NMR-based Metabolic Profiling of Pancreatic Cancer and Response to Glutamine Transport Inhibition

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Background

Glutamine is the most abundant, free amino acid in plasma, utilized by cancer cells for survival and rapid reproduction. Cancer cells employ glutamine through a transporter (ASCT2) and metabolizing it through a catalyzing enzyme. During glutaminolysis, the amino acid transporter, SLC1A5 (ASCT2), metabolizes glutamine with a catalyzing enzyme

Hypothesis

Mouse using the developed inhibitor of the ASCT2 amino acid transporter, V-9302, malignant tumors will be unable to metabolize glutamine leading to difficulties in proliferation and an imbalance of metabolites in the microenvironment.
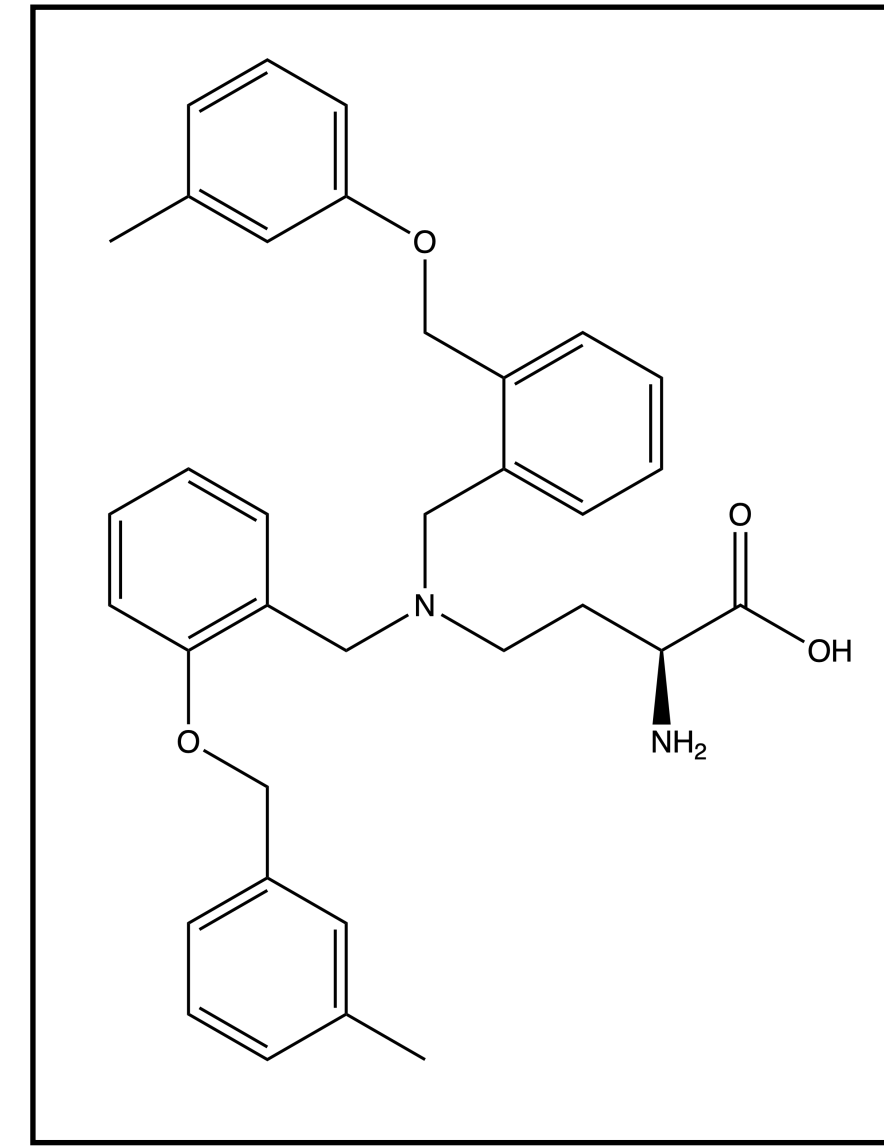

Fig. 1 V-9302.
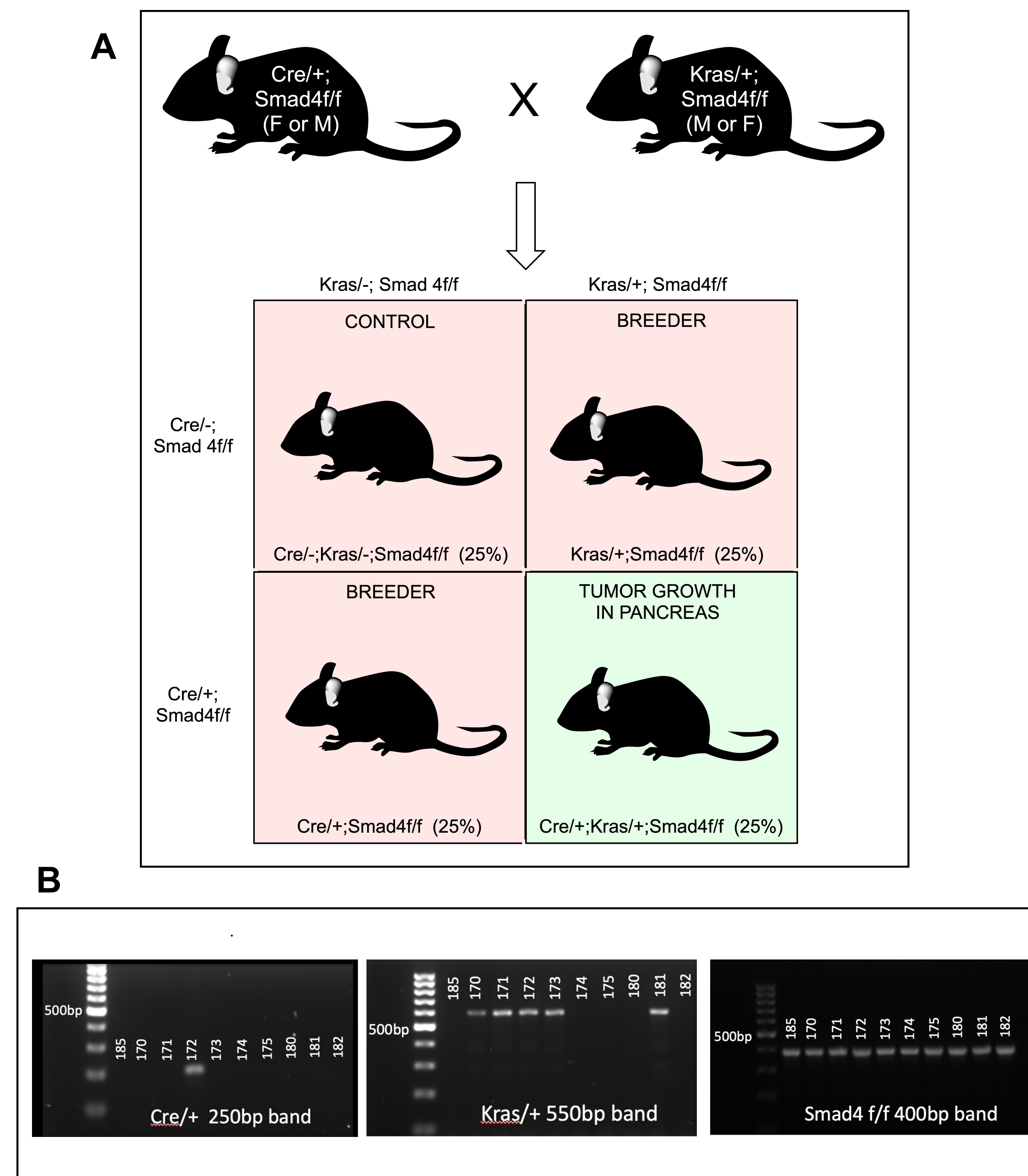
Materials and methods

Mouse selection by genotyping

Fig. 2 Breeding and genotyping. A. Mice with Cre/+;Kras/+;Smad4f/f are the only ones that will grow tumor with only a 25% chance of occurring. **B.** Genotyping through gel electrophoresis allows for the identification of the groups in the study.

V-9302 treatment *in-vivo*

- Mice with with Cre/+; Kras/+; Smad4 -/- were divided into three groups:
    Group 1 (low dose) consisted of 2 male mice and 2 female mice.
    Group 2 (control) consisted of 2 male mice.
    Group 3 (high dose) consisted of 2 male mice and 2 female mice.
- Mice were given intraperitoneal injection of V-9302:
    Group 1 – V9302, 12.5mg/kg, twice a day for 4 weeks
    Group 2 – Vehicle, 2% DMSO, twice a day for 4 weeks
    Group 3 – V9302, 75mg/kg, single dose for 4 hours

Materials and methods (cont.)
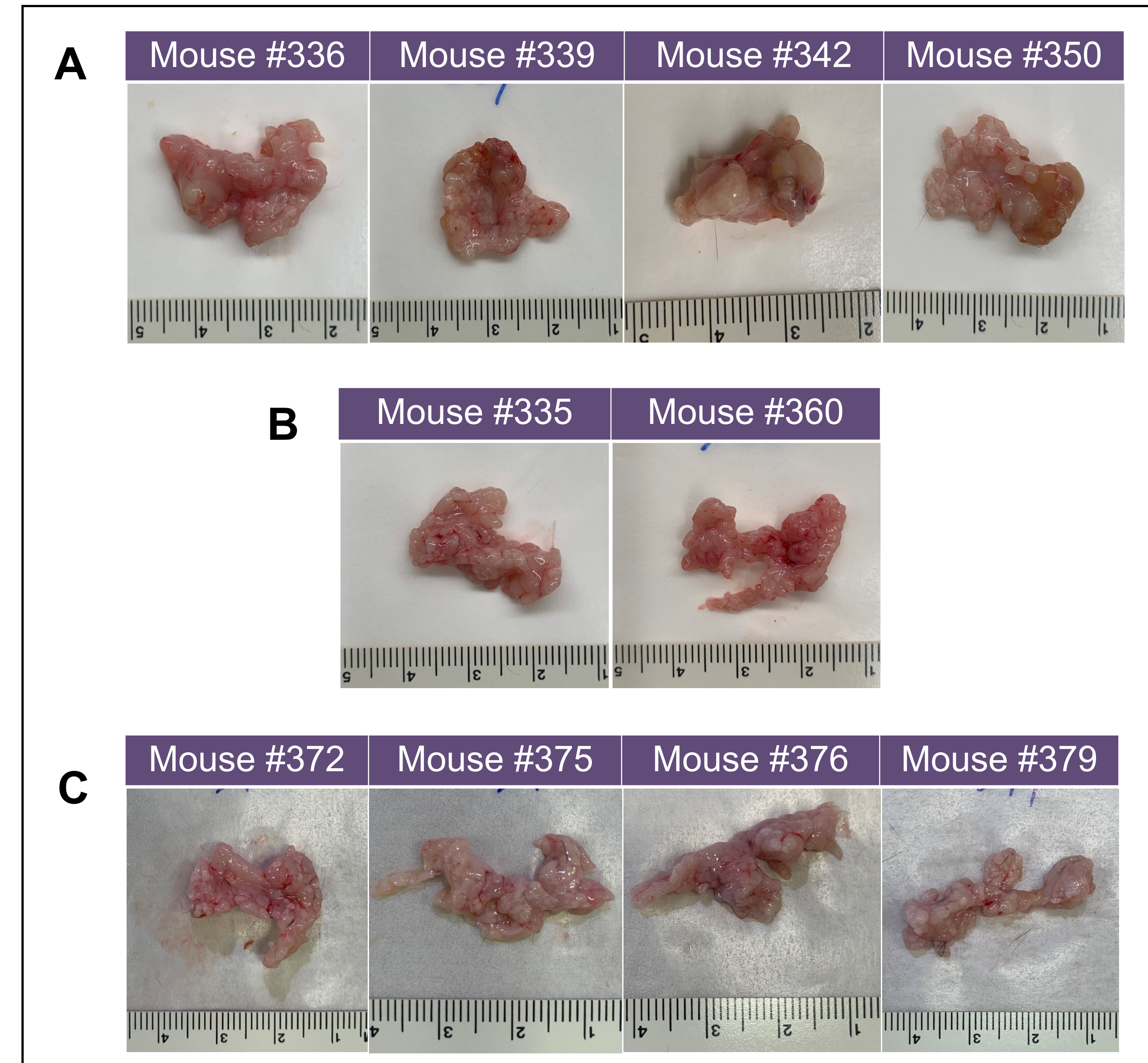
Tumor tissue collection

Fig. 3 Photographs of extracted tumor tissues. A. Group 1. **B.** Group 2 **C.** Group 3

- Mice were euthanized after treatment. Tumors were extracted, half of the tissue was preserved in 10% formalin for histological analysis, the other half was flash frozen in liquid nitrogen for 1HNMR assay. Mice were 11 weeks old at dissection in group 1 (A) and 2 (B), and 9 weeks old in group 3 (C).

Nuclear Magnetic Resonance (NMR)

- Generally utilized to determine the content, molecular structure, and purity of a sample. Allowed us to gather information on the metabolites in the tumor microenvironment.
- Prepared the sample through derivatization and buffering before following usual NMR protocol.

Hematoxylin and eosin staining

- Slides were prepared by preserving, staining, dehydration, infiltration, embedding, mirotoming, and mounting.
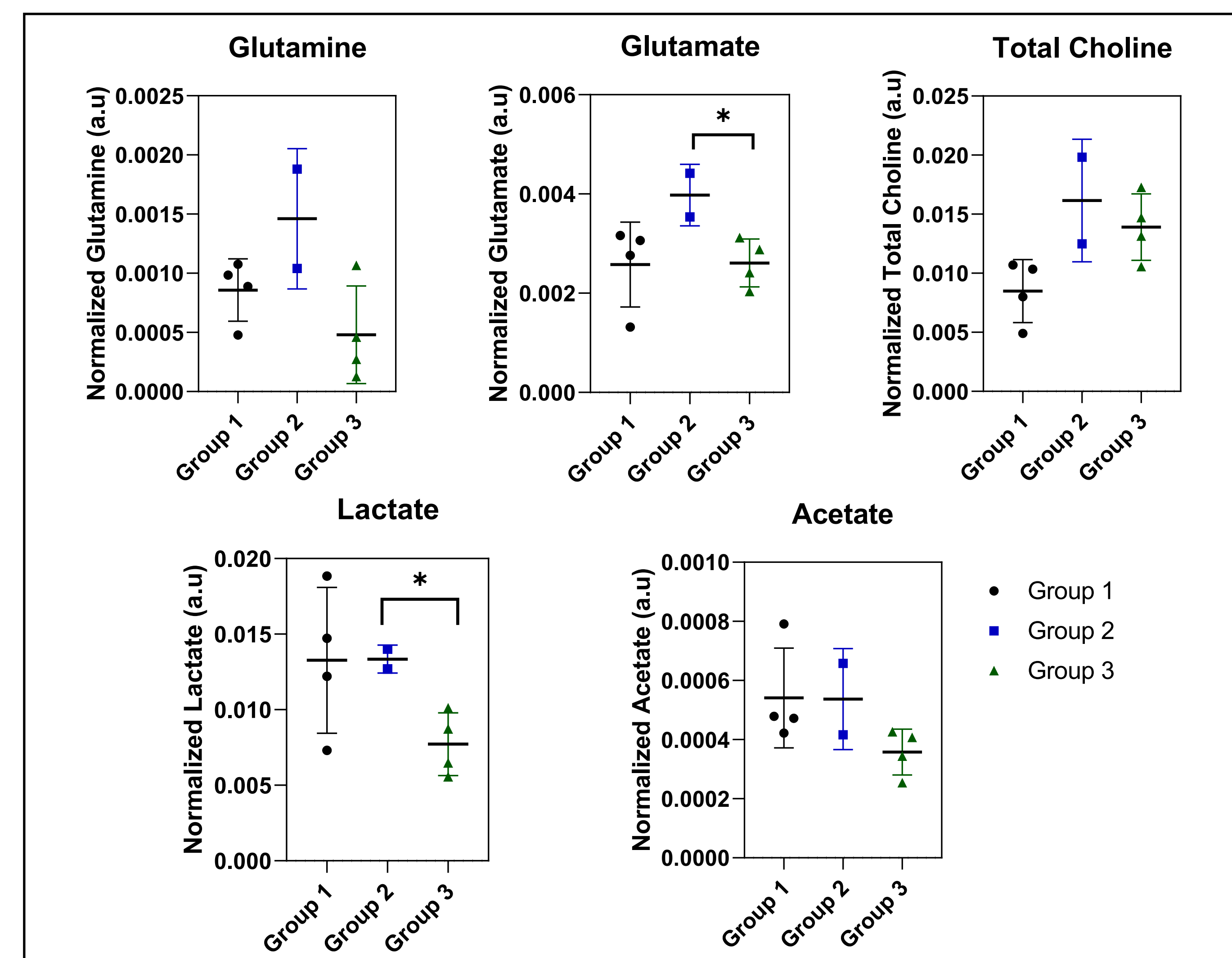
Results

Nuclear Magnetic Resonance (NMR)

Fig. 4 Measurement of Metabolites by Nuclear Magnetic Resonance (NMR). A. Glutamine, **B.** Glutamate, **C.** Total Choline, **D.** Lactate, **E.** Acetate from all three groups in the study.

Results (cont.)

- After treatment, the metabolism profile changed.
- NMR showed lower levels of glutamine, glutamate, and total choline in treated mice compared to the control group.
- Lactate was lower at elevated doses of V9302.
- Acetate displayed lower levels in group 3 while group 1 saw no change.
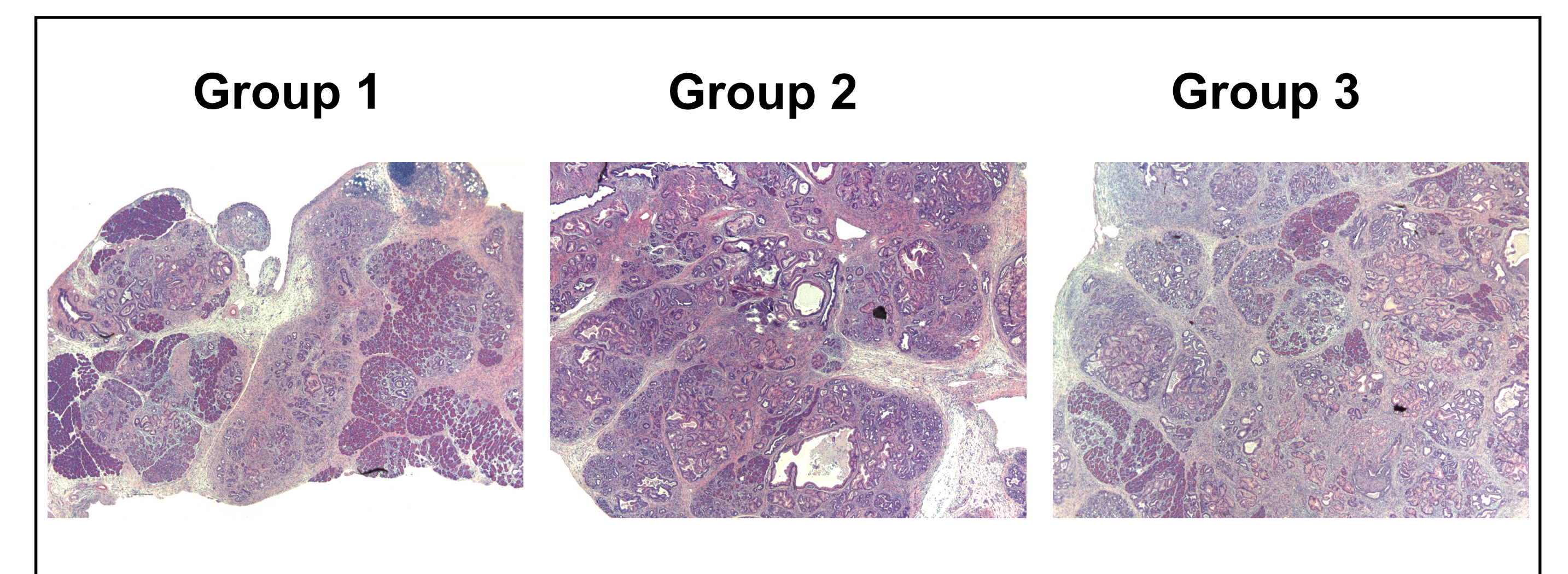
Histology

Fig. 5 Histology of cancer cells (2.5x magnification). Darker purple represents healthy pancreatic cells, lighter purple is fibrosis, and white areas inside the area of the tumor are necrosis.

- Histologically, chronic exposure of V9302 led to tumors that were less advanced than tumors from the acute dose control groups.

Conclusions

- **This investigation attempted to treat malignant cancer cells with V-9302 in different ways and found some changes in metabolic profile.**
- **It is possible that interference with inhibiting glutamine transporter changes cancer characteristics.**
- **However, it is essential to gain a better understanding of glutaminolysis as well as other cancer metabolisms before determining the most effective usage of V-9302 and what manipulations can improve its benefits in treating cancer.**
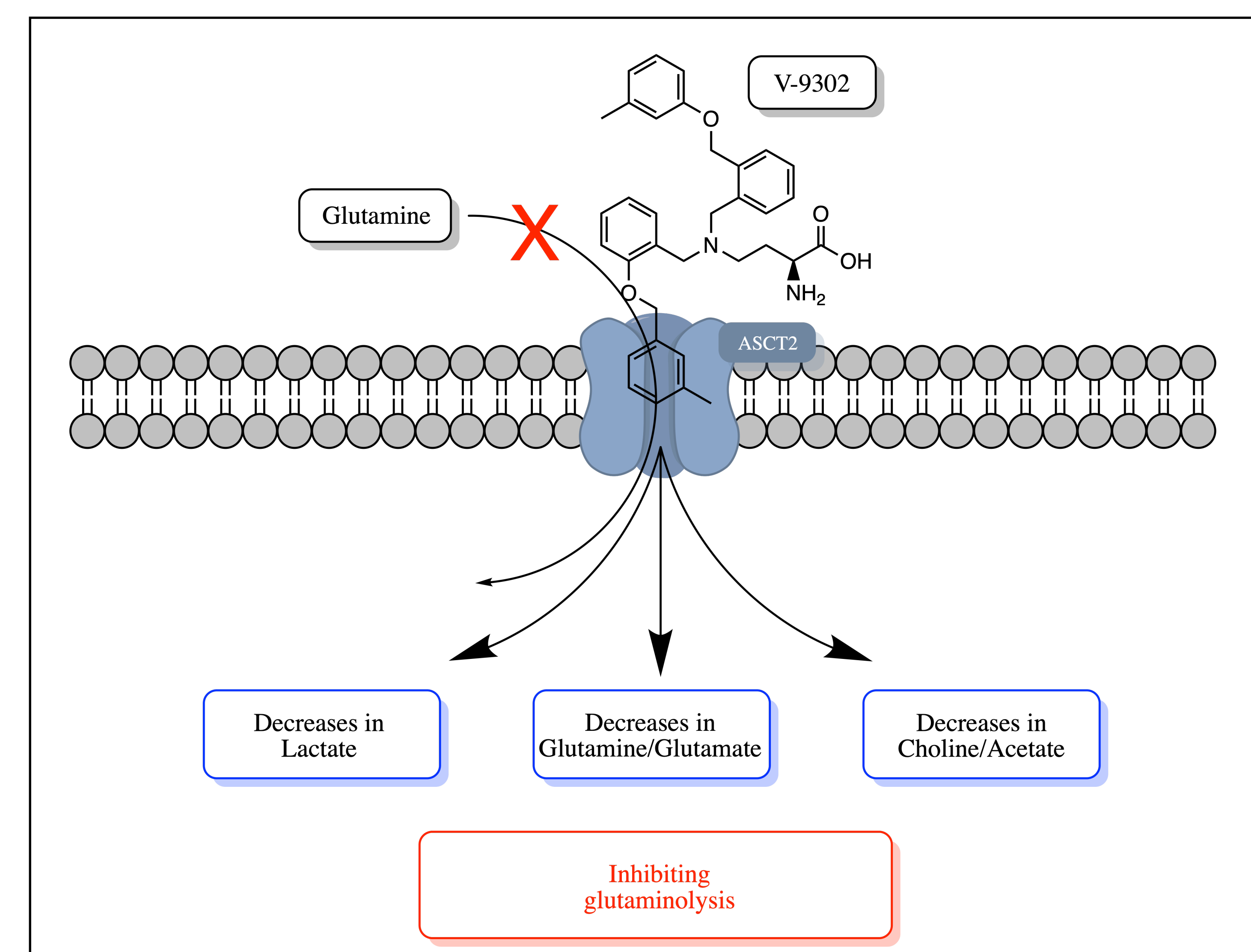

Fig. 6 Representation of general results and takeaways of V-9302 treatment. V-9302 was able to inhibit glutaminolysis and thus decreases were noticed in lactate, glutamine, glutamate, choline, and acetate.

References

1) Choi, Y. K., et al. G. Targeting Glutamine Metabolism for Cancer Treatment. *Biomolecules & therapeutics*, 26(1), 19–28 (2018).
2) Jin, L., et al. Glutaminolysis as a target for cancer therapy. *Oncogene* 35, 3619–3625 (2016).
3) Schulte, M. L., et al. Pharmacological blockade of ASCT2-dependent glutamine transport leads to antitumor efficacy in preclinical models. *Nature medicine*, 24(2), 194–202 (2018).