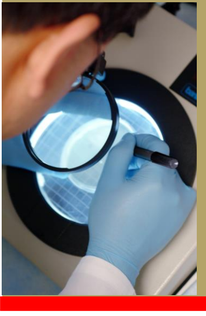

CCR4 Antagonists in Cutaneous T-Cell Lymphoma (CTCL)

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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

Ligand binds to CCR4 → **Regulatory T cells (Treg) accumulate** → **Inhibit immune response to tumor**

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

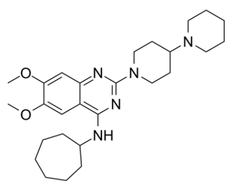


Figure 1. Small molecule CCR4 inhibitor C021, dihydrochloride.

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. NBP1-86584 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

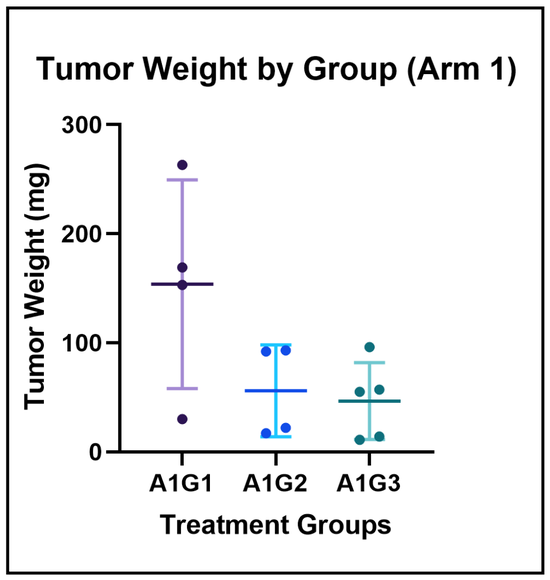


Figure 2. Differences in tumor weight among the three groups (G1: n=4, G2: n=4, G3: n=5) suggest a trend of decreased tumor weight in treated mice.

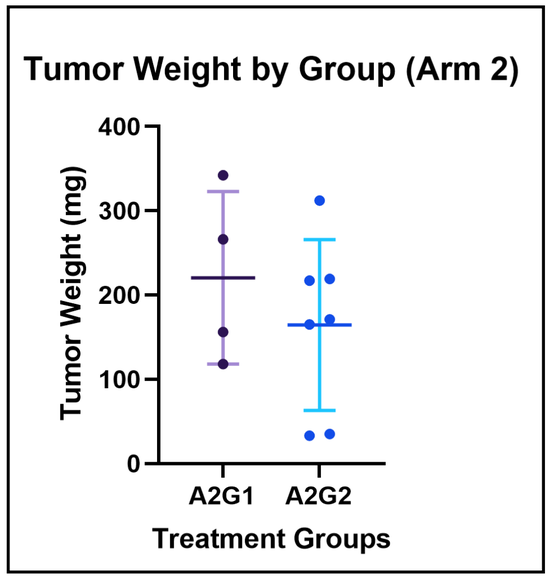


Figure 3. A lower average tumor weight in G2 (low dose, n=7) compared to G1 (control, n=4) suggests treatment may decrease tumor weight. One outlier (A2G24L: 0, 1840mg) was removed.

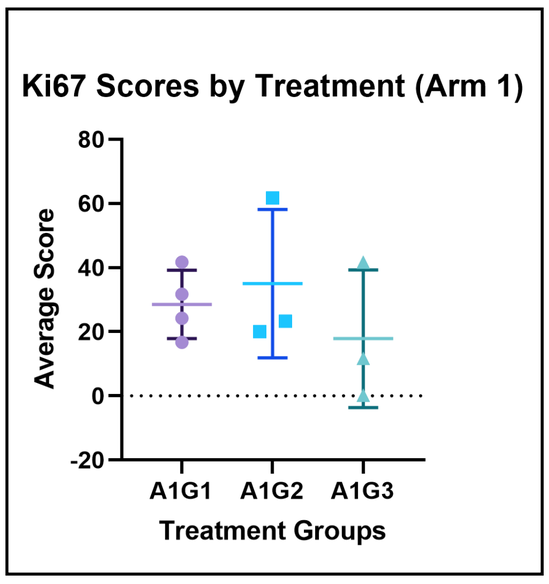


Figure 4a. Arm 1 Ki67 average scores were compared by group: G1 (control, n=4), G2 (low dose, n=3), G3 (high dose, n=3). In Arm 1 samples, there was a slight decrease in proliferation scores between G1 and G3.

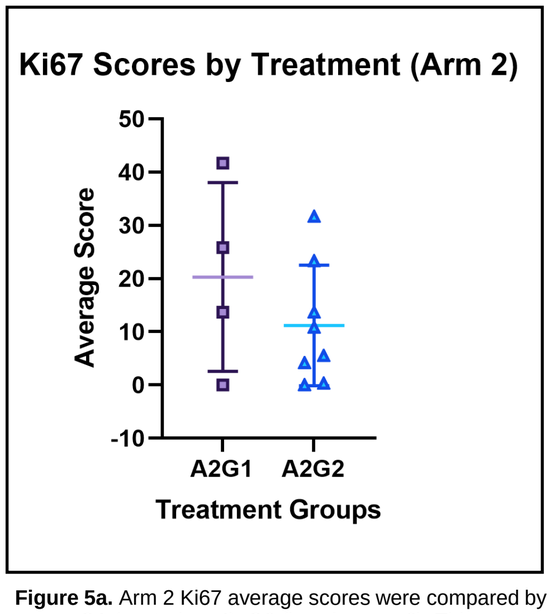


Figure 5a. Arm 2 Ki67 average scores were compared by group: G1 (control, n=4), G2 (low dose, n=8). This suggests a possible trend for a decrease in proliferation when treatment is given.

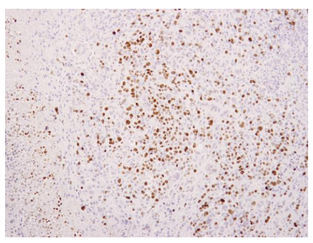


Figure 4b. Ki67 IHC from Arm 1, G1 (control) group.

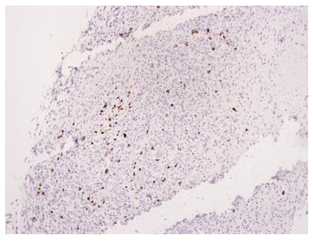


Figure 4c. Ki67 IHC from Arm 1, G2 (low dose) group.

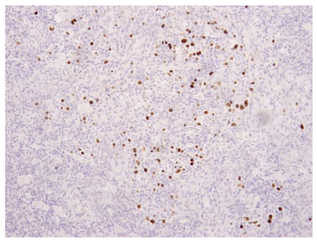


Figure 4d. Ki67 IHC from Arm 1, G3 (high dose) group.

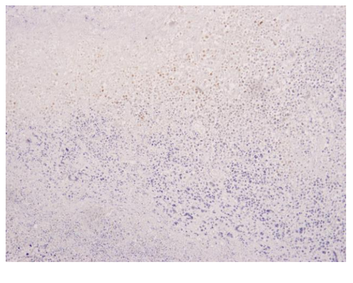


Figure 5b. Ki67 IHC from Arm 2, G1 (control) group.

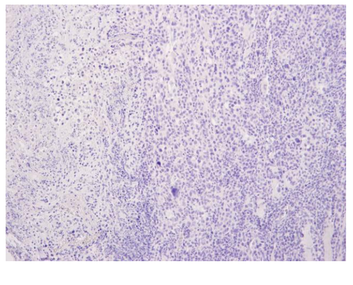


Figure 5c. Ki67 IHC from Arm 2, G2 (low dose) group.

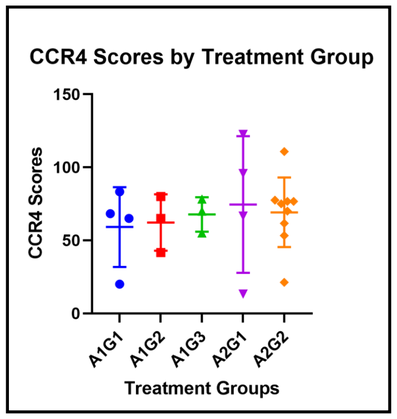
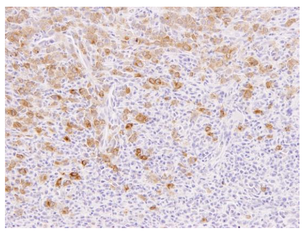


Figure 6a. There was no large difference in CCR4 scores or CCR4+ cells between treated and untreated groups.



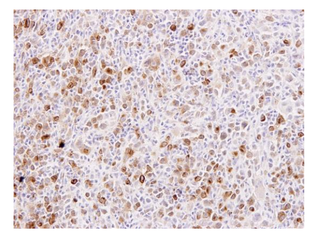


Figure 6b. CCR4 IHC from Arm 1, G1 (control) group.
Figure 6c. CCR4 IHC from Arm 1, G3 (high dose) group.

Summary

- Tumor weight was lower in treatment groups as compared to the control group, especially in the Arm 1 high dose group.
- We observed lower levels of Ki67 expression in C021 treatment groups.
- CCR4+ cells were not drastically decreased, but we observed large necrotic areas in C021 treated tissues.

Conclusion

Our results suggest that C021, a small CCR4 antagonist, has inhibitory effects on CTCL cell proliferation, which may contribute to decreased tumor volume in xenograft CTCL mice.

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.

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