Background

- The CC Chemokine Receptor type 4 (CCR4) is highly expressed on activated Th2 cells, regulatory T cells (Treg), as well as cutaneous T-cell lymphoma (CTCL) cells, making it a prominent therapeutic target for CTCL.

**CCR4 binding leads to immune evasion**

- Thus, inhibiting CCR4 may reduce Treg accumulation and CTCL development and progression.

![Chemokine Receptor Structure](image)

**Hypothesis**

The inhibition of CCR4 with C021 treatment may lead to decreased tumor proliferation and decreased tumor volume in CTCL.

**Methods**

**Study Group:** We analyzed tissues from mice injected with MJ CTCL cells, then followed with treatment (Arm2) and mice that were simultaneously injected with treatment and tumor cells (Arm1).

**Treatment:** Mice were divided into 3 groups based on dosage of C021: G1 (control), G2 (low dose), G3 (high dose).

**Immunohistochemistry (IHC) assay:** A monoclonal rabbit antibody (D3B5) was used to detect Ki67 at a 1:3000 dilution. NBPI-86584 C-terminal polyclonal antibody was used to detect CCR4 at a 1:500 dilution. Dako EnVision System kit was used for staining.

**Grading:** The average of 3 graders was taken for reading the expression of each section.

- **Ki67:** Sections were graded based on percentage of positive and negative stained cells.
- **CCR4:** Sections were graded by intensity (negative, weak, moderate, strong) and percentage of cells within each intensity class.

### Results

**Figure 2.** Differences in tumor weight among the three groups (G1: n=4, G2: n=4, G3: n=5) suggest a trend of decreased tumor weight in treated mice.

![Tumor Weight by Group (Arm 1)](image)

**Figure 3.** A lower average tumor weight in G2 (low dose, n=7) compared to G1 (control, n=4) suggests treatment may decrease tumor weight. One outlier (A2G24L: 0, 1840mg) was removed.

![Tumor Weight by Group (Arm 2)](image)

**Figure 4a.** Arm 1 Ki67 average scores were compared by group: G1 (control, n=4), G2 (low dose, n=3), G3 (high dose, n=3). In Arm 1 samples, there was a slight decrease in proliferation scores between G1 and G3.

![Ki67 Scores by Treatment (Arm 1)](image)

**Figure 4b.** Ki67 IHC from Arm 1, G1 (control) group.

**Figure 4c.** Ki67 IHC from Arm 1, G2 (low dose) group.

**Figure 4d.** Ki67 IHC from Arm 1, G3 (high dose) group.

**Figure 5a.** Arm 2 Ki67 average scores were compared by group: G1 (control, n=4), G2 (low dose, n=6). This suggests a possible trend for a decrease in proliferation when treatment is given.

![Ki67 Scores by Treatment (Arm 2)](image)

**Figure 5b.** Ki67 IHC from Arm 2, G1 (control) group.

**Figure 5c.** Ki67 IHC from Arm 2, G2 (low dose) group.

**Future Work**

- This study will benefit from larger sample sizes in treatment groups, which will decrease variance and lead to more significant results.
- **Tumor necrosis across treatment groups could be analyzed to assess anti-tumor effects.**

**References**


