triphosphate pool, and forodesine inhibits phosphorylase, increasing the deoxyguanosine triphosphate pool. Both agents have shown some efficacy in CTCL. A phase 1/2 study of oral forodesine in relapsed and refractory CTCL patients reported a 53% overall response rate, and a phase 2 trial has been completed.[109]

Proteasome inhibitors

Bortezomib, a proteasome inhibitor, has been well tolerated and active as a single agent in relapsed or refractory CTCL patients.[110] In a phase 2 study of bortezomib in relapsed CTCL or PTCL patients, the ORR was 67% with 2 CR and no grade 4 toxicity.[110] The GELA has conducted a phase 2 study of bortezomib with ACVBP chemotherapy in 57 untreated PTCL patients and has reported that 29 patients were withdrawn prematurely because of

toxicity.[111] The ORRs were similar to ACVBP alone. Bortezomib has been used in combination with gemcitabine plus doxorubicin,[107,108] and recent evidence shows that bortezomib may synergize with pralatrexate in T-cell lymphoma (see antifolates section above).[112]

Signaling inhibitors

Enzastaurin is a selective inhibitor of protein kinase C, which acts in part through the AKT pathway. By targeting the PI3K/AKT pathways, enzastaurin inhibits cell proliferation, induces tumor cell apoptosis, and suppresses tumor-induced angiogenesis in CTCL cell lines.[113] Enzastaurin is currently in 2 phase 2 trials: one for patients with several types of NHL, including PTCL and CTCL, and another for relapsed CTCL patients.

Treatment approaches for HTLV-1-associated ATL and NK-/T-cell lymphomas

Optimal treatment approaches for patients with NK-/T-cell lymphoma have not yet been established. A phase 1/2 study of concurrent chemoradiotherapy for untreated localized NK-/T-cell lymphoma was conducted in Japan.[114] Extranodal NK-/T-cell lymphoma, nasal type, is generally refractory to CHOP associated with a high expression of the multidrug resistance gene, P-glycoprotein. Patients in this trial were concurrently treated with radiotherapy and chemotherapy consisting of carboplatin, etoposide, ifosfamide, and dexamethasone. With a median follow-up of 32 months, the 2-year OS was 78% (95% confidence interval [CI], 57%-89%). This compared favorably with the historical control of radiotherapy alone (45%). Of the 26 patients assessable for a response, 20 (77%) achieved a CR, with one PR. The ORR was 81%. The most common grade 3 nonhematologic toxicity was mucositis related to radiation (30%). The investigators concluded that concurrent chemoradiotherapy using multidrug resistance-nonrelated agents and etoposide is a safe and effective treatment for localized nasal NK-/T-cell lymphoma.

A novel approach for patients with advanced NK-/T-cell lymphomas incorporates L-asparaginase along with ifosfamide, etoposide, dexamethasone, and methotrexate (SMILE). Overexpression of P-glycoprotein in NK-/T-cell lymphomas has contributed to relative chemoresistance and poor outcomes after CHOP-based therapies with 5-year survivals of 20% or less in patients with advanced stage disease. Yong et al treated 18 patients who were refractory to CHOP with L-asparaginase, vincristine, dexamethasone, and involved field radiotherapy and reported a response rate and 5-year OS of 55%.[115] A phase 1 study escalating methotrexate and etoposide was completed and reported a response rate of 67%. A prospective phase 2 trial has been reported with the SMILE regimen in patients with newly diagnosed stage IV or relapsed refractory NK-/T-cell lymphomas.[116] Of 39 enrolled patients, 29 (74%) completed the planned treatment. The responses were complete remission (CR) in 15, partial remission in 14, and early death because of infection in 4. ORR and CR were 74% (95% CI, 58%-87%) and 38%, respectively. The most common grade 3 nonhematologic toxicity was infection (41%).

For patients with ATL, results with conventional chemotherapy regimens have been uniformly poor. A phase 3 Japanese study evaluated a combination regimen of VCAP (vincristine, cyclophosphamide, doxorubicin, and prednisone), AMP (doxorubicin, ranimustine, and prednisone), and VECP (vindesine, etoposide, carboplatin, and prednisone) against CHOP-14 in ATL.[117] The study demonstrated superiority for VCAP-AMP-VECP for newly diagnosed aggressive ATL patients. A phase 2 study is in preparation to investigate the ability of allogeneic stem cell transplantation after induction with the VCAP-AMP-VECP regimen to prolong the median survival time, which is currently 13 months with the VCAP-AMP-VECP regimen.

The use of interferon and zidovudine has been shown to induce responses in up to 50% of patients with acute or lymphomatous ATL.[118] In a recent meta-analysis, 116 patients with acute ATL, 18 patients with chronic ATL, 11 patients with smoldering ATL, and 100 patients with ATL lymphoma were evaluated.[119] Five-year OS rates were 46% for 75 patients who received first-line antiviral therapy ($P = .004$), 20% for 77 patients who received first-line chemotherapy, and 12% for 55 patients who received first-line chemotherapy followed by antiviral therapy. The authors claimed that patients with acute, chronic, and smoldering ATL significantly benefited from first-line antiviral therapy, whereas patients with ATL lymphoma experienced a better outcome with chemotherapy. In acute ATL, 82% of patients were alive at 5 years with antiviral therapy, and 100% of patients with chronic and smoldering ATL were alive at 5 years. Multivariate analysis showed that first-line antiviral therapy significantly improved OS (hazard ratio = 0.47; 95% CI, 0.27-0.83; $P = .021$). However, considering the potential selection bias in this retrospective study, future prospective studies are needed.

Finally, a humanized anti-CCR4 antibody, KW-0761, has shown promise as a single agent in Japan for the treatment of ATL. KW-0761 was used for relapsed patients with CCR4-positive ATL and PTCL in a phase 1 study.[120] The ORR was 31% (5 of 16; 95% CI, 11%-59%). There were no dose-limiting toxicities, and no anti–KW-0761 antibodies were detected.[120] A phase 2 trial for relapsed ATL patients was recently completed. Of 27 enrolled patients (14 acute, 6 lymphomatous, 7 chronic ATL), the ORR was 54% with 7 CR. Toxicities included cytopenias (lymphopenia 96%, neutropenia 33%), skin rash (52%), and mild transaminitis.

Transplantation

Several retrospective studies suggest that there are populations of patients with PTCL that will benefit from transplantation. The National Cancer Consortium Network guideline includes transplantation as an option for consolidation after first remission in patients with histologies other than ALK$^+$ ALCL and in patients with intermediate or high IPI scores. Disease status at transplantation is a major predictor of success, particularly for autologous transplantation.[121] Results are better for patients who are in chemosensitive remission, whereas only 25% to 30% of refractory patients benefit.[122,123] In the studies where 5-year outcomes are reported, OS and FFS average 34% and 18%, respectively.[124] The single-center experience at Stanford reported only a modest benefit after autologous transplantation (5-year OS of 36%) for patients with relapsed disease and a 5-year OS of 76% in patients transplanted in first remission.[125]

A prospective study from Germany using chemotherapy and up-front autologous transplantation for PTCL has been reported. The treatment regimen consisted of 4 to 6 cycles of CHOP, followed by mobilizing therapy with either dexaBEAM (dexamethasone, carmustine, melphalan, etoposide, and cytarabine) or ESHAP (etoposide, methylprednisolone, cytarabine, and cisplatin). Patients in complete or partial remission then underwent myeloablative chemoradiotherapy and ASCT. Two-thirds (66%) of the patients had a disease response to chemotherapy and thus went on

to receive ASCT. At a median follow-up time of 33 months, the estimated 3-year OS and PFS for patients in CR were 48% and 36%, respectively. Patients who did not experience a response to chemotherapy and therefore did not undergo ASCT had a very poor outcome, with a median survival of less than 2 years.[126] The role of autologous transplantation in second or subsequent remission is less well defined.

There are currently no randomized studies comparing outcomes between autologous and allogeneic transplantation in the United States. The Nordic and German lymphoma groups are launching a large, prospective randomized trial to compare the different strategies as consolidation after first-line therapy. In addition, the Center for International Blood & Marrow Transplant Research is currently conducting a database review comparing allogeneic and autologous transplants in PTCL patients. In a retrospective single-institution study that compared autologous and allogeneic transplantation, outcomes for autologous transplantation were best when conducted in first remission, and allogeneic transplantation was better for patients with resistant or relapsed disease.[127] Further prospective studies are needed to define which subsets of PTCL patients will optimally benefit from allogeneic or ASCT.

Evidence-based treatment approaches for PTCL

Because of the inferior outcomes with CHOP-based regimens, novel strategies are needed for patients with aggressive T-cell lymphomas. The National Cancer Consortium Network has established evidence-based treatment approaches for T-cell lymphoma and stratifies patients based on stage. For early-stage patients with localized disease, chemotherapy should be followed by involved field radiotherapy. It is recommended that all patients except for those with low IPI be consolidated with ASCT. ALK^+ ALCL is identified as the one subtype that has an excellent outcome and should not be transplanted in first remission. Recent data suggest that ALK^+ patients with high IPI could be an exception to this rule. In prospective trials where up to 40% of patients do not undergo a complete remission and therefore cannot be consolidated with transplantation, new approaches are necessary.

Selection of first-line therapy based on histopathologic features has not yet been widely used but should be considered. For nodal T-cell lymphomas (PTCL-NOS, AITL, and ALCL), the standard regimen used is a CHOP-based therapy. For extranodal subtypes, regimens may be individualized. For panniculitis-like T-cell lymphoma, distinction should be made between the $\alpha\beta$ type and the $\gamma\delta$ type, which is now included in the category of cutaneous $\gamma\delta$ T-cell lymphoma. The $\alpha\beta$ patients may be treated with single-agent therapies or combination chemotherapy and generally have an excellent outcome. The cutaneous $\gamma\delta$ T-cell lymphomas overall do poorly and should be treated with aggressive chemotherapy followed by transplantation. Likewise, hepatosplenic and intestinal T-cell lymphomas have a poor outcome. In one study, 26 enteropathy-associated T-cell lymphoma patients were treated with CHOP and then methotrexate alternating with ifosfamide, etoposide, and epirubicin.[128] Patients who achieved CR went on to transplantation (n = 33). For the transplanted enteropathy-associated patients, the PFS and OS were 52% and 60%, respectively. NK-/T-cell lymphoma patients have also had inferior outcomes with CHOP-based regimens, and consideration of alternative regimens, such as SMILE and asparaginase combinations, should be strongly considered for these patients.

The role of autologous versus allogeneic stem cell transplantation in patients with poor prognosis subtypes, such as NK-/T-cell and $\gamma\delta$ T-cell lymphomas, has not been ascertained. In retrospective series, results with these subtypes are inferior to those of the more common PTCL-NOS and AITL subtypes after autologous transplantation. Therefore, consolidation with allogeneic stem cell transplantation should be considered in patients who have appropriate donors.

In conclusion, from this summary of the current state of classification, prognosis, and therapeutic approaches in PTCL, it is apparent that much progress has been made. Although the problem with PTCL treatment in the past was a lack of available therapies, the development of novel therapies necessitates the development of paradigms to combine these agents to improve response rates and durability of responses. Based on the success of the chronic lymphocytic leukemia and the mantle cell lymphoma consortiums, investigators from North America have formed a North American PTCL Collaborative Group, which will collaborate with the International PTCL Project to develop new treatment strategies to improve outcome in PTCL. An international registry is currently open and registering patients with newly diagnosed PTCL. In addition, an international tissue bank has been initiated to capture sufficient quantities of clinically annotated and well-characterized specimens.

Authorship

Contribution: F.M.F. prepared the manuscript for publication; and all authors wrote and reviewed the manuscript.

Conflict-of-interest disclosure: F.M.F. was a consultant for Eisai and Celgene and received research funding from Merck and Bayer. J.M.V. received research funding from Allos. S.T.R. was on the Scientific Advisory Board of Celgene and Genzyme, the Speakers Bureau of Merck, and the Advisory Panel of Eisai and Allos. K.T. received research funding from Kyowa Kirin, Mundipharma, and Merck. The remaining authors declare no competing financial interests.

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