

Immune Checkpoint Inhibitor–mediated Cancer Theranostics with radiolabeled anti-Granzyme B Peptide

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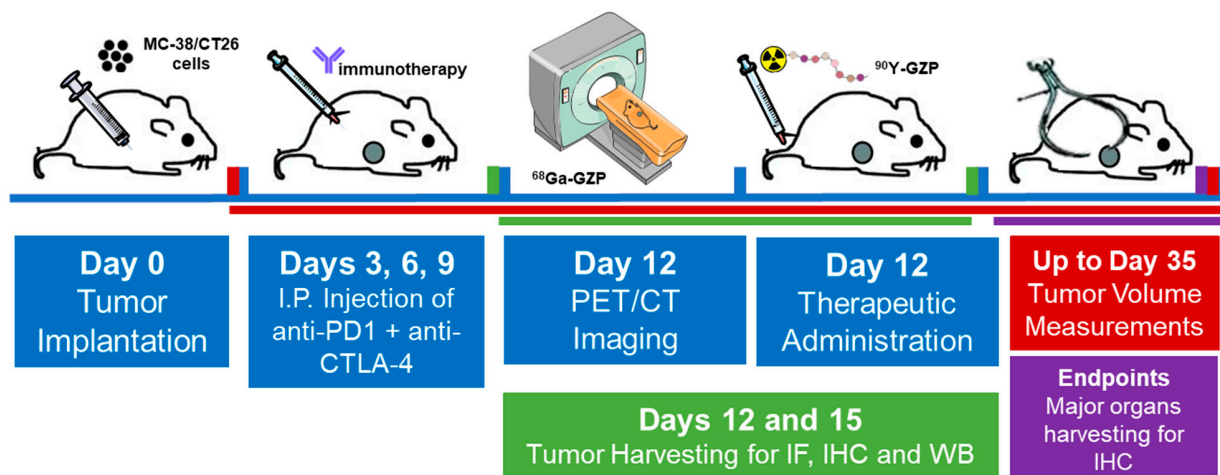


Figure S1. Schematic representation of studies workflow. MC38 and CT26 xenografts were implanted at Day 0. ICI injections were carried out on days 3, 6 and 9. ^{68}Ga -GZP and PET imaging or ^{90}Y -GZP for therapy studies were administered on Day 12. Tumor harvesting for IF, IHC and WB was carried out 3 days after last treatment (days 12 or 15).

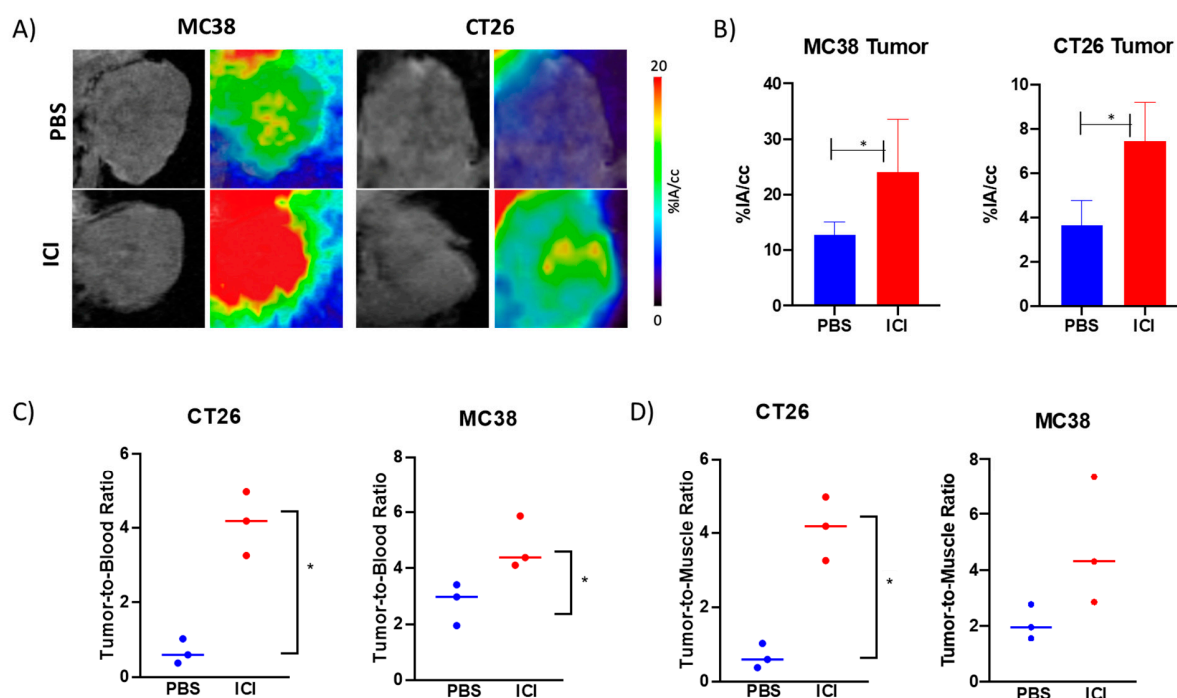


Figure S2. A) PET/MR insert of tumor region and B) ROI values of tumor uptake. C) Tumor-to-blood and D) Tumor-to-Muscle ratios for both tumor models. * $p < 0.05$

Table S1. Groups and Doses for the Therapeutic Study

Group	Treatment	Administration	Day of administration
PBS	PBS (100μL)	i.v.	12
ICI	Anti-PD1 (250μg) Anti-CTLA-4 (100μg)	i.p.	3, 6 and 9
ICI + GZP	Anti-PD1 (250μg) Anti-CTLA-4 (100μg) GZP	i.p. (ICI) i.v. (GZP)	3, 6 and 9 (ICI) 12 (GZP)
ICI + ⁹⁰ Y-GZP (Low Dose)	Anti-PD1 (250μg) Anti-CTLA-4 (100μg) ⁹⁰ Y-GZP (2.2MBq)	i.p. (ICI) i.v. (⁹⁰ Y-GZP)	3, 6 and 9 (ICI) 12 (⁹⁰ Y-GZP)
ICI + ⁹⁰ Y-GZP (High Dose)	Anti-PD1 (250μg) Anti-CTLA-4 (100μg) ⁹⁰ Y-GZP (22.2MBq)	i.p. (ICI) i.v. (⁹⁰ Y-GZP)	3, 6 and 9 (ICI) 12 (⁹⁰ Y-GZP)
ICI + ⁹⁰ Y (High Dose)	Anti-PD1 (250μg) Anti-CTLA-4 (100μg) ⁹⁰ Y (22.2MBq)	i.p. (ICI) i.v. (⁹⁰ Y)	3, 6 and 9 (ICI) 12 (⁹⁰ Y)

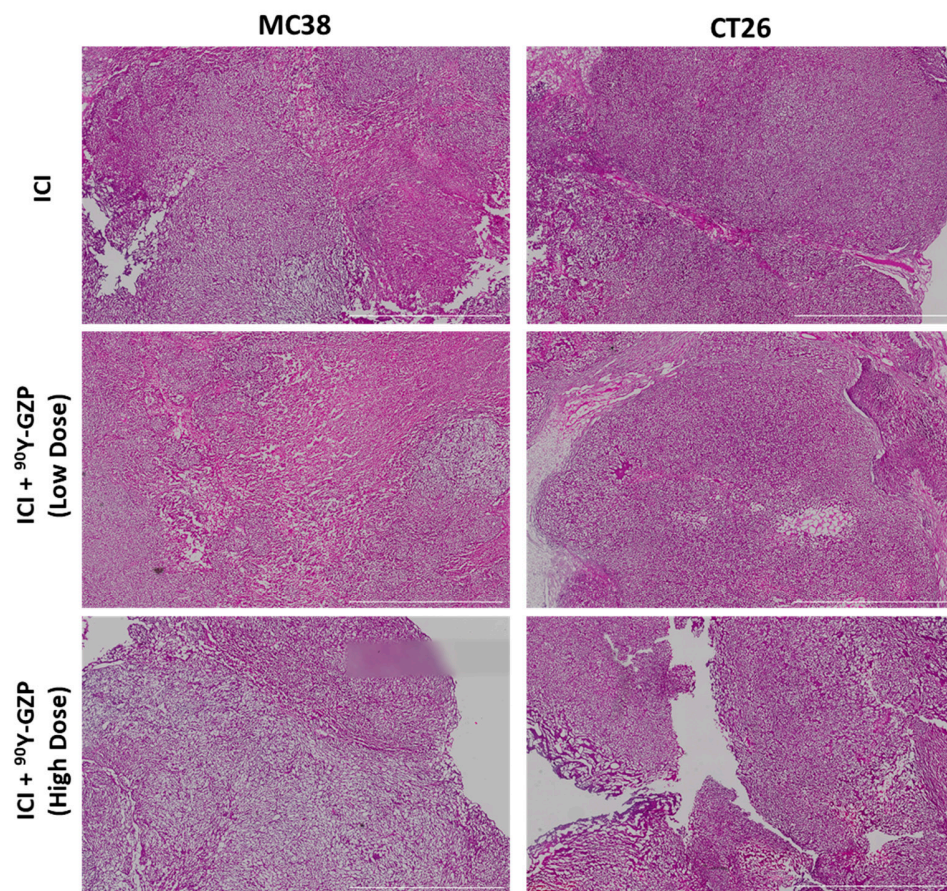


Figure S3. H&E staining of MC38 and CT26 tumor tissue, collected at day 15 demonstrating increased necrotic areas in animals injected with ICI + ⁹⁰Y-GZP (high dose).

CT26

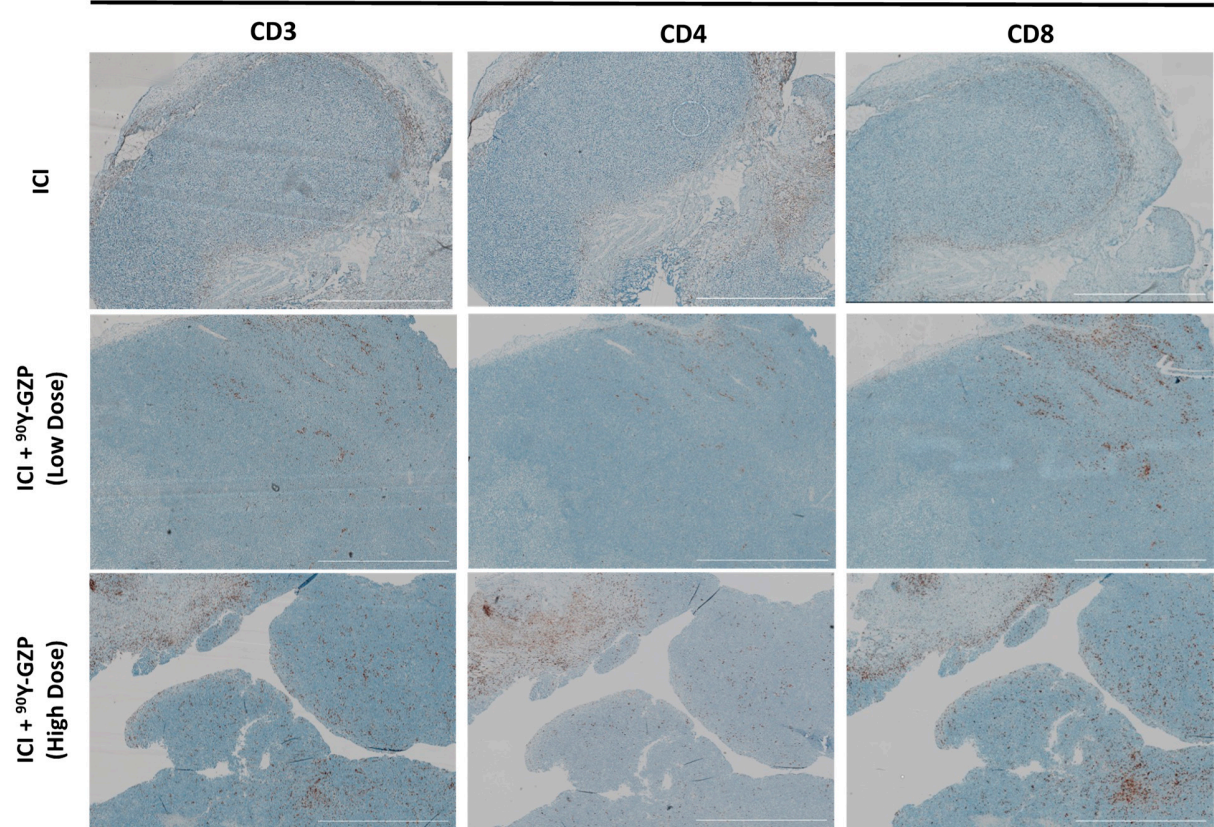


Figure S4. Immunohistochemistry staining of CD3, CD4 and CD8 on CT26 tumor tissue collected 3 days after therapeutic administration (day 12 for ICI and day 15 for ICI + ⁹⁰Y-GZP).

MC38

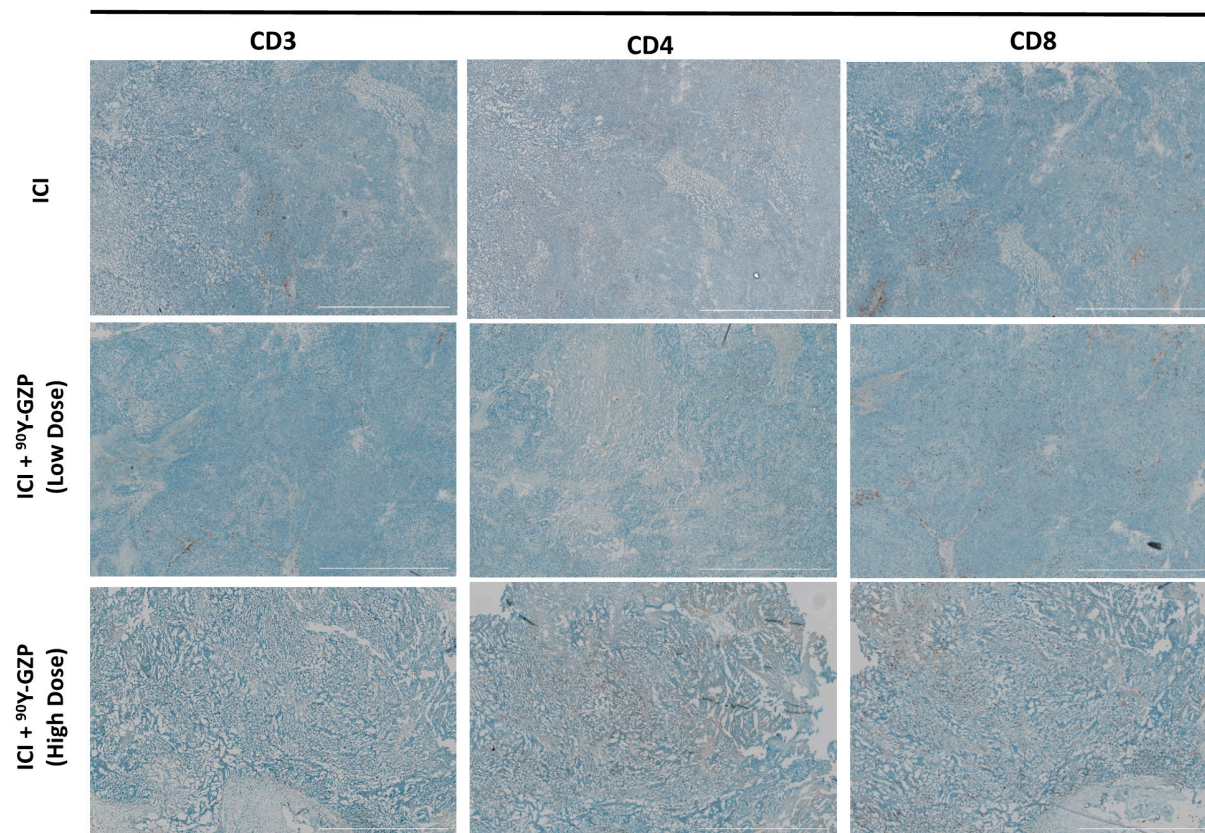


Figure S5. Immunohistochemistry staining of CD3, CD4 and CD8 on MC38 tumor tissue collected 3 days after therapeutic administration (day 12 for ICI and day 15 for ICI + ⁹⁰Y-GZP).

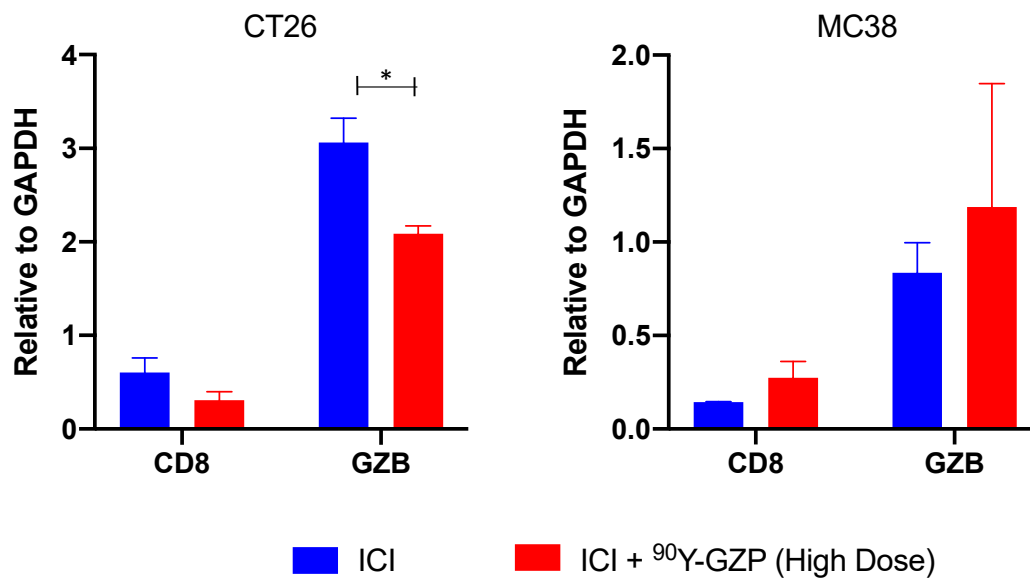


Figure S6. Western Blot quantitative data depicting CD8 and GZB expression in CT26 and MC38 tumor bearing mice that received either ICI or ICI + ⁹⁰Y-GZP (High dose). *p < 0.05

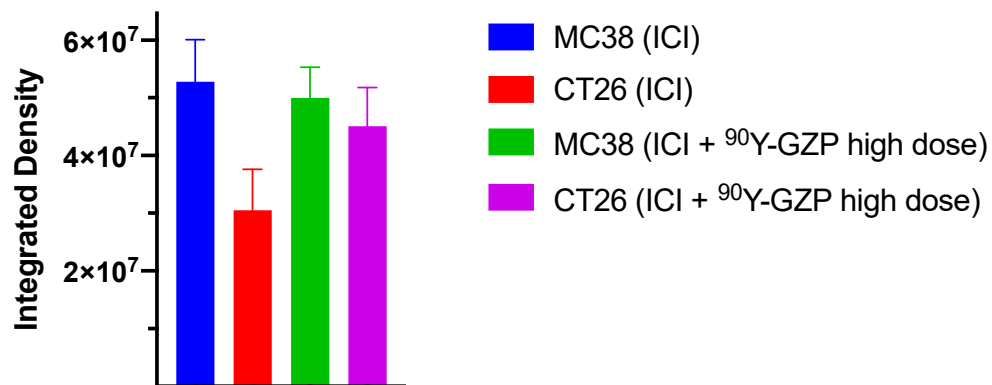


Figure S7. Semi-quantitative analysis of immunofluorescence staining of granzyme B of tumor samples of CT26 and MC38 tumor bearing mice after ICI and ICI + ⁹⁰Y-GZP high dose.

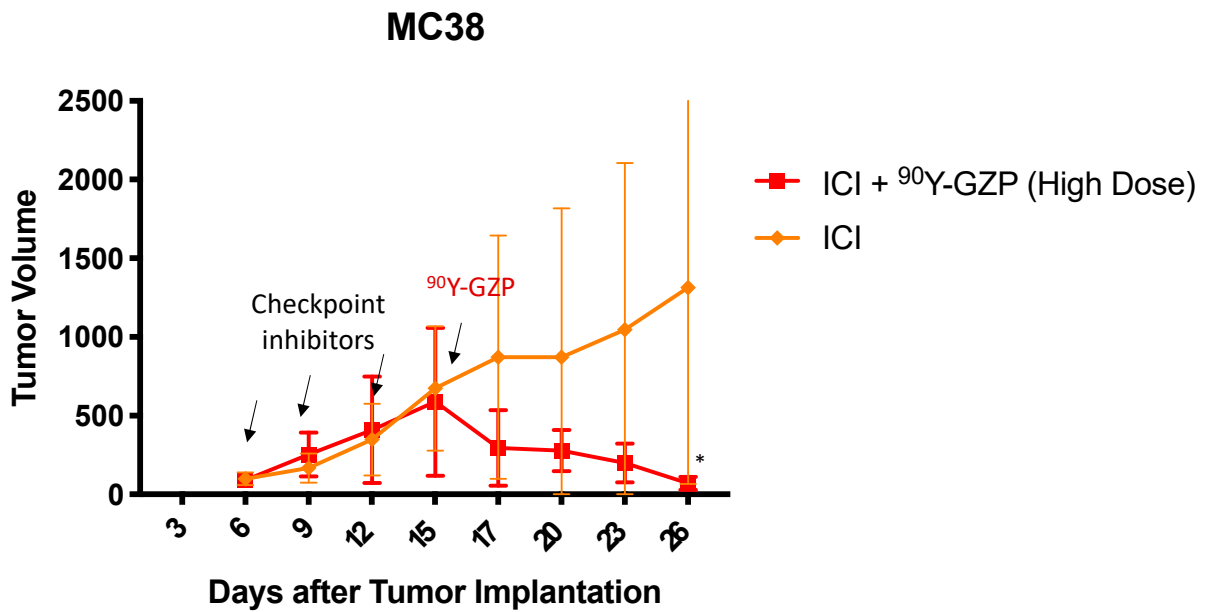


Figure S8. Tumor Growth Curves of MC38 tumor bearing mice after administration of the different therapeutic groups. Checkpoint inhibitors were given days 6, 9 and 12 after tumor implantation and ⁹⁰Y-GZP was given on day 15 (indicated by arrows). *Statistically significant than all the other groups ($p < 0.05$).