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621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

North American Adult T-Cell Leukemia/Lymphoma Has Frequent Mutations in CCR4 and Responds in Vitro to a Small Molecule CCR4 Antagonist, CCR4-351

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Adult T-cell leukemia/lymphoma (ATLL) is a rare and aggressive disease of malignant CD4+ T cells that develops in human T-lymphotropic virus-1 (HTLV-1) carriers. Patients diagnosed with ATLL have the worst survival among all peripheral T-cell lymphomas. There is an urgent need for in-depth understanding of its pathogenesis and development of new treatment strategies. We and others have shown that ATLLs diagnosed in the Japanese (J-ATLL) and North American (NA-ATLL) patients have differences in clinical characteristics and immune-genetic landscape. Among the most frequently mutated genes in J-ATLL cohorts is CCR4, a chemokine receptor that is targeted by C-terminal truncation mutations in one third of the cases. These mutations result in a more stabilized surface CCR4 and have been reported to cause elevated Akt activation following chemokine stimulation. Since our previous targeted exon sequencing study did not include *CCR4*, this work was initiated to assess *CCR4* mutation frequency in NA-ATLL cases and to evaluate the preclinical activity of a small molecule CCR4 antagonist. This is because, although an anti-CCR4 mAb, mogamulizumab, has been approved in Japan to treat ATLL, this therapy failed in a Phase 2 trial among patients outside of Japan. Thus, mogamulizumab is not available as a routine treatment for NA-ATLL patients.

We compiled *CCR4* gene status data from bulk RNA-seq (n=10) and whole genome sequencing (WGS) analysis (n = 10). Out of a total of 17 NA-ATLL cases, 8 (47.1%) carried *CCR4* gene alterations that included in-frame and frameshift indels, as well as nonsense mutations targeted to the C-terminus cytoplasmic tail and also the trans-membrane domain. In subtype analysis, 6 out 14 acute cases (42.9%) and 2 out of 3 (66.7%) of chronic cases carried at least one copy of mutated *CCR4* gene, indicating that the mutation frequency in NA-ATLL is possibly even higher than that reported for the Japanese cohorts (**Figure 1**). We then evaluated cell surface CCR4 expression using flow cytometry. The primary ATLL cells ($CD4^+CD7^-CD8^-$) from all 7 diagnostic samples uniformly express markedly increased levels of CCR4 relative to healthy control CD4 T cells. Interestingly, CCR4 expression among 8 ATLL cell lines is highly variable, implying that in vivo, tumor microenvironment factors may also contribute to sustained CCR4 upregulation in addition to the influence from the HTLV-1 oncoprotein, HBZ.

FLX475 is an orally active small molecule CCR4 antagonist that is currently in a Phase 1/2 study (NCT03674567) in several types of advanced cancer including EBV+ NK/T cell lymphoma. So far, favorable clinical antitumor activity has been observed that includes complete responses with FLX475 monotherapy and encouraging combination activity (Lin C.-C. et al ESMO-IO 2022 Dec; #187P). Using several CCR4-positive cell lines, we examined in vitro anti-ATLL activity of CCR4-351, a CCR4 antagonist that is a highly related analog of FLX475. CCR4 is the exclusive homing receptor for two chemokine molecules, CCL17 and CCL22. In a transwell assay, both chemokines can elicit strong chemotaxis activity in a J-ATLL cell line ED40515(+). However, only CCL22 but not CCL17 can induce significant chemotaxis in two NA-ATLL cell lines, ATL#13 and ATL#29. The exact cause of this difference between these cell lines awaits future studies. In these transwell-based assays, CCR4-351 exhibited potent chemotaxis inhibitory activity with IC_{50} values ranging from 7-60 nM (**Figure 2**). In addition, when the cell lines were cultured

under growth factor-deprivation conditions, CCR4-351 treatment caused a mild reduction in total cell viability, suggesting an additional anti-ATLL mechanism of action.

In summary, in this first study of the CCR4 pathway status in NA-ATLL, we found that nearly half of the cases in our ATLL cohort carried a mutated *CCR4* gene. Contrary to primary ATLL cells, ATLL cell lines exhibit a wide variation in CCR4 expression. CCL22 but not CCL17 induced strong chemotaxis behavior in NA-ATLL cell lines, which was potently inhibited by a small molecule CCR4 antagonist, CCR4-351. Since extramedullary presentation is frequently seen in NA-ATLL and central nervous system involvement is an adverse prognostic feature, inhibiting chemotaxis with a CCR4 antagonist such as FLX475 may be an effective therapeutic approach.

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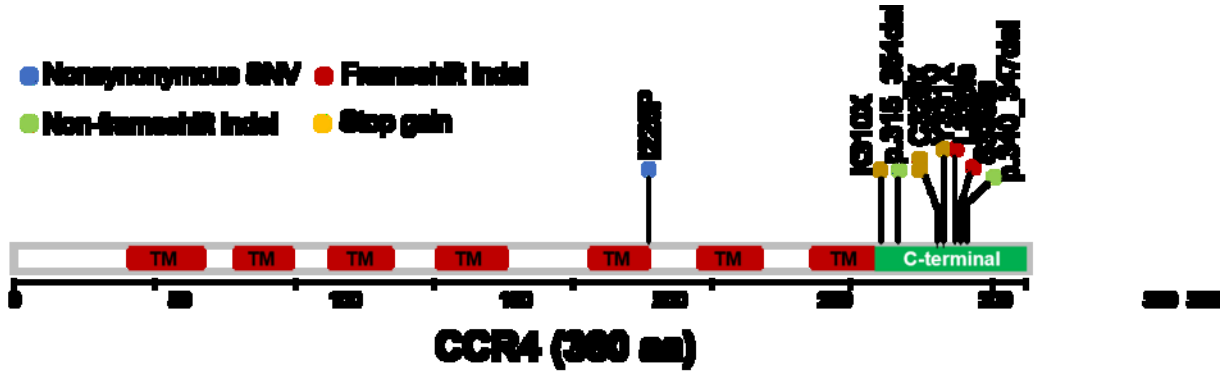

Figure 1. Distribution of alterations in the CCR4 protein structure. A total of 9 nonsynonymous changes and 1 synonymous SNV were found in 8 cases. Two cases carried bi-allelic alterations.

Figure 2. The CCR4 inhibitor CCR-351 can potently inhibit chemotaxis of ATLL cells in vitro. A 30 min pretreatment with the indicated concentration of CCR-351 inhibited CCL22-driven chemotaxis with an IC50 concentration of ~ 7 nM and 32 nM for ED40515(+) and ATL#29 cell line, respectively.

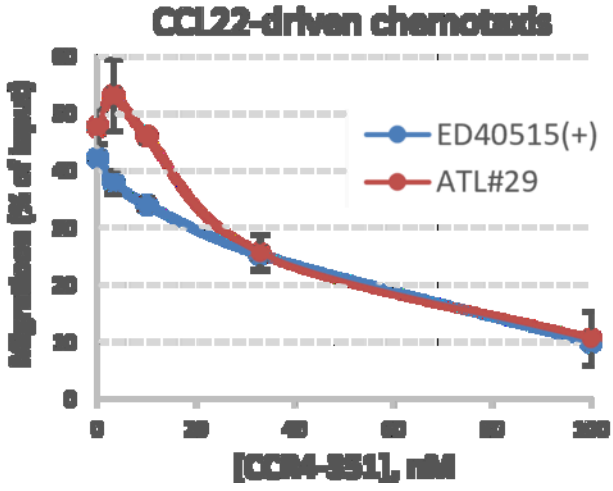

Figure 1

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