Feinstein Institutes for Medical Research
Northwell Health®

December 2023

Dear Swim Across America Friends,

Our laboratory is truly honored and very grateful to be a beneficiary of Swim Across America. The major focus in our laboratory remains to be therapeutically targeting tumor microenvironment (TME) of B cell leukemia and lymphoma. With your support, we have significant findings from the project “Targeting the Tumor Microenvironment to Treat Chronic Lymphocytic Leukemia (CLL)”. We found that the molecular CLECL1 not only marks the aggressive clone of CLL, activates T cells to produce IL-4, but also interact with TME, specifically stromal cells within spleens and lymph nodes (LNs). The activated CLL cells enriched of CLECL1+ cells produce lymphotoxin and promote B-cell zone specific stromal cells (follicular dendritic cells, FDCs), but suppress T cell zone fibroblastic reticular cells (FRCs). As the result of these actions, T cell functions are impaired and leukemic B cells retain and gain growth signal in TME. These data highlight how B-cell tumors educate and restructure the stromal cells to promote disease progression. In addition, we have made a huge progress in the generation and production of CLECL1 antagonist antibodies for therapeutic purposes. These studies have accelerated our pattern application, created new collaboration opportunities including those with the industry, and provided us the key preliminary data for grant applications.

We are very excited by the preliminary data and all the proposed experiments. This work is highly translational, the results are highly applicable to almost all the B-cell tumors. Using the established models, we are now initiating our work in Mantle cell lymphoma. We are thankful for your support that accelerates our research, and truly hoping to continue our partnership with SAA to fight cancer.

Sincerely,

Shihshih Chen

Assistant professor
The Feinstein Institute for Medical Research at Northwell Health

Progress Report

SUMMARY STATEMENT

Chronic lymphocytic leukemia (CLL) is the most common blood cancer in the western. CLL, similar to other leukemia and lymphoma, requires the support of lymph node (LN) niches for disease progression and transformation. CLL patients, especially subset #8 patients frequently have transformed Richter's syndrome and have poor prognosis with overall survival of less than 6-months. Although the current frontline therapies of Bruton's tyrosine kinase inhibitors (BTKi) have greatly improved the treatment outcome in CLL, these treatments are less effective in Richter's patients, and resistance occurs.

Since the last report in December, we focused on our goal of understanding the contribution of tumor microenvironment (TME) in CLL transformation and drug resistance, especially stromal cells including follicular dendritic cells specialized in the formation of B-cell follicles (BRCs) and fibroblastic reticular cells (FRCs) that regulate T cell function (TRCs). We established *in vitro* Matrigel and *in vivo* mouse models mimicking human secondary lymphoid tissue niches and focused on the three aims we proposed, **Aim 1:** Target the IL-4/IL-4R axis to develop an effective treatment for BTKi-resistant patients. **AIM 2:** Understand the cellular and molecular mechanisms whereby infiltration of CLL B cells changes stromal cells. **AIM 3:** Determine how CLL-exposed FRCs alter T cells and create an immune suppressive TME. With your support, we made the following discoveries: for Aim 1, we have generated hybridomas that secret anti-CLECL1 antagonist antibodies to target IL-4/IL-4R axis for CLL therapy. In Aim 2, we found CLL-induced expansion of BRCs. Finally in Aim 3, we demonstrated that CLL cell infiltration eliminates TRCs and impairs T-cell function.

Overall, for our initial goal of understanding and manipulating the cellular interactions in the TME that promote CLL cell survival and growth, our preliminary results provide evidence of the interactions between FRCs, T and malignant B cells that can be targeted to improve CLL therapy. These results have accelerated our pattern application, created new collaboration opportunities including those with the industry, and provided us the key preliminary data for grant applications. Using the established models, we are now also initiating our work in Mantle cell lymphoma. Taken together, our work is highly translational, the results are highly applicable to almost all the B-cell tumors.

Specific Aims

Aim 1: Target the IL-4/IL4R axis in the TME of BTKi ibrutinib-resistant CLL to develop an effective treatment for drug resistant patients.

Hypothesis: targeting IL-4 receptor (IL4R) signaling in CLL TME will treat BTKi ibrutinib resistant CLL.

Preliminary data generated in the past year: We previously reported a surface-expressing molecular CLECL1 that is highly on only activated but not resting CLL cells. In the past year, we further confirmed that: [1] CLECL1 expression is associated with BTKi ibrutinib responses. BTKi resistant CLL patients have increased CLECL1 expression on cell surface. [2] The surface level of CLECL1 on CLL cells also correlates with time to first treatment. [3] CLECL1 expressing CLL cells stimulate autologous T cell proliferation and IL-4 production. [4] The *in vitro* treatment of

commercial IgG antibody against CLECL1, or *in vitro* administration of dupilumab that targets IL-4R led to reduced Th2 T cells and CLL B-cell survival. The commercial antibody is however lack of specificity. We therefore aim to produce therapeutic antibody against CLECL1 with high specificity and high efficacy. Total 5 mice and 2 rats were therefore immunized with CLECL1 peptide; within these, mouse #2 showed best antibody titer (**Fig. 1**). The serum collected from mouse #2 prior or post immunization was then applied in CLL B cells. Interestingly, the post-immunization serum is sufficient to eliminate CLECL1 positive B cells (**Figure 2A).** In contrast, this serum has no effect in CLECL1 negative B cells or T cells, suggesting the high specificity of this antibody. Importantly, the treatment of mouse #2 serum blocks CLECL1-induced Th2 T cell expansion and cytokine production (**Figure 2B**). Similar results were seen in the supernatant collected from hybridoma culture (data not shown). Overall, these preliminary data suggest CLECL1$^+$ CLL clone as the key of tumor clone that shapes TME; and the new anti-CLECL1 antagonist antibody may serve as a novel therapeutic approach in CLL.

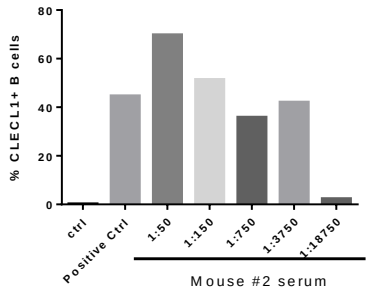

Fig. 1 CLECL1-peptide immunized mice produce antibodies against CLELC1 with high specificity in CLL.

Next steps: We next aim to expand the scale of hybridoma supernatant production, and use these supernatants to repeat *in vitro* experiments shown in **Figure 2.** This cell-culture experiment will use both treatment naïve and ibrutinib-resistant CLL patient cells. We will also purify the antibody and repeat the PDX model to demonstrate the *in vivo* effect of anti-CLECL1 antagonist antibody. As our preliminary data shown above highly support using CLECL1 as therapeutic target, the proposed function assays using this antibody will facilitate the production of therapeutic antibody targeting CLECL1 and IL4/IL4R axis in ibrutinib-resistant CLL cells for our goal of improving CLL therapy.

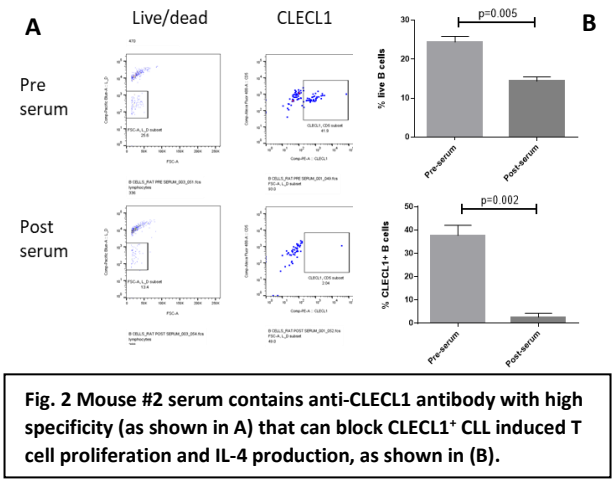

Fig. 2 Mouse #2 serum contains anti-CLECL1 antibody with high specificity (as shown in A) that can block CLECL1$^+$ CLL induced T cell proliferation and IL-4 production, as shown in (B).

AIM 2: Understand the cellular and molecular mechanisms whereby infiltration of CLL B cells change FRCs.

Hypothesis: CLL B cells regulate the structure and function of FRC networks in an activation dependent manner. Here, we originally proposed to confirm and build upon initial findings analyzing [1] molecular and cellular changes in FRCs upon CLL B cell interaction in vitro and in vivo; [2] determine the extent that CLL B cells differing in activation status and responsiveness to BTK inhibitors regulate these processes; and [3] define key factors that regulate CLL modulated FRCs for potential therapeutic targets.

P A ninary data generat B in the past year: CLECL1-expressing CLL cells are the activated ce..e .ocated in the prolifer...e center of LNs. Previously we have demonstrated that [1] *In vitro*, CLL cells, especially CLECL1$^+$ cells attached to FRCs better than the CLECL1$^-$ counterparts. [2] CLL cells remodeled stromal cell structure in a 3-D culture system using mouse CLL cells. We now know that CLL cells stimulated via BCR and IL-4R secret lymphotoxin and stimulate the

expansion of stromal cells to produce CXCL13, the key chemokine that controls B-cell follicle formation (**Figure 3**). Importantly, while CLL alters FRCs, FRCs exposure also results in the decreased lymphotoxin production in CLL B cells (data not shown).
As the result, CLL cell infiltration induces BRCs expansion in both TCL-1 mouse model of CLL (**Figure 3-4)** and PDX (**Figure 5**).

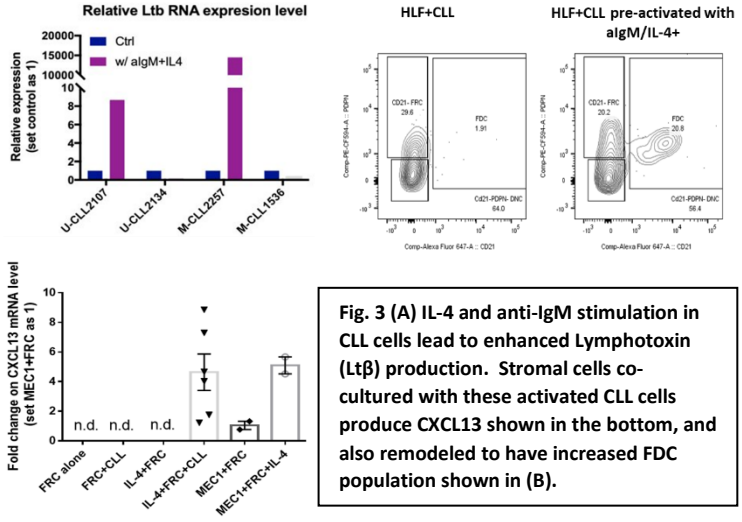

Fig. 3 (A) IL-4 and anti-IgM stimulation in CLL cells lead to enhanced Lymphotoxin (Ltβ) production. Stromal cells co-cultured with these activated CLL cells produce CXCL13 shown in the bottom, and also remodeled to have increased FDC population shown in (B).

Next steps: In the next year, we will continue investigating the morphology, molecular/transcriptomic and cellular changes in FRCs upon CLL cell interaction in vivo using both mouse and human CLL samples, including activated vs resting CLL cells. These studies will allow us to identify factors critical for CLL-stromal cell interaction that facilitates BRC expansion and Th2 TME generation. Finally, we will repeat these experiments and explore whether BTKi and IL-4R blocking agents can suppress the expansion and function of BRCs in both BTKi sensitive and resistant patients.

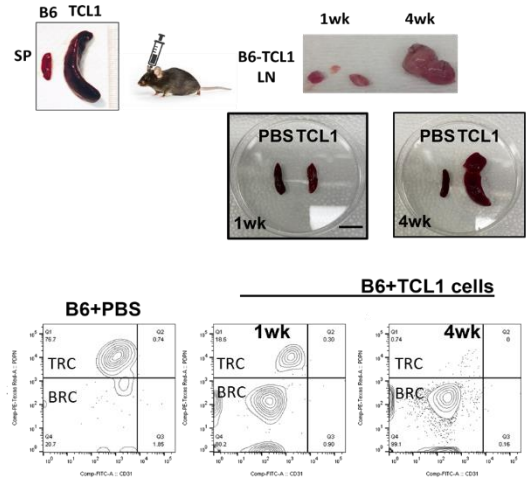

Fig. 4 TCL1-CLL cell injected mice have expanded BRCs but eliminated TRCs during CLL disease development.

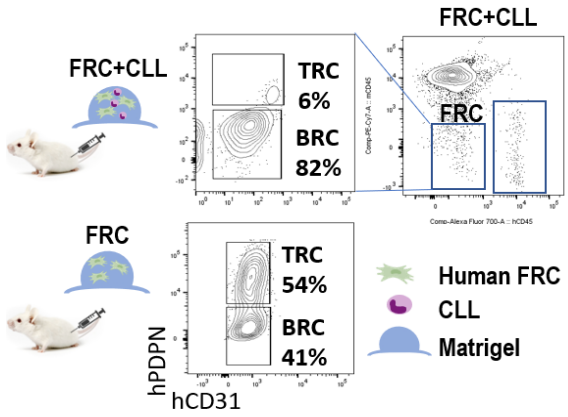

Fig. 5 Remodeled stromal cells in immune-deficient mice injected with human lymph node stromal cells mixed with CLL PBMCs in matrigels. CLL cell infiltration induced the expansion of BRCs and suppressed TRC in mouse spleens.

AIM 3: Determine how CLL-exposed FRCs alter T cells and create an immune suppressive TME.

Hypothesis: CLL cell-exposed FRCs modulate T-cell functions and create a pro-tumor TME that supports CLL B cells, both ultimately impacting disease progression. In this Aim, I proposed to [1] decipher changes in T-cell populations and functions in vitro mediated by CLL-exposed FRCs; [2] determine the extent that CLL cells differing in responsiveness to ibrutinib regulate these processes; and [3] target FRCs to improve T-cell immunity and CAR-T treatment in CLL.

Preliminary data generated in the past year: Different from patients with diffused large B-cell lymphoma (DLBCL) that have impaired TRC network but intact expression of podoplanin (PDPN), a marker for T-cell regulator FRCs, CLL patients have stromal cells within LNs that reveal altered morphology and strikingly almost completely loss of PDPN; similar results were also seen in wild-type C57/B6 mouse LN stromal cells upon CLL cell infiltration (**Figure 6**). Importantly, our *in vivo* model suggests the reduction of PDPN+ FRCs during CLL disease progression (**Figure 4**). The CLL-disrupted T-cell network might contribute to the dysregulated T cell functions and altered T cell subsets that we previously demonstrated. Hence, recovery of T cell function via restructuring stromal network might improve CLL therapy especially the engineered chimeric antigen receptor (CAR)-T cell therapy.

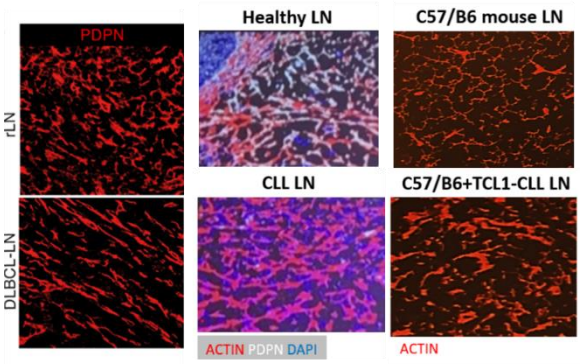

Fig. 6 Remodeled LN stromal network in DLBCL patients (left), CLL patients (middle) and TCL-1 CLL cell injected wild type C57/B6 mice. Strikingly, a notable reduction of PDPN is seen in CLL but not DLBCL LNs.

Next steps: Because of the observation of CLL-suppressed TRCs overtime during the disease progression, we would perform mechanistic assays, such as transcriptomic analysis on FRC and other TRC subsets in LNs before and after tumor infiltration to identify key factors (soluble or membrane) presented by CLL cells to inhibit T cells. We would investigate the outcome of changes in stromal cells on T cell activity, function and subsets; and then we would investigate whether recovering TRC network by directly targeting lymphotoxin receptor expressed on stromal cells, or by removing CLL suppression using either BTKi/IL-4Ri or indirectly by blocking CLECL1-expressing CLL could improve T cell activities in CLL. Finally, we would repeat these experiments in ibrutinib resistant CLL using the above treatments. For the ultimate goal of understanding TRC network on T cell function, we would also include CAR-T cell therapy.

Timeline and Milestones. We will finish determining the role of IL-4- IL4R in CLL by producing the effective anti-CLELC1 antibody in the next year (Aim 1), and also focus on cellular and molecular mechanisms whereby CLL B cell infiltration changes BRCs (Aim 2) during the third year. In the following two years, we will investigate how CLL alters TRCs and see how CLL-exposed FRCs change T cells and create a protumor TME (Aim 3). Finally, we will test potential targets identified in the first two aims in mice. At the end, we expect to have pre-clinical studies finished for the goal of recovering disrupted stromal network to improve treatment.

Expected results and clinical relevance. The proposed work is innovative because it will be the first to explore the underlying mechanisms of the dynamic interaction between CLL cells and normal T cells and FRCs for disease progression. It will also identify potential therapeutic targets for not only CLL but likely other B cell leukemias and lymphomas. Aim 1 will provide novel data on the effects of anti-CLELC1 and anti-IL4R antibody in CLL. Aim 2 will show for the first time how CLL cells stimulate BRCs in LN and spleens to support tumor growth. Aim 3 will provide novel knowledge on how stroma contributes to the impaired T cell immunity in CLL. Results of these studies will identify key players in these processes that can be targeted for better treatment and possibly prevention of drug resistance. Importantly, since our preliminary data suggest the association of CLECL1 with DLBCL disease progression. Findings here can be translated to not only CLL but also other B-cell leukemia/lymphoma and autoimmune diseases.