COX-2/PGE2-driven, photoimmunotherapy-induced edema is reduced by prophylactic NSAIDs in mouse tumor models

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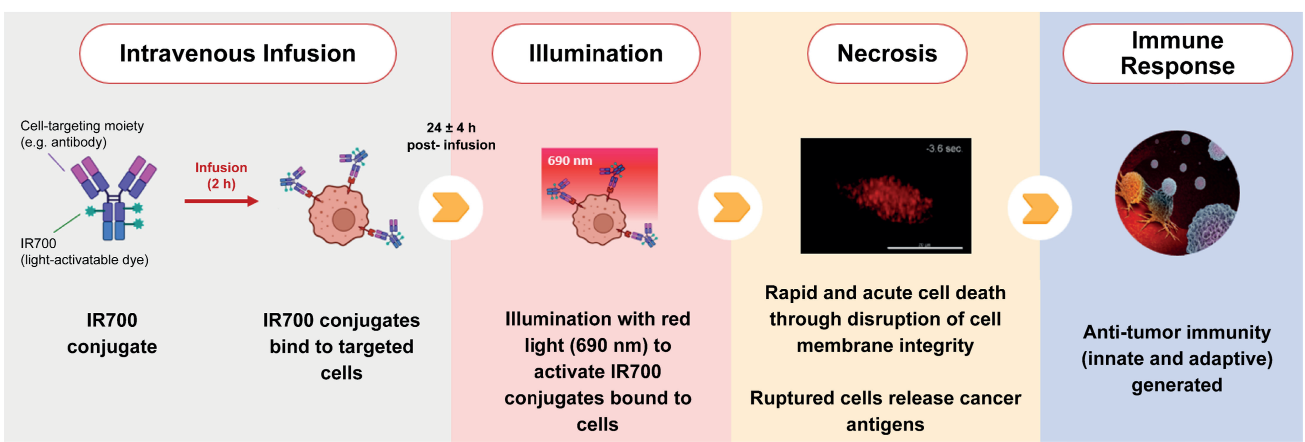
Background

- ASP-1929 photoimmunotherapy (PIT) is an investigational therapy that combines binding of ASP-1929, an anti-EGFR antibody conjugated to IRDye®700DX (IR700), with red light laser focal illumination for selective cell killing.
- ASP-1929 PIT has shown clinical activity for head and neck cancer (HNC) and although it has a manageable safety profile, edema is a commonly reported adverse event, with severe laryngeal edema noted in some patients.[1–4]
- Thus, a critical need exists to reduce ASP-1929 PIT-associated edema without compromising therapeutic efficacy.
- In this poster we elucidate the underlying mechanism of PIT-induced edema *in vitro* and *in vivo*, expand upon our previous finding for the use of prophylactic COX-2 inhibition to reduce edema, and translate our findings to a clinically relevant HNC xenograft model.

Figure 1. Mechanism of action of ASP-1929 photoimmunotherapy



*Based on pre-clinical observations

Methods

- CT26-EphA2 or LL/2-EphA2 cells were incubated with anti-EphA2-IR700 ± light and evaluated for changes in COX-2 expression using real-time PCR and Western blot.
- Considering prostaglandin E2 (PGE2) is downstream of COX-2 and a known effector of vascular permeabilization, we evaluated PGE2 secretion in cell culture media by ELISA.
- To evaluate the effect of pan COX vs selective COX-2 inhibition on edema reduction post-PIT, mice bearing CT26-EphA2 or LL/2-EphA2 tumors were administered anti-EphA2-IR700 ± light and the absence or presence of COX inhibitors.
- To confirm the role of COX-2, we evaluated tumor lysates to determine if there was an increase in COX-2 expression post-PIT. Secretion of PGE2 was evaluated by measuring PGE2 metabolites (PGEM) in circulation post-PIT.
- Lastly, we developed a HNC xenograft model using human hypopharyngeal cancer cells and evaluated the effect of COX inhibition on edema formation post-PIT.

Conclusions

- Prophylactic administration of NSAIDs reduced PIT-induced edema in two syngeneic mouse models.
- *In vitro* and *in vivo* data in the CT26-EphA2 and LL/2-EphA2 cell lines suggest PGE2 secretion as a potential driver of PIT-induced edema.
- COX-2 selective and pan-COX inhibitors showed similar reductions in PIT-induced edema formation.
- Prophylactic NSAID administration in the HNC xenograft model, HPCM2, reduced PIT-induced edema without affecting therapeutic efficacy.
- In conclusion, this is the first report showing increased COX-2/PGE2 post-PIT, linking PGE2 secretion with edema generation, and suggests COX-2 inhibition to combat ASP-1929 PIT-induced edema may lead to improved patient safety. The translation of this result to the clinical setting is under investigation at treatment centers throughout Japan (jRCTs031250140).

References

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3. Kushihashi Y, et al. Case Rep Oncol 2024; 17:471–6.
4. Cognetti DM, et al. Head Neck 2026; 48: 160–74.

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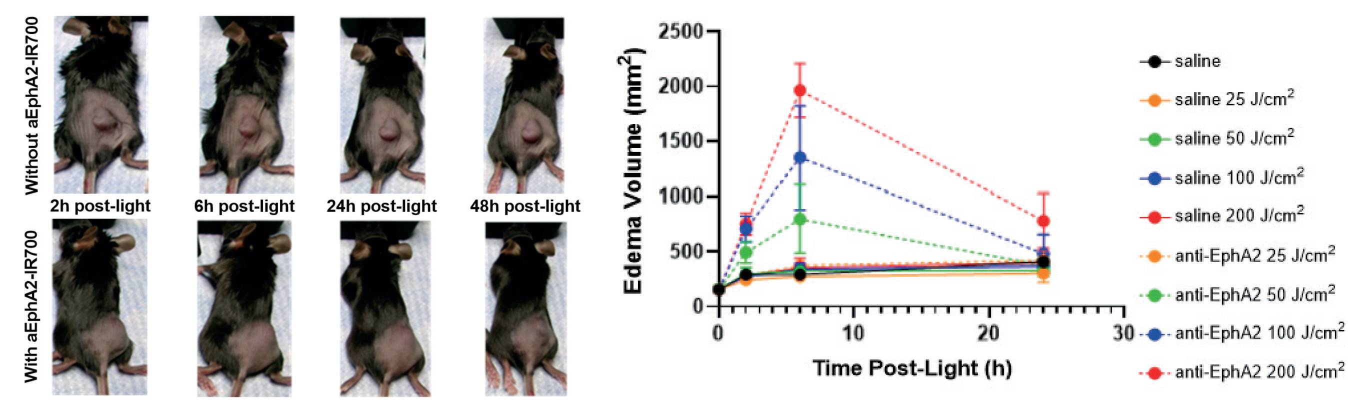
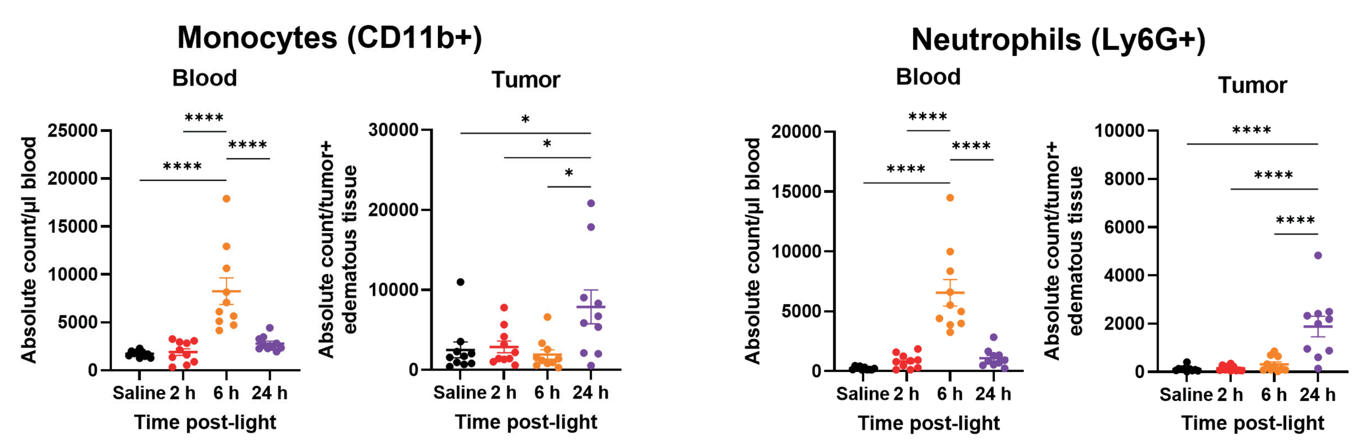
Results

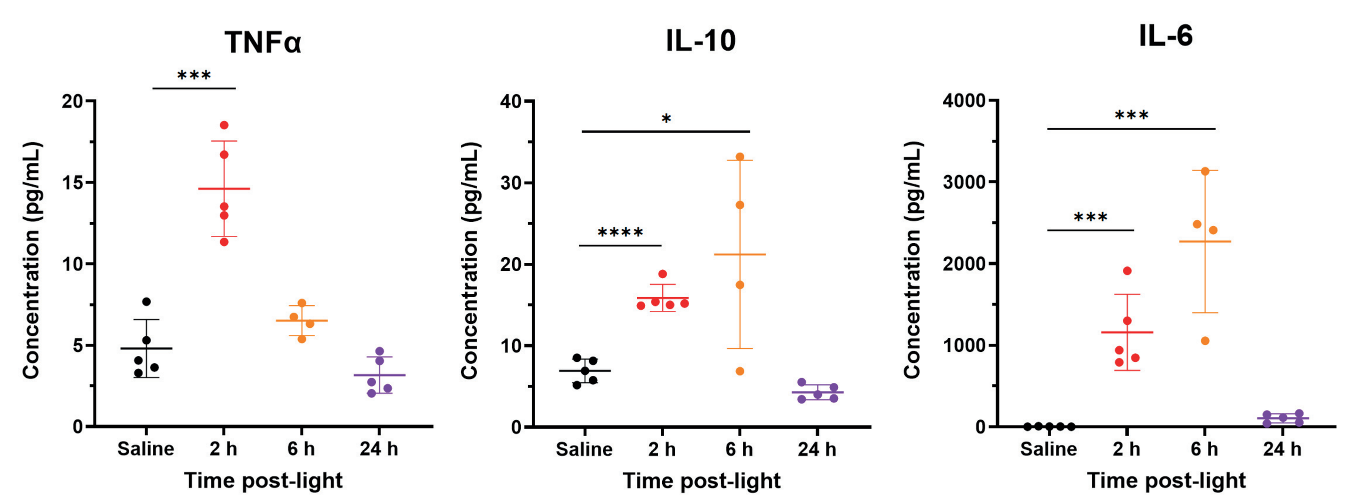
Figure 2. Kinetics of edema formation and related immune response post-PIT in LL/2-EphA2 tumor-bearing mice



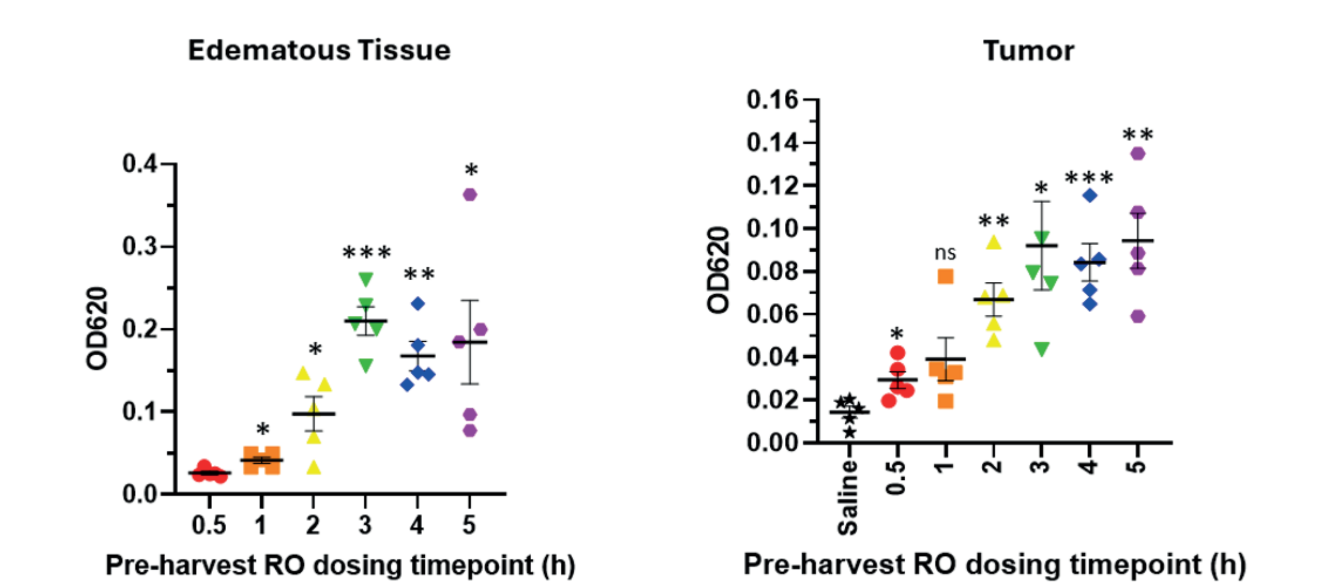
Representative time-course images and quantification of edema formation in LL/2-EphA2 syngeneic mice following anti-EphA2-IR700 (aEphA2-IR700) PIT treatment using light doses between 25 to 200 J/cm^2 at 150 mW/cm^2 (n=10/group).



Induction of edema in PIT-treated mice corresponded with increases in monocyte and neutrophil cell populations in blood that were 500-fold over populations in control mice ($p<0.0001$) as detected by flow cytometry (n=10/group). A delayed increase in these immune cell populations followed in the TME at 24 hours post-PIT (n=10/group). *=$p<0.05$, ****=$p<0.0001$

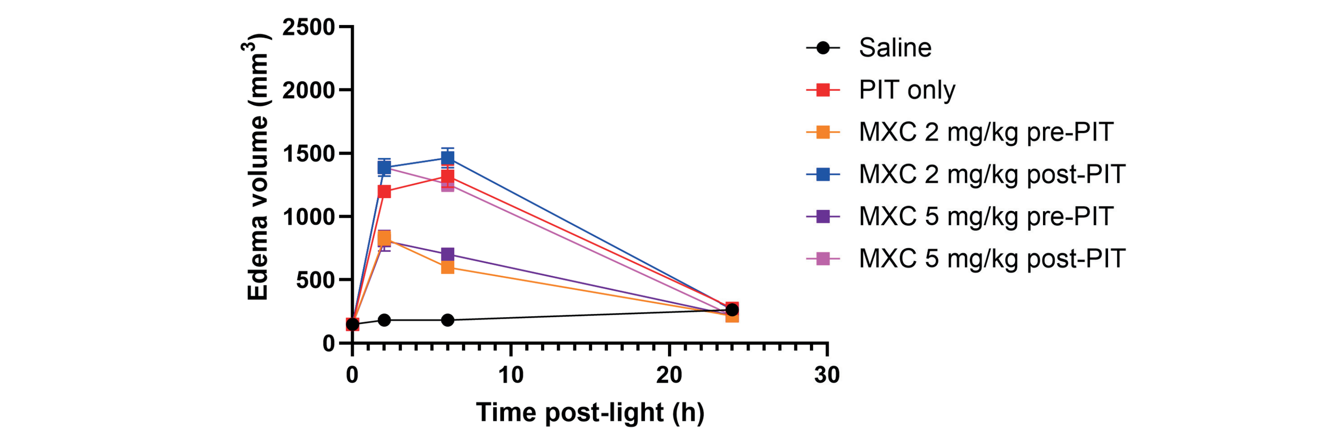


Similarly, a cytokine response followed edema kinetics, with statistically significant increases in the inflammatory mediators TNFα ($p<0.001$), IL-10 ($p<0.05$), and IL-6 ($p<0.001$) in plasma throughout the onset and peak of edema formation as measured by sandwich immunoassay (Meso Scale Discovery [MSD]) (n=5/group). *=$p<0.05$, ***=$p<0.001$, ****=$p<0.0001$

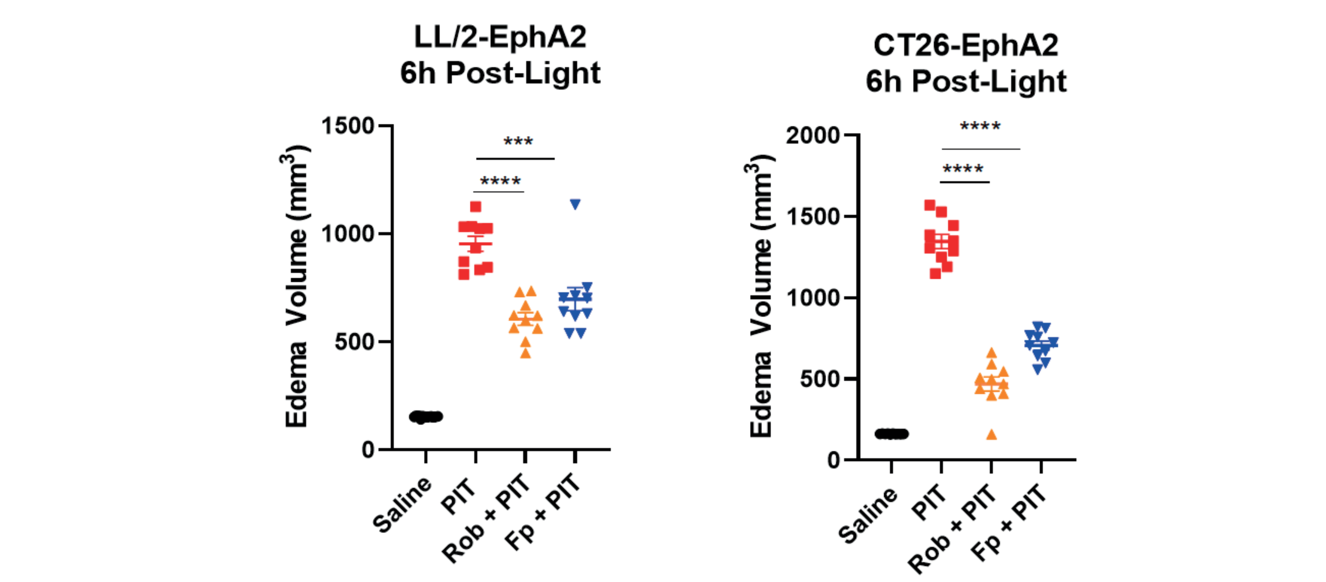


An immediate increase in Evans blue levels was measured in both edematous tissue (left panel) and LL/2-EphA2 tumor tissue (right panel) out to 3 hours post-PIT, at which time the absorbance signal plateaued (n=5/group). ns=not significant, *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$

Figure 3. Prophylactic administration of NSAIDs reduced PIT-induced edema in two syngeneic mouse models

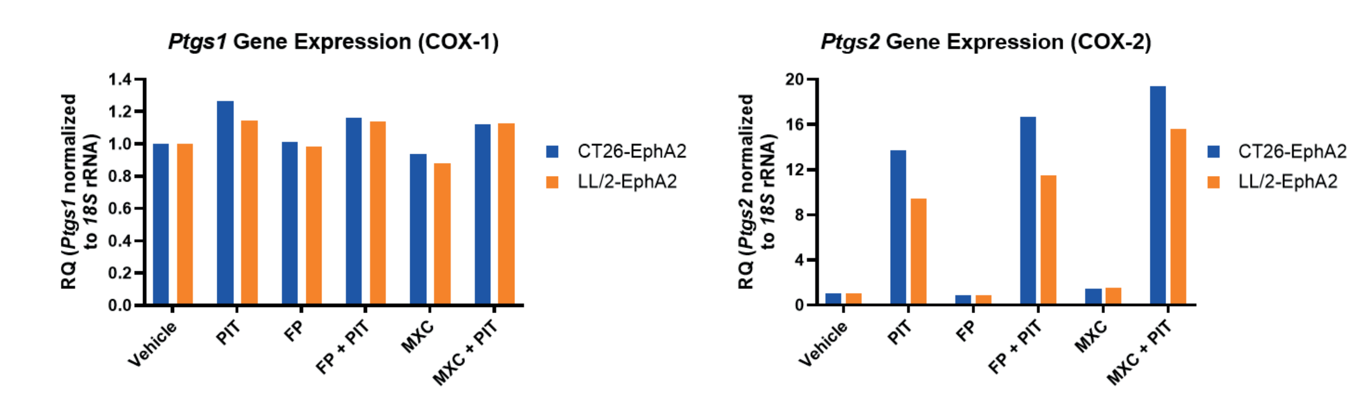


Meloxicam (MXC) dosed at 2 and 5 mg/kg pre-PIT (-2 hours), but not post-PIT (+3 hours), reduced edema in LL/2-EphA2 tumor-bearing mice compared to mice that received PIT alone at a light dose of 50 J/cm^2 (n=10/group; $p<0.0001$).

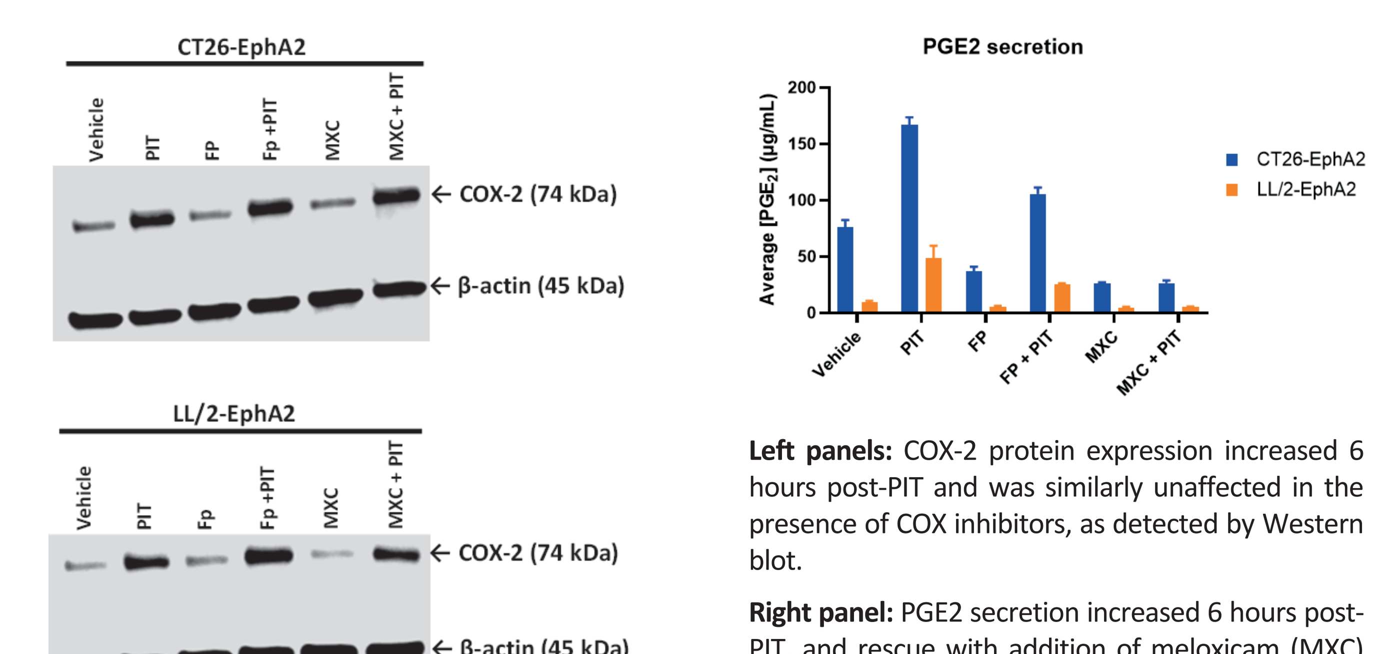


The selective COX-2 inhibitor robenacoxib (Rob) dosed at 50 mg/kg and the pan-COX inhibitor flurbiprofen (Fp) dosed at 10 mg/kg showed similar reductions in PIT-induced edema in both LL/2-EphA2 (left panel) and CT26-EphA2 (right panel) syngeneic mouse models at a light dose of 50 J/cm^2 (n=10/group). ***=$p<0.001$, ****=$p<0.0001$

Figure 4. *In vitro* data in the CT26-EphA2 and LL/2-EphA2 cell lines suggest PGE2 secretion as a potential driver of PIT-induced edema



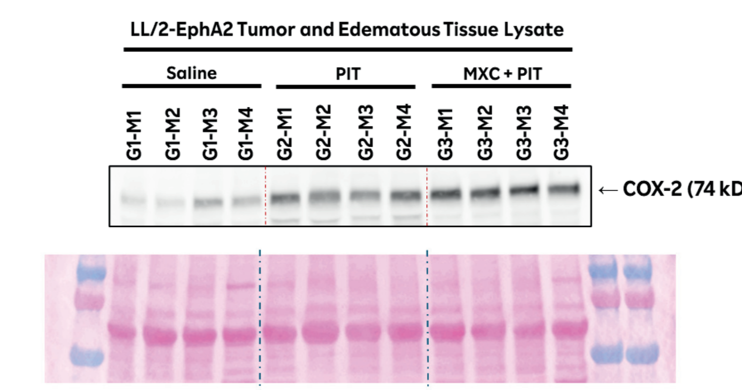
No change in *Ptgs1* (COX-1 gene) transcription was observed 6 hours post-PIT, as detected by RT-qPCR (n=3). *Ptgs2* (COX-2 gene) transcription increased post-PIT, but no change was observed with prophylactic (1 hour prior to PIT) addition of 10 μM meloxicam (MXC) or 100 nM flurbiprofen (Fp) (n=3).



Left panels: COX-2 protein expression increased 6 hours post-PIT and was similarly unaffected in the presence of COX inhibitors, as detected by Western blot.

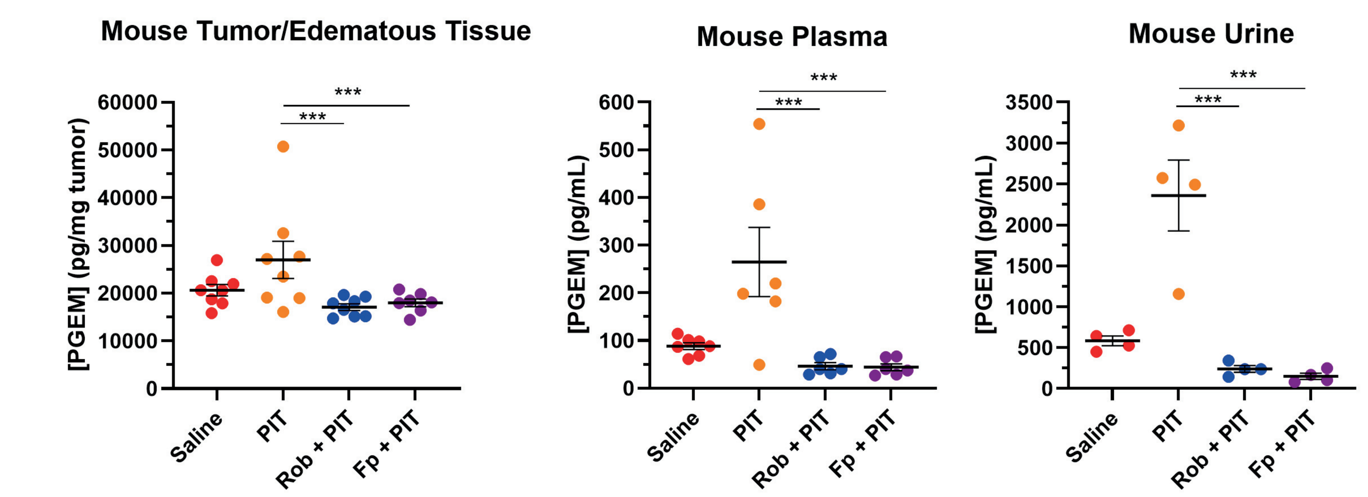
Right panel: PGE2 secretion increased 6 hours post-PIT, and rescue with addition of meloxicam (MXC) and flurbiprofen (Fp) was detected by ELISA (n=3).

Figure 5. Results in LL/2-EphA2 tumor-bearing mice support the COX-2/PGE2 pathway as the mechanistic driver of PIT-induced edema and show similar reductions in PGE2 metabolites with pan-COX and selective COX-2 inhibition



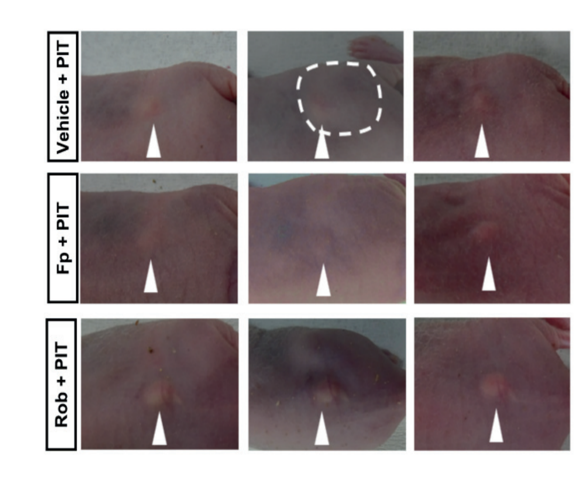
An increase in COX-2 protein expression in tumors and edematous tissue post-PIT was visualized by Western blot (top panel) and Ponceau staining (bottom panel). Samples were analyzed 6 hours post-PIT at a light dose of 50 J/cm^2 in LL/2-EphA2 tumor-bearing mice.

Consistent with *in vitro results*, pre-treatment with the COX-2 inhibitor meloxicam (MXC) does not rescue PIT-induced COX-2 expression *in vivo*.



Both robenacoxib (Rob) and flurbiprofen (Fp) showed statistically significant ($p<0.001$) and similar reductions of PGE2 metabolites (PGEM) in LL/2-EphA2 mouse tumor (n=8), plasma (n=6), and urine (n=8 double-caged mice for 4 readouts) samples 24 hours post-PIT at a light dose of 50 J/cm^2. ***=$p<0.001$

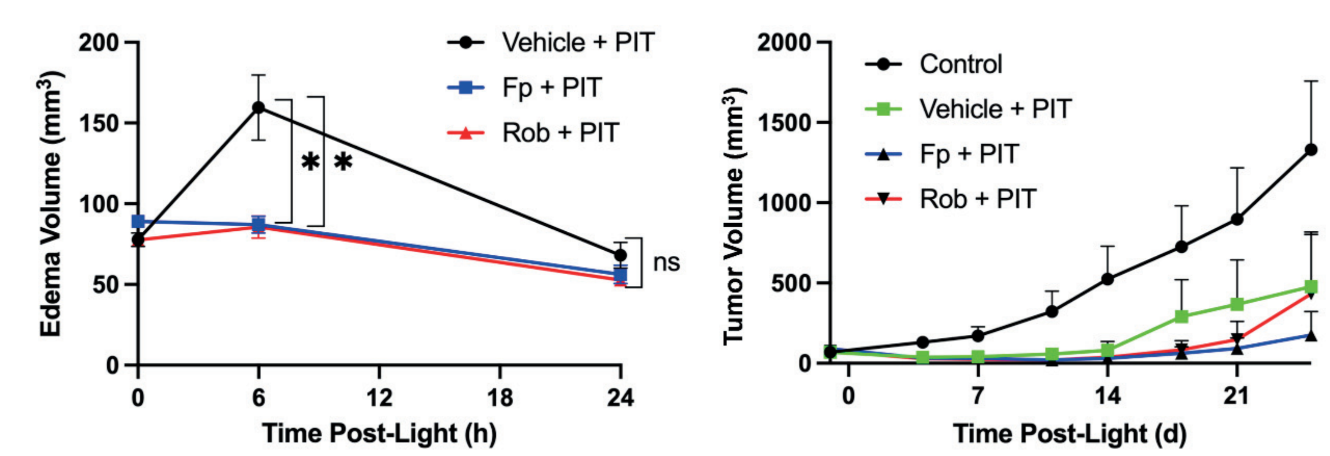
Figure 6. Reduction in edema with prophylactic administration of NSAIDs in the clinically relevant HPCM2 xenograft model



Representative time-course images of tumors prior to treatment (left images) and following cetuximab-IR700 PIT at peak (6 hours post-PIT; center images) and resolution (24 hours post-PIT; right images) of edema formation.

HPCM2 tumor-bearing mice were dosed with cetuximab-IR700, and flurbiprofen (Fp) or robenacoxib (Rob), 1 hour prior to PIT (50 J/cm^2 at 150 mW/cm^2).

Arrows mark tumor sites, and the white dotted circle outlines the diffuse margin of tumor/edema 6 hours post-PIT in a representative mouse that received PIT but no COX inhibitor.



Edema volume peaked at 6 hours post-PIT and showed a statistically significant reduction with both COX inhibitors compared with the vehicle control group (n=6/group). Statistical significance was determined by two-way RM ANOVA followed by Tukey's test. ns=not significant, *=$p<0.05$

Treatment efficacy, as measured by tumor volume post-PIT, was not affected by addition of COX inhibitors flurbiprofen (Fp) and robenacoxib (Rob). Volume was measured twice a week post-PIT for 4 weeks, and no significant differences were detected at any timepoint. Cetuximab-IR700 was administrated on day -1, and light was applied on day 0.

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