Title. Examining the host immune response to cutaneous squamous cell carcinoma in chronic lymphocytic leukemia

Specific Aims. This pilot study will examine the host immune response to cutaneous squamous cell carcinoma (cSCC) in patients with chronic lymphocytic leukemia (CLL).

Aim 1: To compare the host immune response between CLL and immunocompetent patients.
Aim 2: To measure the density of CLL B cells surrounding cSCC from CLL patients.
Aim 3: To evaluate whether the host immune response correlates with clinical outcomes.

Background and Significance. Hematologic malignancies are an important risk factor in the development of cSCC. The most common type of leukemia, CLL is characterized by proliferating monoclonal B cells that are immunologically disruptive and that accumulate in organs, disrupting organ function[1]. Patients are immunocompromised from the leukemia itself, as well as from treatment. CLL patients have a 3-8 fold higher incidence of cSCC and have higher rates of local recurrence, metastasis, and death after Mohs surgery[2-6]. A high CLL stage is a risk factor for poor outcomes[7]. Furthermore, CLL patients with locally advanced or metastatic cSCC have a reduced response rate to immunotherapy compared to immunocompetent patients[8].

Mohs surgeons have published findings of a dense lymphocytic infiltrate surrounding cSCC from CLL patients[9-11]. This infiltrate reflects the host's immune response against tumor, a well-documented interaction mediated by host T cells. Modulating this T cell response is the target of immune checkpoint inhibitors, which have transformed the treatment of advanced cSCC[12]. However, the host T cell response to cSCC in CLL patients has not been well characterized.

Cytotoxic $CD8^+$ T cells (Tc) drive the immune response against cancer[13]. $FOXP3^+$ regulatory T cells (Treg) suppress Tc and the host immune response, and cSCC recruit Treg to the tumor site, promoting a favorable growth environment[14,15]. Data from organ transplant recipients indicate that Tc and Treg activity is aberrant in immunocompromised patients; specifically, organ transplant recipients have a lower number of peri-tumor Tc, as well as a higher ratio of Treg:Tc, favoring cSCC growth[16,17]. To our knowledge, there is no data on whether this aberrant response is also present in CLL.

Hypotheses. (1) CLL patients have a higher Treg:Tc ratio compared to immunocompetent controls, indicating an impaired host immune response; (2) Among CLL patients, there is a greater density of CLL B cells around high-risk cSCC compared to low risk cSCC, suggesting a pro-tumorigenic role of CLL B cells.

Preliminary Studies. Otley et al. evaluated the lymphocytic infiltrate around non-melanoma skin cancers from 56 CLL and 105 immunocompetent patients[9]. They showed that CLL patients are more likely to have a dense peri-tumor lymphocytic infiltrate, that this infiltrate contains CLL B cells, and that tumors from CLL patients were more likely to have subclinical extension, suggesting that CLL B cells may be cSCC-supportive.

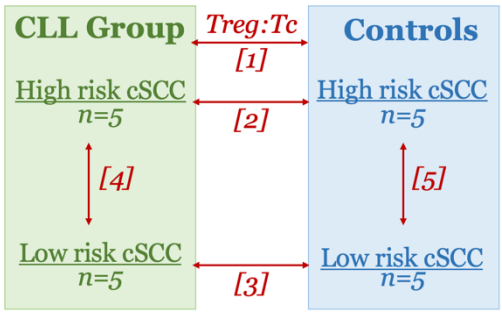
Portions of this project will be performed in Chrysalyne Schmults' lab, which studies desmoplastic cSCC and the role of desmoplasia in blocking T cells from reaching the cSCC. Their data show that desmoplasia is associated with fewer peri-tumor T cells and greater T cell exhaustion[18]. The lab has optimized using multispectral immunofluorescence to compare T cell subsets on cSCC samples. This includes using a machine learning approach to identify T cell types on immunofluorescence images.

Methods. The Brigham and Women's Hospital (BWH) Mohs database collects comprehensive information on tumor characteristics and clinical outcomes. The database has been queried to identify 10 CLL patients who have had both a high-risk SCC (defined as BWH stage T2b or T3) and a low-risk SCC treated between 2016 and 2021. This design allows us to compare high risk cSCC to low risk

cSCC, with patients acting as their own internal control. We will also identify 10 healthy controls matched for age, gender, and history of SCC. The database has a corresponding repository which contains cSCC tissue. To determine study inclusion, we will review the corresponding H&E slides and include only cSCC with a dense peri-tumor lymphocytic infiltrate. For each included tumor, two additional cuts will be obtained for immunofluorescence and imaging mass cytometry.

We will first confirm the presence of T cells and CLL B cells in the peri-tumor infiltrate using immunofluorescence performed in Chrysalyne Schmults' lab. Next, we will quantify peri-tumor Treg, Tc, and CLL B cells using imaging mass cytometry. Compared to immunofluorescence, imaging mass cytometry provides much higher resolution (at the subcellular level) and preserves the tissue architecture, allowing us to visualize the spatial relationship between T cells, CLL B cells, and cSCC. The technique uses antibodies labeled with heavy metals; up to 40 antibodies are added to a single slide, which is divided into 1 μm^2 pixels. A laser ablates each pixel sequentially, releasing the heavy metals, which are identified by mass spectrometry. Pixel-level data are combined to form a map of the cSCC microenvironment[19,20]. We will use Standard BioTools, a company that produces imaging instruments and that provides imaging mass cytometry services.

Aim 1: To compare the host immune response between CLL and immunocompetent patients.
To evaluate the host immune response, we will use imaging mass cytometry to measure the density of Treg and Tc in the cSCC microenvironment and will calculate a Treg:Tc ratio for each cSCC. We will use the T test to compare the Treg:Tc ratio between cSCC from CLL patients and matched controls (comparison [1]). We hypothesize that CLL patients have a higher Treg:Tc ratio, indicating an impaired immune response. For sensitivity analysis, we will compare the Treg:Tc ratio between high risk cSCC from CLL patients and controls [2], as well as between low risk cSCC from CLL patients and controls [3]. Furthermore, to determine whether the Treg:Tc ratio correlates with tumor aggressiveness, we will compare the Treg:Tc ratio between high-risk and low risk cSCC among CLL patients [4] and among controls [5].



Aim 2: To measure the density of CLL B cells surrounding cSCC from CLL patients.
To investigate the role of CLL B cells in the host immune response, we will use imaging mass cytometry to measure the density of peritumor CLL B cells. We will use T test to compare the density of CLL B cells between high-risk SCC and low-risk SCC from CLL patients (n=5 per group). We will also measure the distance between each lymphocyte subset (Treg, Tc, CLL B cell) and the cSCC to examine whether CLL B cells form a physical barrier between Tc and cSCC.

Aim 3: To evaluate whether Treg:Tc ratio and CLL B cell density correlate with clinical outcomes.
Retrospective chart review will be performed for all patients to determine tumor characteristics (including diameter, depth of invasion, differentiation, and presence of perineural invasion) and presence/absence of a poor clinical outcome (defined as local recurrence or regional metastasis). For the CLL and control group (n=10 each), logistic regression will be used to evaluate whether Treg:Tc ratio and/or CLL B cell density correlates with poor clinical outcomes.

Organizational Structure. This research will be conducted at Brigham and Women's Hospital (BWH). Emily Ruiz, the director of the Dana Farber Cancer Institute (DFCI) High-Risk Skin Cancer Clinic, will serve as the primary mentor. The following faculty will also advise: Chrysalyne Schmults, who runs a translational research lab at BWH; Adriano Piris, a BWH Dermatopathologist who specializes in cutaneous oncology; and Inhye Ahn, an oncologist at DFCI who treats CLL. The BWH Dermatology department provides wide-ranging support for research, including a dedicated statistician and faculty who oversee both a basic science and a clinical research program.

Literature Cited

1. Hallek M. Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. Am J Hematol 2019;94(11):1266-1287. (In eng). DOI: 10.1002/ajh.25595.
2. Mehrany K, Weenig RH, Lee KK, Pittelkow MR, Otley CC. Increased metastasis and mortality from cutaneous squamous cell carcinoma in patients with chronic lymphocytic leukemia. J Am Acad Dermatol 2005;53(6):1067-71. (In eng). DOI: 10.1016/j.jaad.2005.08.055.
3. Mehrany K, Weenig RH, Pittelkow MR, Roenigk RK, Otley CC. High recurrence rates of squamous cell carcinoma after Mohs' surgery in patients with chronic lymphocytic leukemia. Dermatol Surg 2005;31(1):38-42; discussion 42. (In eng). DOI: 10.1111/j.1524-4725.2005.31006.
4. Hock BD, McIntosh ND, McKenzie JL, Pearson JF, Simcock JW, MacPherson SA. Incidence of cutaneous squamous cell carcinoma in a New Zealand population of chronic lymphocytic leukaemia patients. Intern Med J 2016;46(12):1414-1421. (In eng). DOI: 10.1111/imj.13261.
5. Ishdorj G, Beiggi S, Nugent Z, et al. Risk factors for skin cancer and solid tumors in newly diagnosed patients with chronic lymphocytic leukemia and the impact of skin surveillance on survival. Leuk Lymphoma 2019;60(13):3204-3213. (In eng). DOI: 10.1080/10428194.2019.1620941.
6. Zheng G, Chattopadhyay S, Sud A, et al. Second primary cancers in patients with acute lymphoblastic, chronic lymphocytic and hairy cell leukaemia. Br J Haematol 2019;185(2):232-239. (In eng). DOI: 10.1111/bjh.15777.
7. Velez NF, Karia PS, Vartanov AR, Davids MS, Brown JR, Schmults CD. Association of advanced leukemic stage and skin cancer tumor stage with poor skin cancer outcomes in patients with chronic lymphocytic leukemia. JAMA Dermatol 2014;150(3):280-7. (In eng). DOI: 10.1001/jamadermatol.2013.6249.
8. Leiter U, Loquai C, Reinhardt L, et al. Immune checkpoint inhibition therapy for advanced skin cancer in patients with concomitant hematological malignancy: a retrospective multicenter DeCOG study of 84 patients. J Immunother Cancer 2020;8(2) (In eng). DOI: 10.1136/jitc-2020-000897.
9. Mehrany K, Byrd DR, Roenigk RK, et al. Lymphocytic infiltrates and subclinical epithelial tumor extension in patients with chronic leukemia and solid-organ transplantation. Dermatol Surg 2003;29(2):129-34. (In eng). DOI: 10.1046/j.1524-4725.2003.29034.x.
10. Walters M, Chang C, Castillo JR. Diagnosis of chronic lymphocytic leukemia during Mohs micrographic surgery. JAAD Case Reports 2023;33:1-3. DOI: 10.1016/j.jdcr.2022.12.012.
11. Wilson ML, Elston DM, Tyler WB, Marks VJ, Ferringer T. Dense lymphocytic infiltrates associated with non-melanoma skin cancer in patients with chronic lymphocytic leukemia. Dermatol Online J 2010;16(3):4. (In eng).
12. Migden MR, Rischin D, Schmults CD, et al. PD-1 Blockade with Cemiplimab in Advanced Cutaneous Squamous-Cell Carcinoma. N Engl J Med 2018;379(4):341-351. (In eng). DOI: 10.1056/NEJMoa1805131.
13. Raskov H, Orhan A, Christensen JP, Gögenur I. Cytotoxic CD8(+) T cells in cancer and cancer immunotherapy. Br J Cancer 2021;124(2):359-367. (In eng). DOI: 10.1038/s41416-020-01048-4.
14. Lai C, August S, Albibas A, et al. OX40+ Regulatory T Cells in Cutaneous Squamous Cell Carcinoma Suppress Effector T-Cell Responses and Associate with Metastatic Potential. Clin Cancer Res 2016;22(16):4236-48. (In eng). DOI: 10.1158/1078-0432.Ccr-15-2614.
15. Clark RA, Huang SJ, Murphy GF, et al. Human squamous cell carcinomas evade the immune response by down-regulation of vascular E-selectin and recruitment of regulatory T cells. J Exp Med 2008;205(10):2221-34. (In eng). DOI: 10.1084/jem.20071190.
16. Zhang S, Fujita H, Mitsui H, et al. Increased Tc22 and Treg/CD8 ratio contribute to aggressive growth of transplant associated squamous cell carcinoma. PLoS One 2013;8(5):e62154. (In eng). DOI: 10.1371/journal.pone.0062154.
17. Frazzette N, Khodadadi-Jamayran A, Doudican N, et al. Decreased cytotoxic T cells and TCR clonality in organ transplant recipients with squamous cell carcinoma. npj Precision Oncology 2020;4(1). DOI: 10.1038/s41698-020-0119-9.
18. Hirakawa Y, Zhan Q, Essien S, et al. Desmoplasia is associated with decreased cytotoxic and helper T cells and increased T cell exhaustion in cutaneous squamous cell carcinoma. J Invest Dermatol 2024 (In eng). DOI: 10.1016/j.jid.2024.01.019.
19. Elaldi R, Hemon P, Petti L, et al. High Dimensional Imaging Mass Cytometry Panel to Visualize the Tumor Immune Microenvironment Contexture. Front Immunol 2021;12:666233. (In eng). DOI: 10.3389/fimmu.2021.666233.
20. Veenstra J, Ozog D, Loveless I, et al. Distinguishing Keratoacanthoma from Well-Differentiated Cutaneous Squamous Cell Carcinoma Using Single-Cell Spatial Pathology. J Invest Dermatol 2023;143(12):2397-2407.e8. (In eng). DOI: 10.1016/j.jid.2023.06.192.