# ALK Fusion-Driven Pediatric Gliomas Escape Targeted Therapy Through Transdifferentiation to Pericytes

Sybella Ssewakiryanga[1,2], Julia Walker[1], Zachary Russell[1], Eric Holland[1]

[1]Fred Hutchinson Cancer Center, Seattle, WA, [2] Whitman College, Walla Walla, WA

## Background

- Pediatric and adult gliomas are histologically similar but have different behavioral and molecular characteristics. Pediatric gliomas are usually a result of gene fusions, whereas adult gliomas can develop because of one's complex genetic landscape as mutations accumulate over time.
- The EML4-ALK fusion causes the formation of an abnormal protein that drives uncontrolled cell proliferation and tumor formation. This can lead to the development of high-grade pediatric gliomas which carry a high morbidity and mortality rate, with a 5-year survival under 20%.
- RCAS/tva system allows somatic gene transfer of selected oncogenes into targeted brain cells engineered to express the tv-a receptor. By delivering an oncogene via the RCAS virus this mechanism can mimic glioma initiation and progression.
- ALK inhibitors such as Crizotinib (1st line) and Lorlatinib (2nd or 3rd line) are targeted therapies designed to block the activity of ALK fusion proteins. Their efficiency in mouse models has been investigated here to inform future clinical use.

## Objective

- Investigate the molecular mechanisms of tumor cell targeted therapy evasion in EML4-ALK fusion driven pediatric gliomas.

## *In Vivo* Methodology

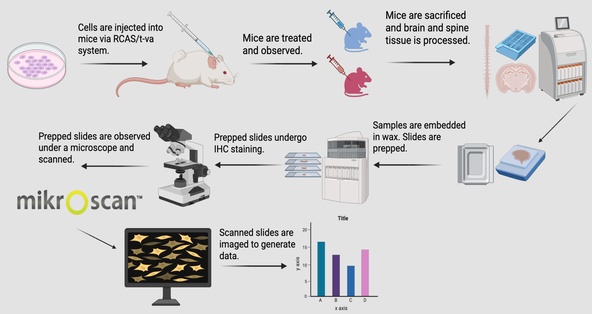



Figure 1: **Chronological Flowchart of Research Methodology.**

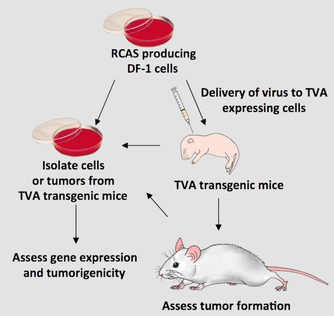
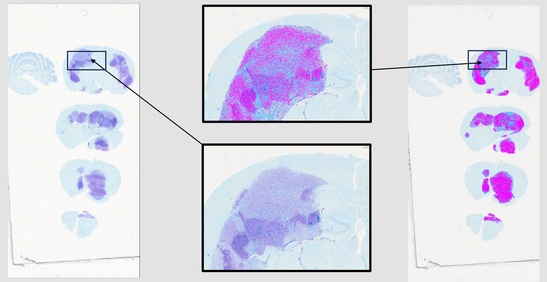



Figure 2: **Diagram of RCAS/tva System.** Images were produced by MediaLab at the Department of Biochemistry, University of Wisconsin at Madison.

## Quantification Methodology

Figure 3. **Quantification of Samples Using QuPath Software.** *Object Classification* feature was used to count number of cells in a particular region expressing the biomarker of interest. Dark purple indicates tumor cells positive for ALK protein, blue indicates Iba1-positive microglia and macrophages in the tumor region, light purple represents negative cells, cells that did not meet the threshold to be classified as Iba1 or ALK positive.



## IHC and Fluorescence Results

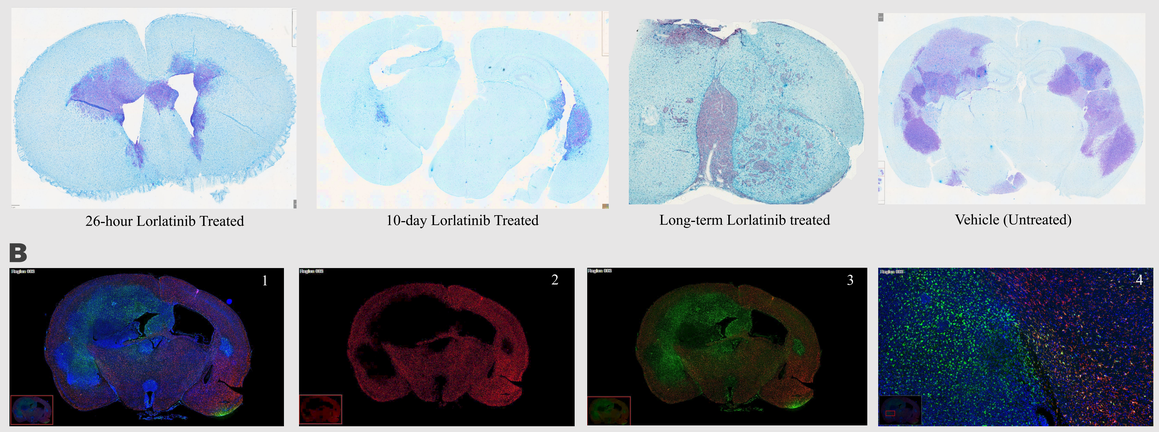



Figure 4. **Immunohistochemical (IHC) and Fluorescent Imaging of ALK and Immune Cell Markers in Mouse Brain Tissue**. A) IHC staining of Lorlatinib-treated mouse brain sections stained for ALK (purple) and Iba1 (dark blue) across three time points. As time progresses tumor region shrinks with less ALK-positive tumor cells. This indicates the efficacy of the Lorlatinib medication. While tumor cells do decrease, residual cells remain in long-term treated subjects. B) Fluorescence imaging of an untreated mouse brain section stained with DAPI (blue, nuclei, B1), P2Y12 (red, microglia, B2), and Iba1 (green, glioma-associated macrophages/microglia, B3). Tumor region stains positively for Iba1 (green, B3) but not for P2Y12 (red, B2) indicating the presence of macrophages not microglia within the region. This confirms that macrophages are likely interacting with residual tumor cells within their microenvironment after long-term treatment.

## IHC Results

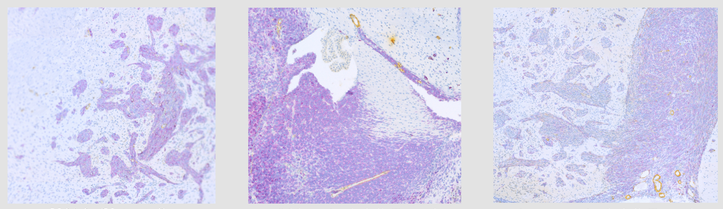



Figure 5: **Immunohistochemical (IHC) Staining in Mouse Brain Tissue of Vehicle (Untreated), 26-hour Lorlatinib-treated, and Long-term Lorlatinib-treated Subjects.** IHC stained for ALK-positive tumor cells (purple) and SMA (yellow) staining marks blood vessel smooth muscle/pericytes and confirms pericyte-like cells within the microenvironment.

- Surviving tumor cells retreat to a perivascular niche and shift their phenotype to become more pericyte-like.
- Tumor cells are likely producing CSF1 that stimulates macrophage growth. This binds to CSF1 receptors on macrophages promoting proliferation.
- Macrophages will produce PDGF which acts on receptors on tumor cells leading to CSF1 production. This creates and reinforces a loop relationship between macrophages and tumor cells.

## Quantification Results

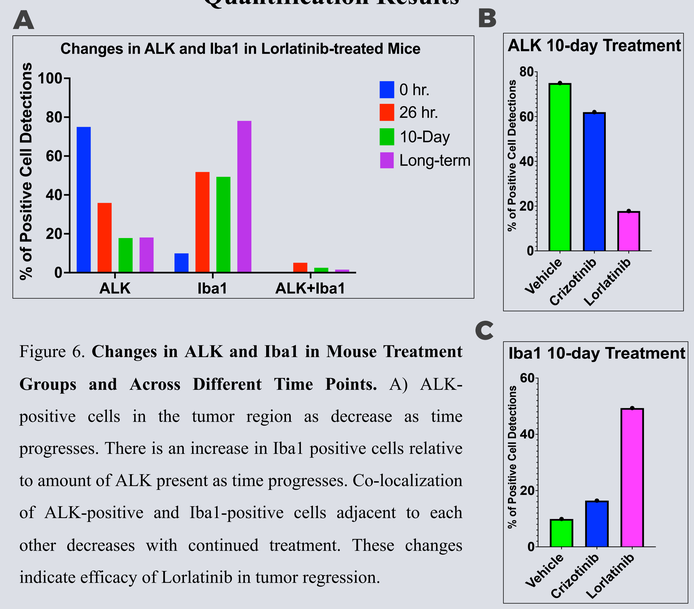



Figure 6. **Changes in ALK and Iba1 in Mouse Treatment Groups and Across Different Time Points.** A) ALK-positive cells in the tumor region as decrease as time progresses. There is an increase in Iba1 positive cells relative to amount of ALK present as time progresses. Co-localization of ALK-positive and Iba1-positive cells adjacent to each other decreases with continued treatment. These changes indicate efficacy of Lorlatinib in tumor regression.

B) Differences in ALK across three treatment groups (Vehicle (untreated) Lorlatinib, and Crizotinib) along a 10-day period. Lorlatinib-treated mice have the lowest levels of ALK-positive tumor cells indicating greater efficacy compared to Crizotinib. C) Differences in Iba1 across three treatment groups along a 10-day period. Lorlatinib-treated mice have the highest amount of Iba1 positive cells relative to the number of ALK-positive cells as treatment progresses. All Graphs were created from quantification in QuPath.

## Conclusion & Future Directions

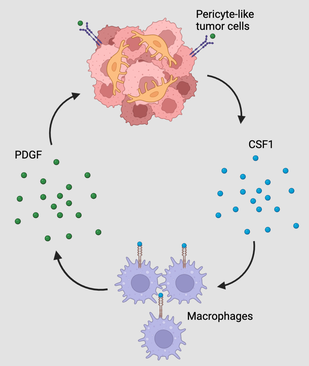



Figure 7: **Diagram of Loop Relationship Between Pericyte-like Tumor Cells and Macrophages.**

- Future therapeutic targeting would involve introducing small molecule inhibitors that can block PDGF receptor signaling on pericyte-like tumor cells and reduce glioma growth following long-term Lorlatinib treatment.
- Additional approaches could involve introducing a CSF1 receptor inhibitor to block macrophage stimulation.
- Introducing these inhibitors can conclusively confirm the presence of the loop relationship and inform future therapeutic interventions.

## Acknowledgements

This work is funded by the NIH, R35 CA253119. The Summer Undergraduate Research Program is supported in parts by Whitman College, the Fred Hutch Internship Program, and individual labs/research groups.