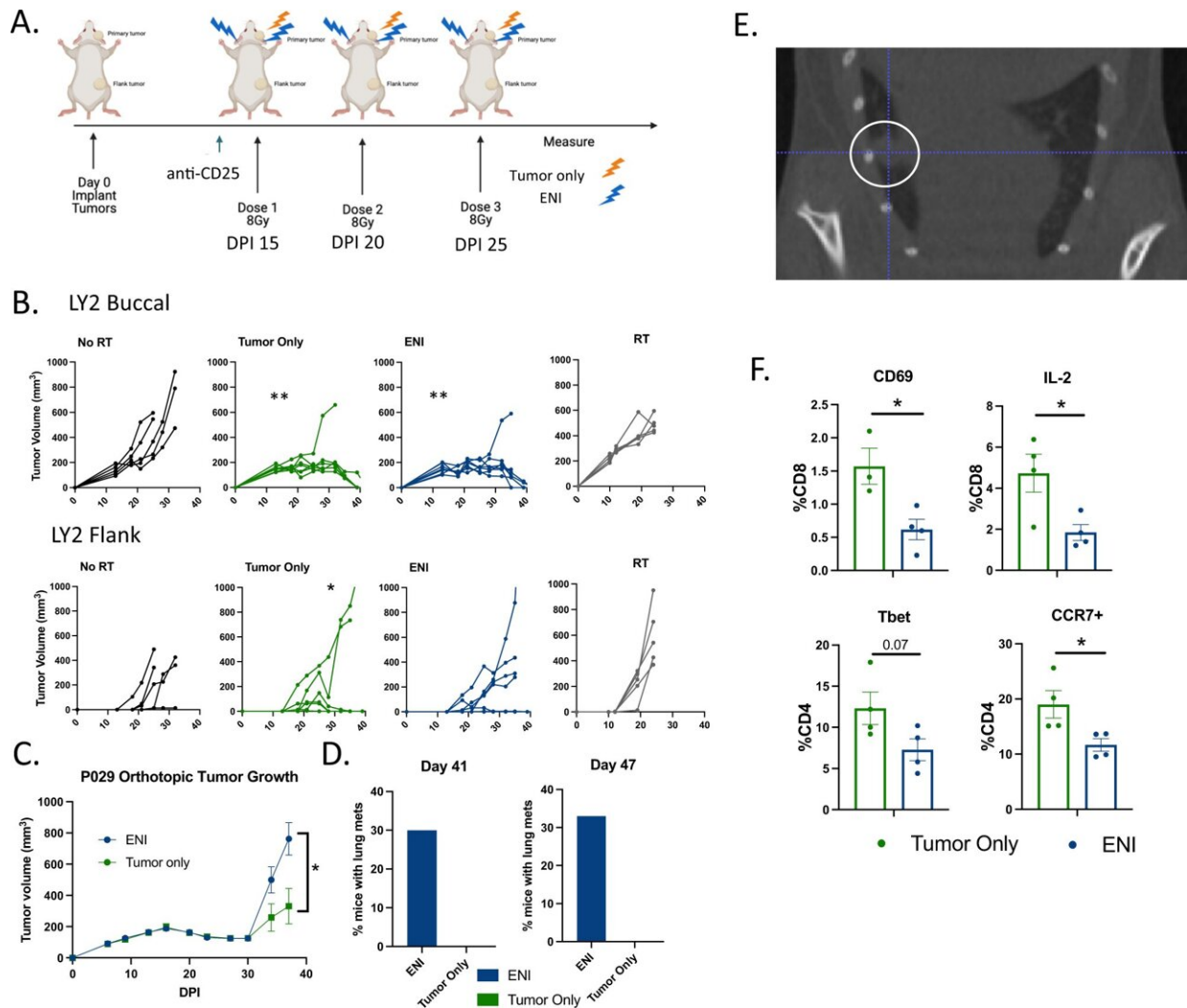


# Selective nodal radiation may be a more effective approach in cancer treatment

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ENI ablates the immune response to combined radiation and immunotherapy. A Schematic of the experimental design for gross tumor irradiation with or without elective nodal irradiation (ENI). Mice were implanted both in the buccal and in

the flank on day 0 post-implantation (DPI). Stereotactic body irradiation (SBRT) was given when tumors reached  $\sim 150\text{mm}^3$  and anti-CD25 was given a day before SBRT. Created with BioRender.com. B Tumor growth curves, from the experiment depicted in (A), Buccal tumor (top) and flank tumor growth curves (bottom) for mice treated with anti-CD25 ( $n = 5$ ), anti-CD25 and tumor only SBRT ( $n = 7$ ), anti-CD25 and ENI ( $n = 7$ ), and tumor only SBRT alone ( $n = 5$ ). C Buccal tumor growth curves for mice implanted with the P029 cell line ( $n = 10$  per group). Mice were implanted in the buccal on day 0 post-implantation (DPI). SBRT was given when tumors reached  $\sim 150\text{mm}^3$  and anti-CD25 was given a day before SBRT and once a week thereafter. The doses of SBRT were spaced by 4–5 days. D Quantification of the percentage of mice with P029 tumors that had radiographically detectable lung metastases at days 41 (ENI,  $n = 10$ ; tumor only,  $n = 7$ ) and 47 (ENI,  $n = 9$ ; tumor only,  $n = 7$ ) post-tumor cell implantation. Lung metastases were evaluated by microCT images. E A representative microCT image of a lung metastasis identified in a mouse treated with ENI in the P029 model. A metastasis is highlighted with a white circle. F Flow cytometry analysis of blood taken from mice at day 24 DPI in the experiment depicted in (A) (ENI,  $n = 4$ ; tumor only,  $n = 4$ ). CD8 T cells were defined as  $\text{CD45}^+\text{CD3}^+\text{CD8}^+$  and CD4 T cells were defined as  $\text{CD45}^+\text{CD3}^+\text{CD4}^+$ . For tumor growth at different time points, 3 or more groups differences were determined by a One-Way ANOVA test with Tukey's post hoc comparisons, with only 2 groups a Two-Way ANOVA was used. To test if there is a difference between tumor only SBRT and ENI treatment groups in reducing the number of mice that grew flank tumors, we used a Fischer's Exact test. For the flow cytometry analysis, a two-tailed student's t-test was used. Significance was determined if the p-value was

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