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Targeting PAI-1 as a Novel Strategy to Enhance Radiotherapy Efficacy in Non-Small Cell Lung Cancer

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Background

- Concurrent chemoradiotherapy (CRT) followed by anti-PD-L1 antibody therapy is the current standard treatment for locally advanced non-small cell lung cancer (NSCLC), yet **locoregional recurrence remains common**, highlighting the need to improve local control.

Spigel DR, et al. J Clin Oncol 2022;40:1301-1311.

- Novel strategies could improve radiotherapy efficacy and prevent local recurrence.

Background

- Emerging evidence suggests that **radiation-tolerant persister cells (RTPs)**, characterized by a reversible, non-genetic, slow-cycling state after fractionated radiotherapy (FRT), may play an important role in radiation tolerance and tumor recurrence.

Gu J, et al. Neuro Oncol 2022;24:1056-1070.
Zhao Y, et al. Adv Sci (Weinh) 2023;10:e2204177.

Background

- We previously reported that **Plasminogen activator inhibitor-1 (PAI-1)** contributes to the survival of drug-tolerant persister cells during the initial phase of treatment with anti-PD-1 antibodies and EGFR tyrosine kinase inhibitors, thus mediating therapeutic tolerance in NSCLC.

Tokumo K, et al. Cancers (Basel) 2023;15.

Sumii M, et al. Mol Cancer Ther 2025;25:435-447.

- However, the role of PAI-1 in the survival of **radiation-tolerant persister cells (RTPs)** remains unclear.

Aim

This study aimed to investigate the role of **PAI-1** in the survival of **RTPs** generated by FRT and its potential as a therapeutic target to **enhance FRT** efficacy.

Methods

Postoperative Specimens Analysis

- We retrospectively enrolled patients treated with or without preoperative chemoradiotherapy at Hiroshima University Hospital, with six cases included in each group after propensity score matching (PSM).
- PAI-1 expression was evaluated in human tumor specimens from both groups.

in vitro analysis

- Twenty-four hours after seeding in 6-well plates, cells were irradiated with 2 Gy/fr.
- RTPs were defined as cells surviving four FRT fractions in Human lung adenocarcinoma cell lines (A549 and PC-9) and three FRT fractions in a murine lung adenocarcinoma cell line (LLC), and PAI-1 expression in RTPs was evaluated. The effects of a PAI-1 inhibitor, TM5614, on cell proliferation and apoptosis in RTPs were examined.

Methods

in vivo analysis

- The *in vivo* antitumor efficacy of TM5614 was investigated in lung cancer subcutaneous tumor mouse models.
- Apoptosis and tumor angiogenesis were evaluated by immunohistochemical (IHC) staining for cleaved caspase-3 and CD31, respectively.

Reagent

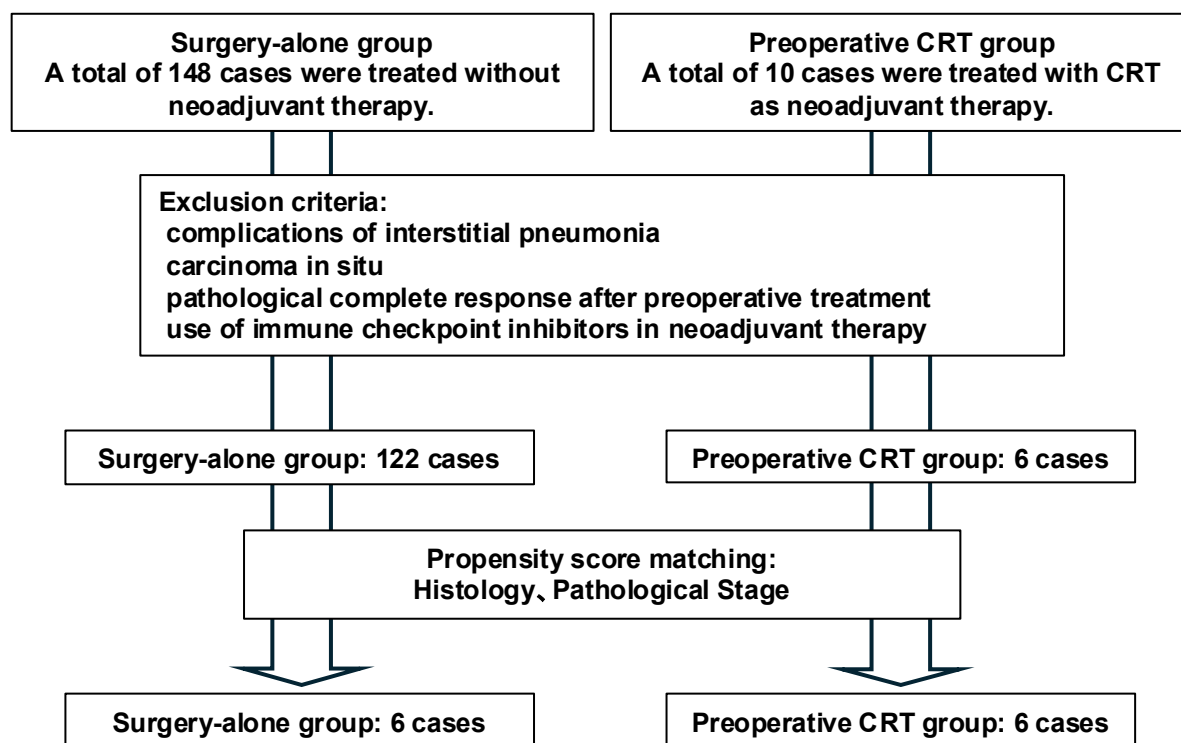
- TM5614 (PAI-1 inhibitor) was used at a concentration of 62.7 $\mu\text{mol/L}$ (in vitro) and 10 mg/kg (in vivo) based on previous study.

Sumii M, et al. Mol Cancer Ther 2025;25:435-447.
Sasaki K, et al. Tohoku J Exp Med 2022;257:211-224.

Result- Postoperative Specimens Analysis

High PAI-1 Expression in Human NSCLC Specimens After Radiation Treatment

Flow chart



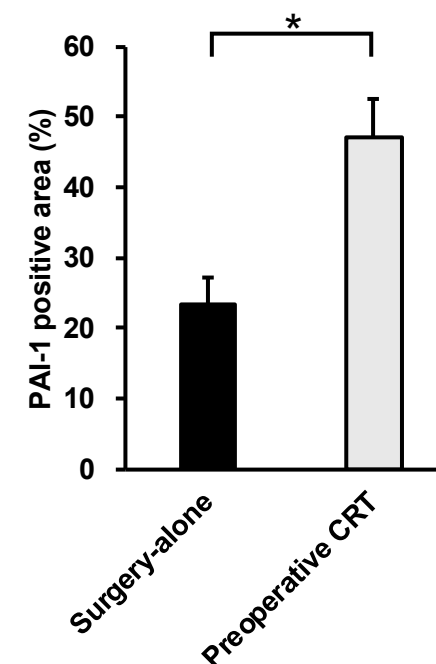
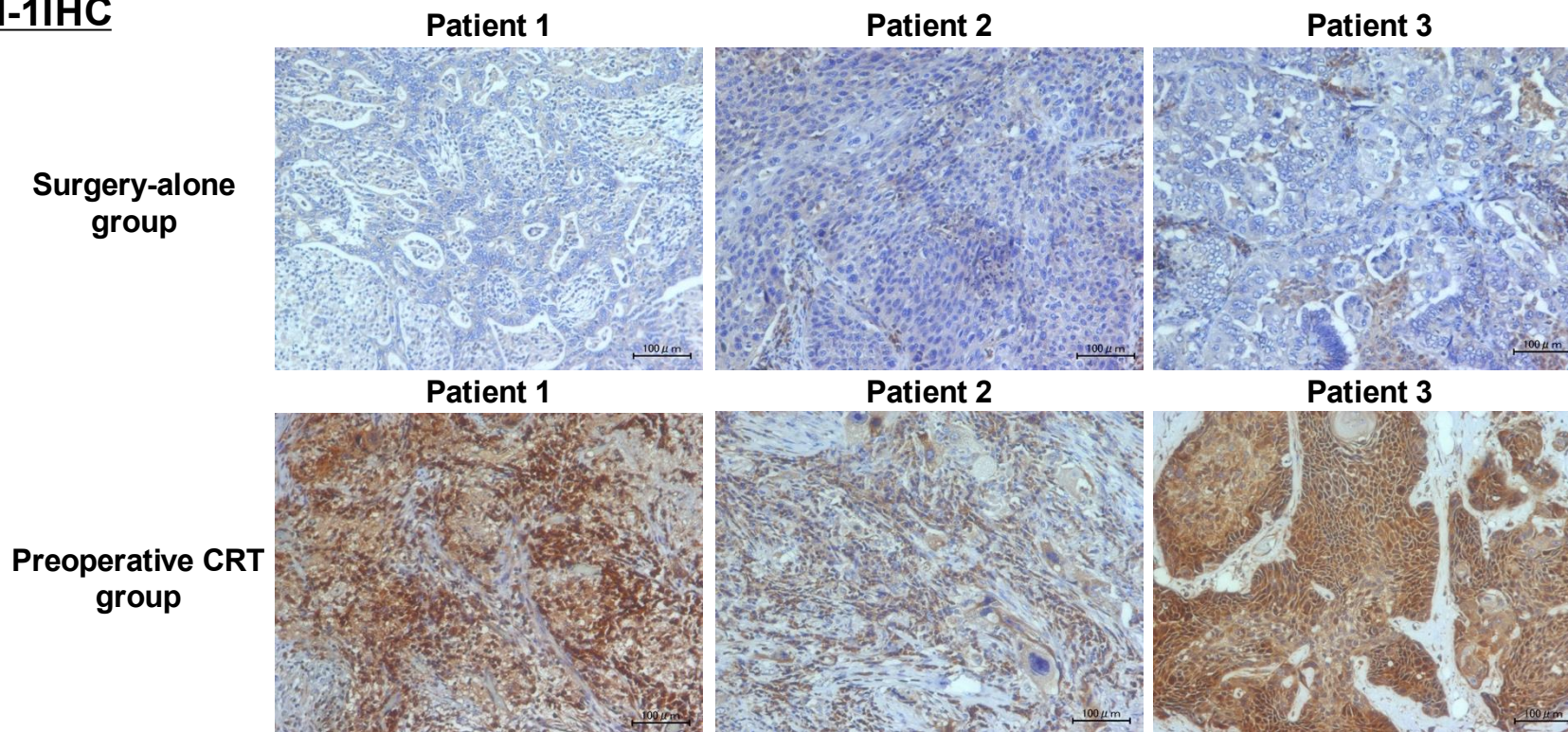
Patient characteristics

		After PSM Adjustment			
Patient characteristics		Surgery-alone (N=122)	Surgery-alone (N=6)	Preoperative CRT (N=6)	p-value
Age	Median (range)	74 (41-88)	67 (41-88)	70 (51-75)	0.83
Sex (%)	Male/Female	58.2 / 41.8	66.7 / 33.3	83.3 / 16.7	0.51
Smoking Status (%)	Former or current/Never	64.8 / 35.2	83.3 / 16.7	66.7 / 33.3	0.51
ECOG-PS (%)	0-1/≥ 2	99.2 / 0.8	100 / 0	100 / 0	-
Histology (%)	Ad/Sq/Other	80.3 / 16.4 / 3.3	33.3 / 16.7 / 50.0	33.3 / 16.7 / 50.0	1.00
Surgical procedure (%)	Lobectomy/ Segmental resection/ partial resection	41.8 / 40.2 / 18.0	100 / 0 / 0	100 / 0 / 0	-
Pathological Stage or y Pathological Stage (%)	I / II / III	86.1 / 9.0 / 4.9	33.3 / 33.3 / 33.3	33.3 / 33.3 / 33.3	1.00

Result- Postoperative Specimens Analysis

High PAI-1 Expression in Human NSCLC Specimens After Radiation Treatment

PAI-1IHC



The proportion of the PAI-1-positive area was significantly higher in the preoperative CRT group than in the surgery-alone group.

Result- Postoperative Specimens Analysis

Summary

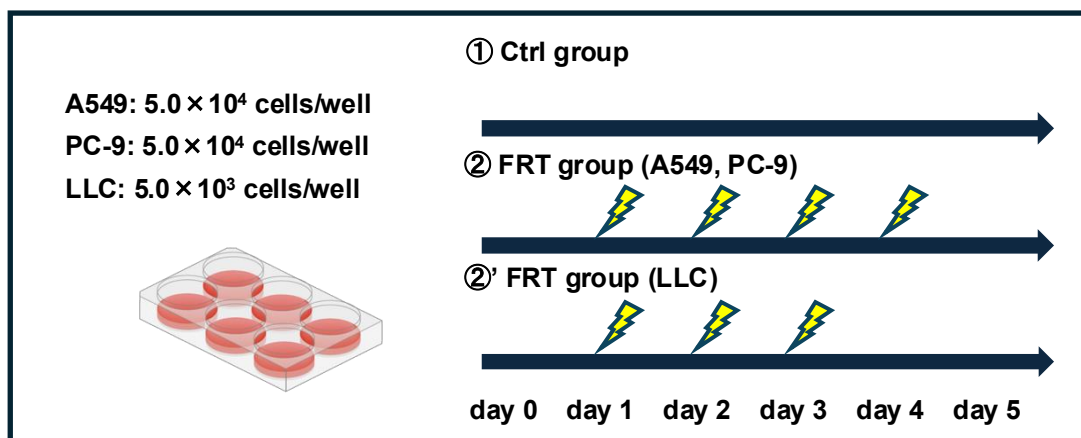
- These findings suggested increased PAI-1 expression in residual NSCLC tumors after preoperative RT.
- Although a contribution from chemotherapy cannot be excluded, radiotherapy is the primary treatment modality in CRT, while chemotherapy mainly serves as a radiosensitizer.

Asmara OD, et al. Breathe (Sheff) 2024;20:240047.

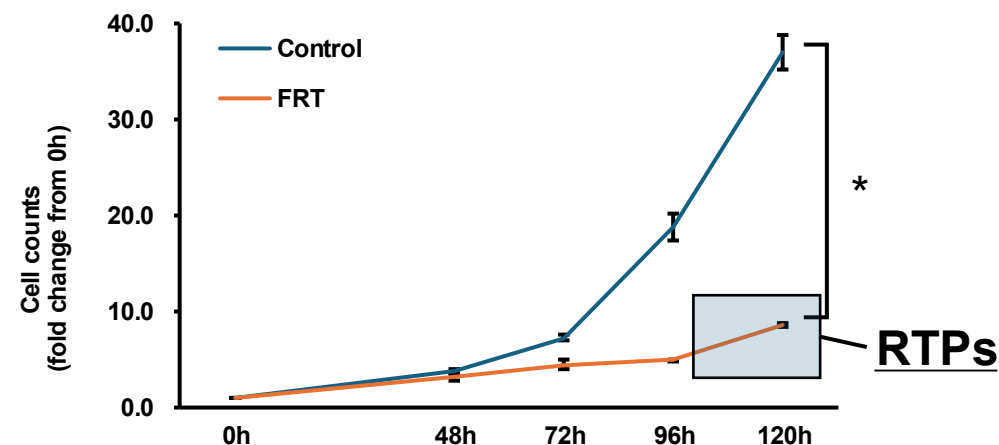
Result- *in vitro* analysis

PAI-1 Expression Level in RTPs at the Early Phase of Radiation Treatment, *in vitro*

Experimental scheme



Cell proliferation with or without FRT (A549)

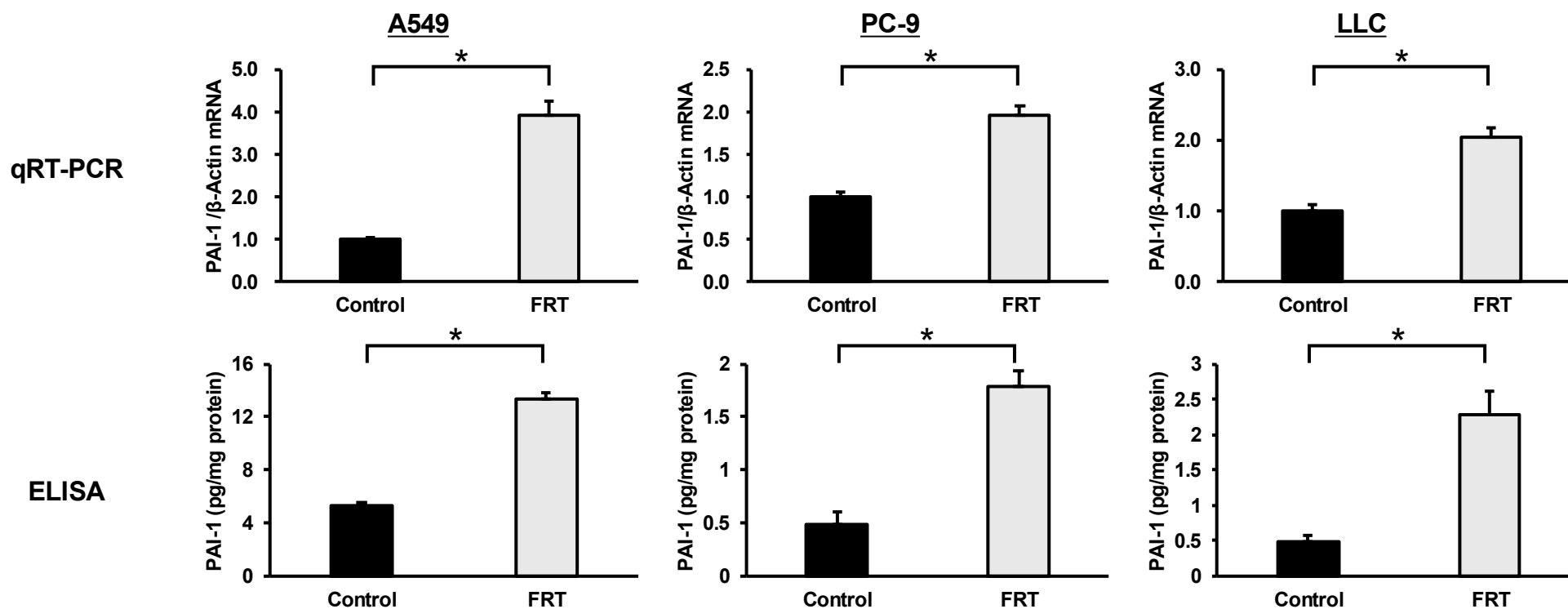


RTPs were defined as cells surviving four FRT fractions in A549 and PC-9 cells and three FRT fractions in LLC cells.

Result- *in vitro* analysis

PAI-1 Expression Level in RTPs at the Early Phase of Radiation Treatment, *in vitro*

PAI-1 Expression of mRNA and protein

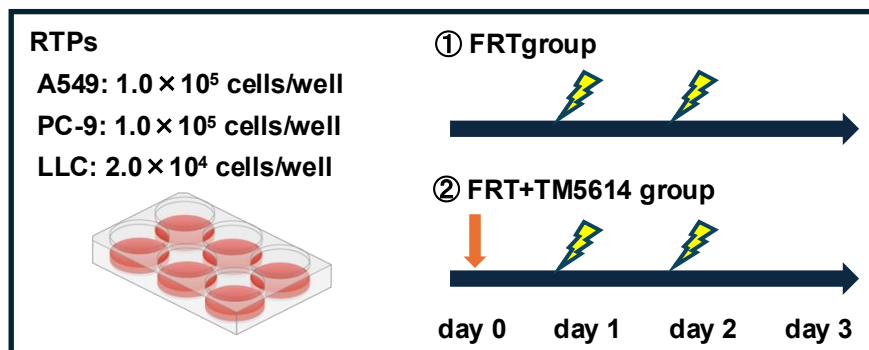


Across all three cell lines, RTPs exhibited significantly higher PAI-1 mRNA expression and secreted protein levels than the corresponding controls.

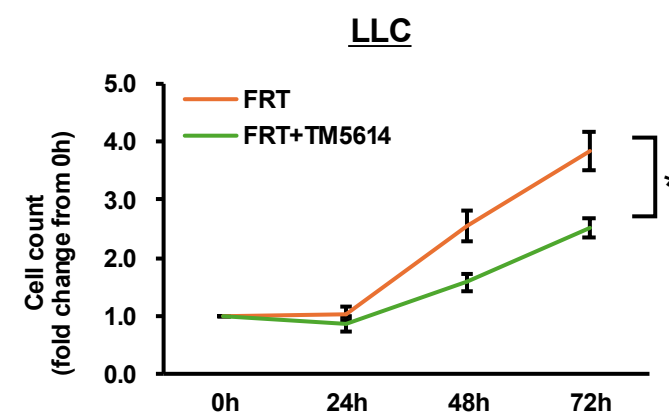
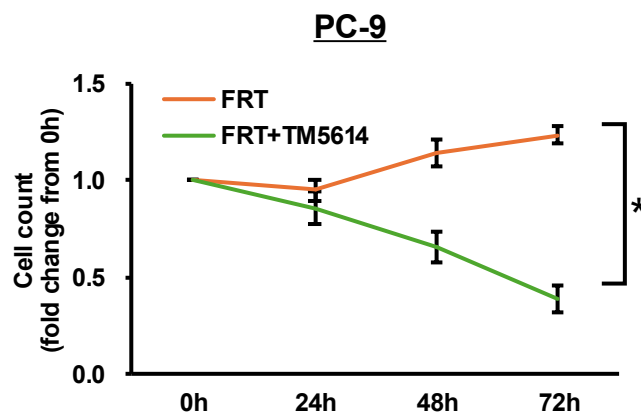
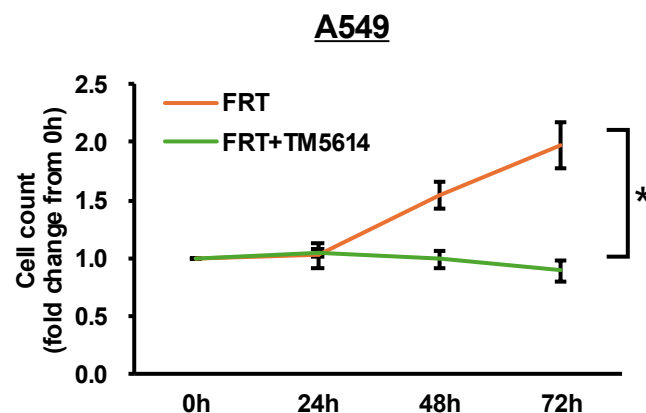
Result- *in vitro* analysis

PAI-1 plays a role in the survival of RTPs

Experimental scheme



Proliferation assay

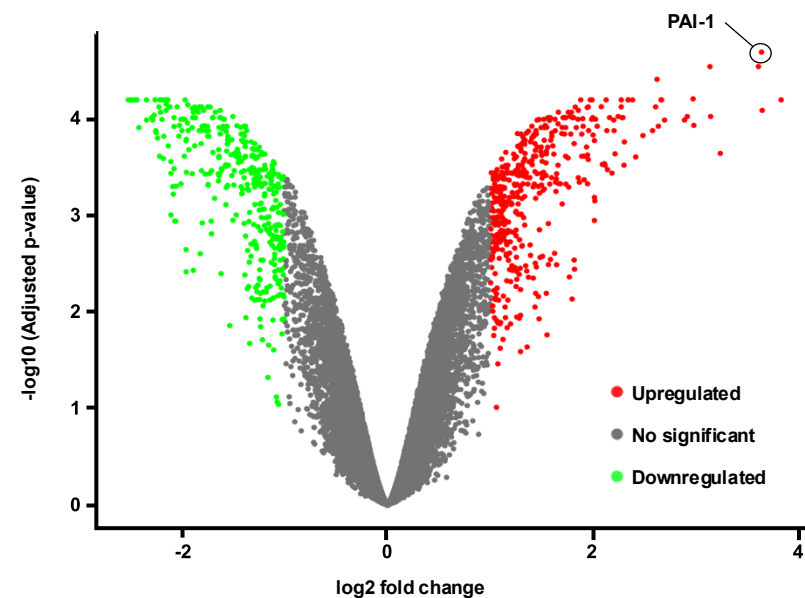
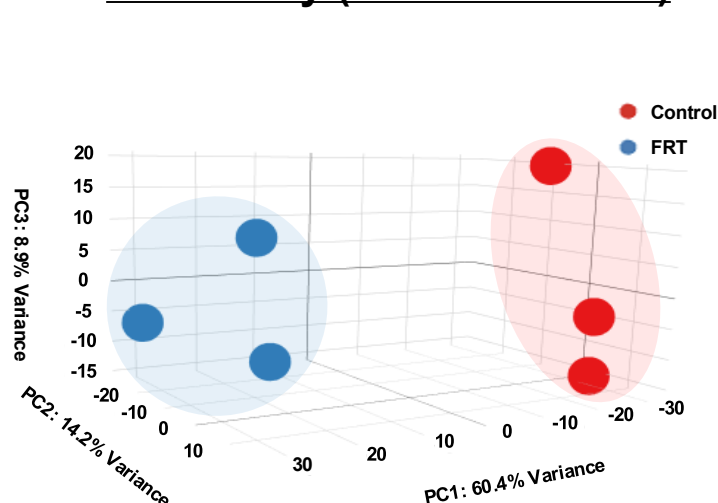


Cell proliferation was significantly suppressed by TM5614 compared with continued FRT alone.

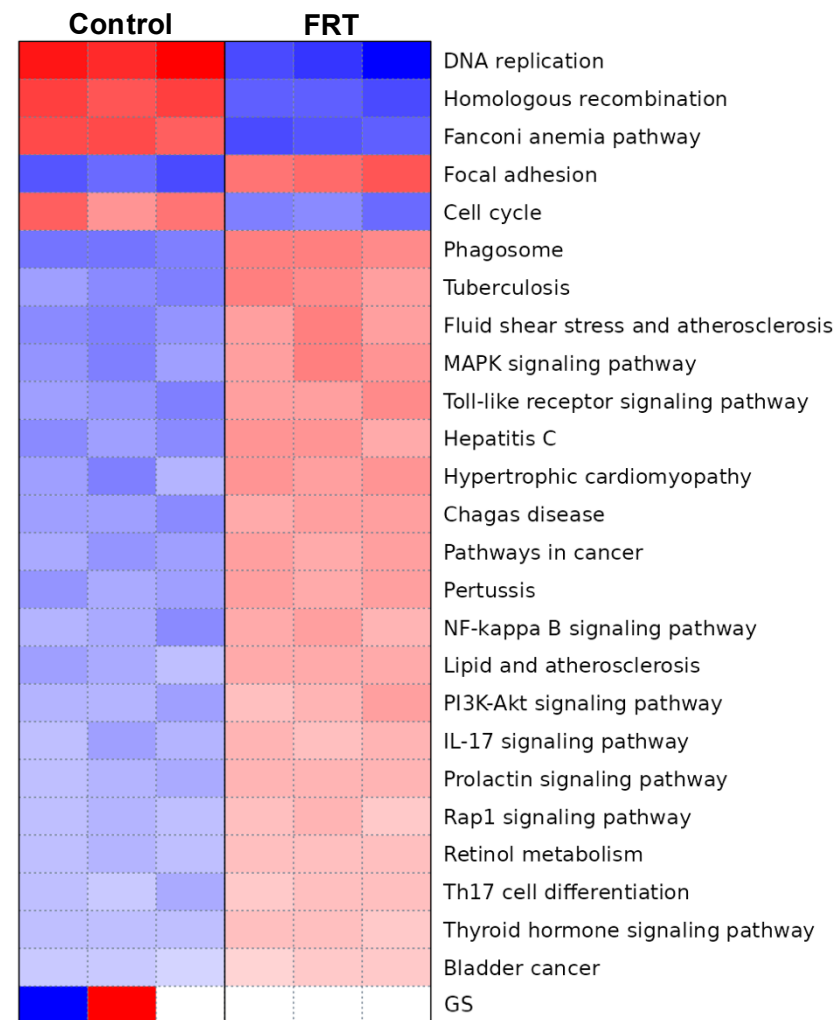
Result- *in vitro* analysis

PAI-1 plays a role in the survival of RTPs

Microarray (FRT vs Control)



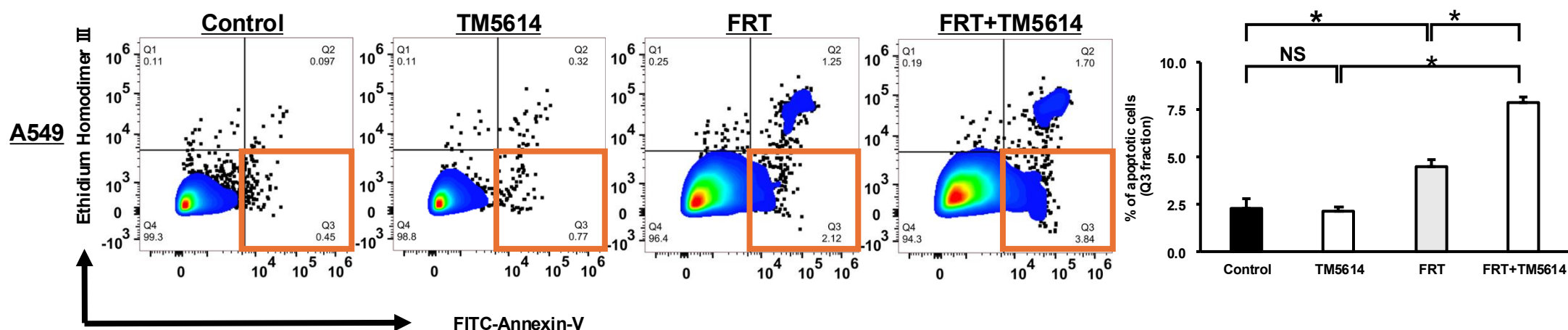
KEGG pathway analysis revealed downregulation of cell cycle and DNA replication pathways and upregulation of stress response and cell survival pathways.



Result- *in vitro* analysis

PAI-1 promotes RTP survival by suppressing apoptosis

Annexin V assay

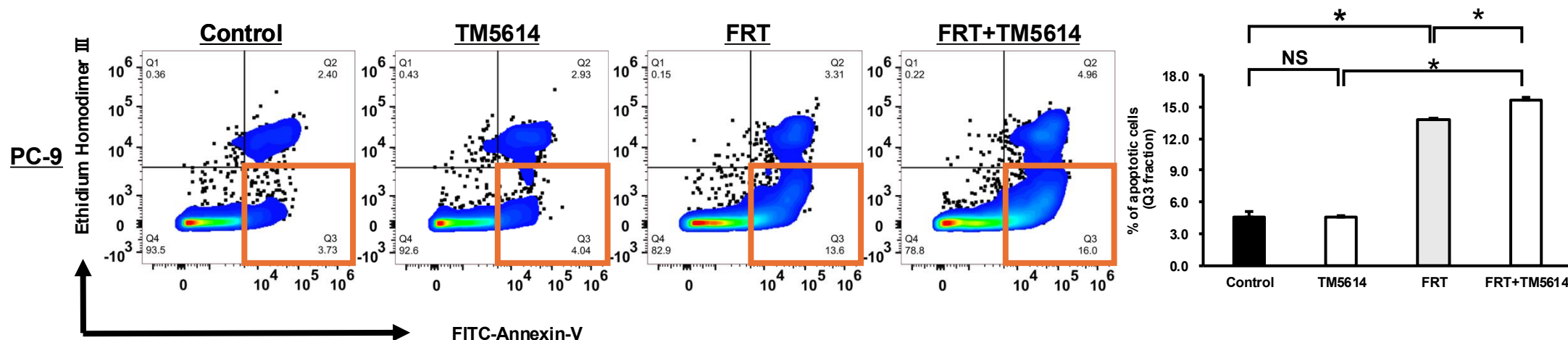


The proportion of apoptotic cells was significantly higher with FRT alone than in the control group and was further increased by TM5614 treatment in A549 cells.

Result- *in vitro* analysis

PAI-1 promotes RTP survival by suppressing apoptosis

Annexin V assay

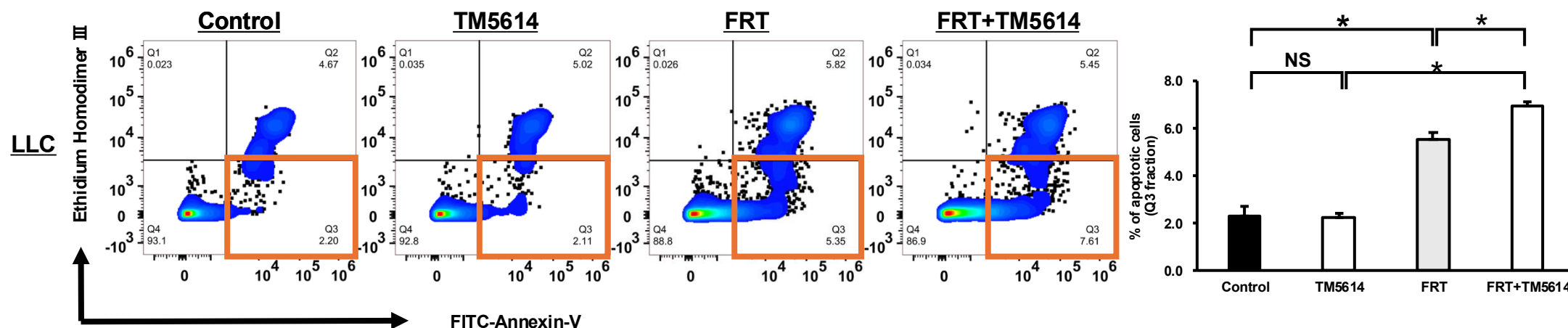


The proportion of apoptotic cells was significantly higher with FRT alone than in the control group and was further increased by TM5614 treatment in PC-9 cells.

Result- *in vitro* analysis

PAI-1 promotes RTP survival by suppressing apoptosis

Annexin V assay

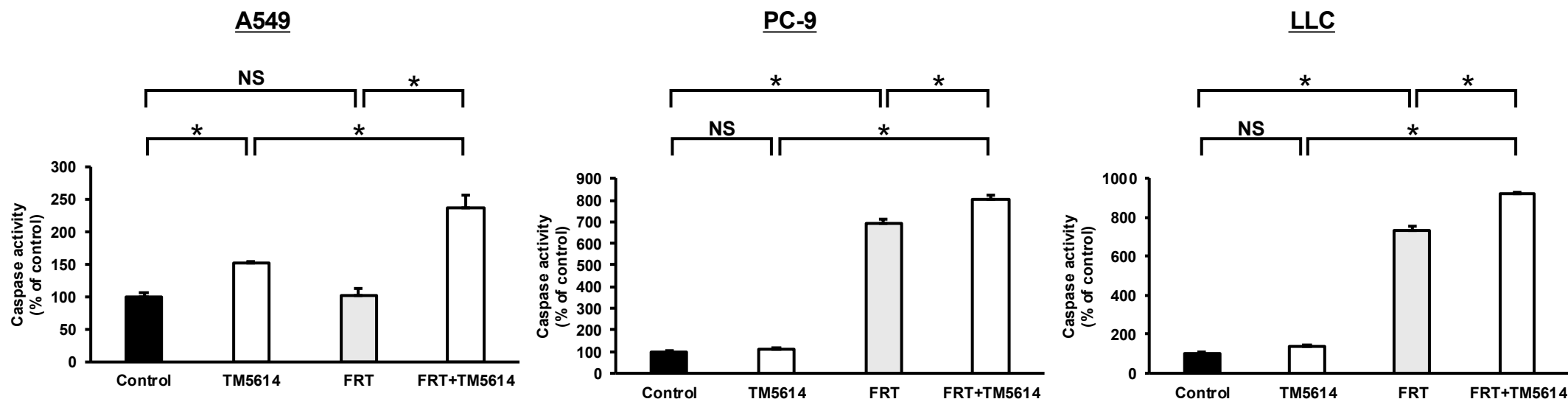


The proportion of apoptotic cells was significantly higher with FRT alone than in the control group and was further increased by TM5614 treatment in LLC cells.

Result- *in vitro* analysis

PAI-1 promotes RTP survival by suppressing apoptosis

Caspase-3/7 activity assay

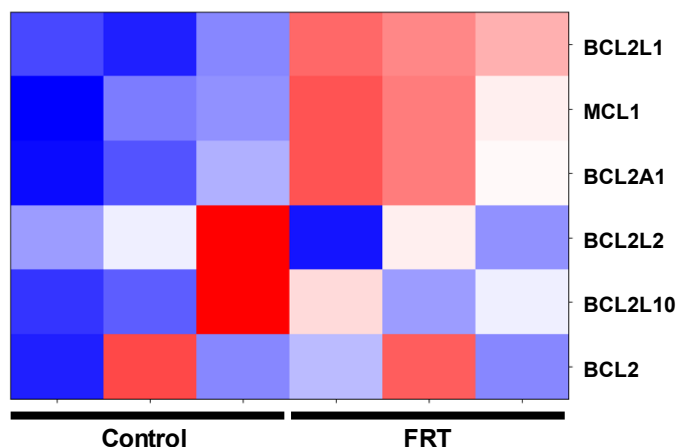


Across all three cell lines, Caspase-3/7 activity was increased by the addition of TM5614 to FRT compared with FRT alone.

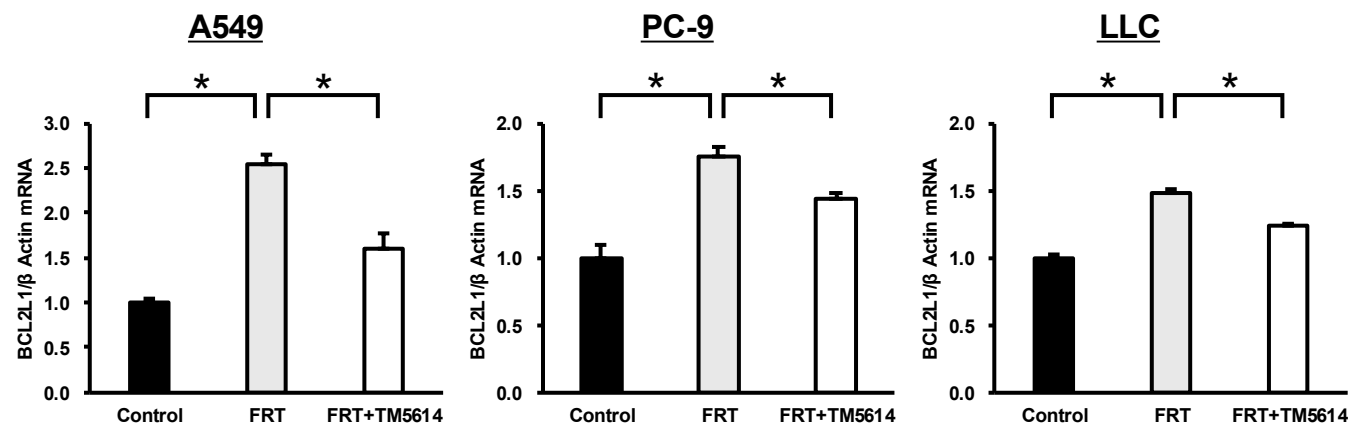
Result- *in vitro* analysis

PAI-1 suppresses RTP apoptosis through regulation of BCL2L1

The mRNA levels of BCL-2 family genes from microarray



BCL2L1 mRNA expression



BCL2L1 mRNA expression was significantly upregulated in the FRT group and significantly downregulated by TM5614 in all three cell lines.

Result- *in vitro* analysis

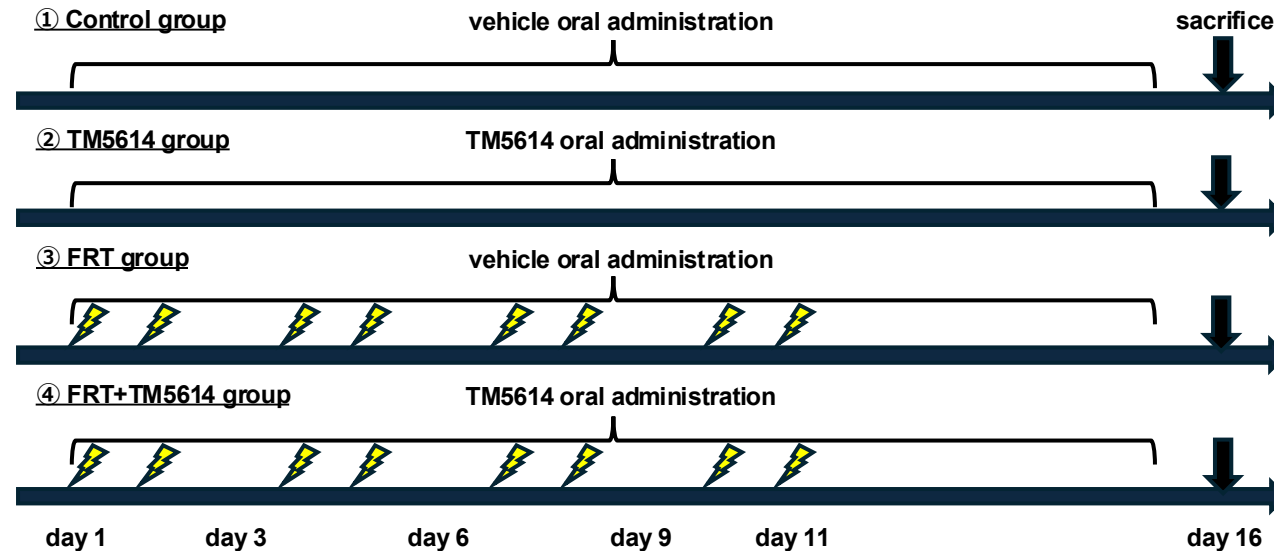
Summary

- PAI-1 is upregulated in RTPs, and its inhibition enhances FRT efficacy.
- PAI-1 contributes to RTP survival by suppressing apoptosis, at least in part, through the regulation of BCL2L1.

Result- *in vivo* analysis

PAI-1 inhibition enhances FRT antitumor efficacy *in vivo*

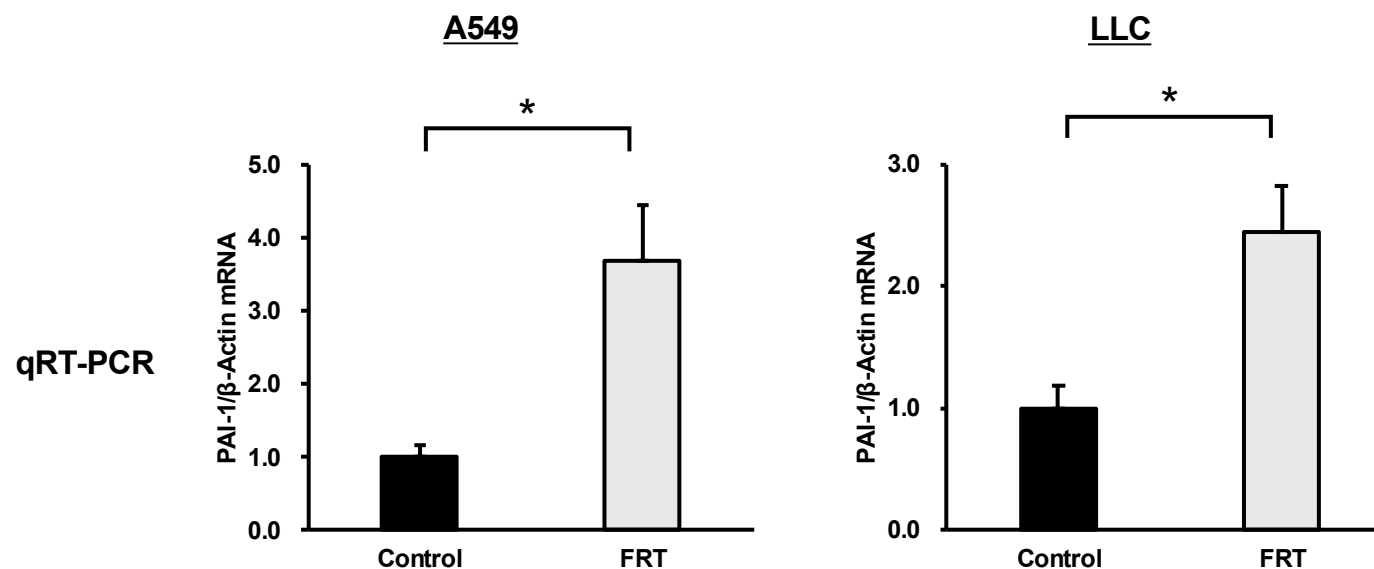
Experimental scheme



Result- *in vivo* analysis

PAI-1 inhibition enhances FRT antitumor efficacy *in vivo*

PAI-1 expression of mRNA in tumors

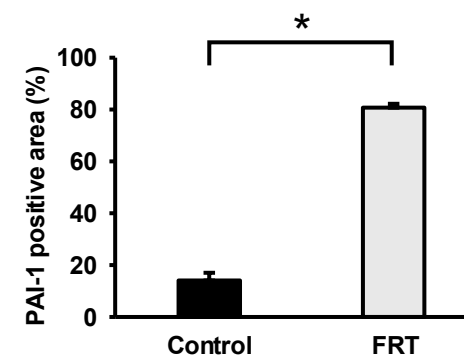
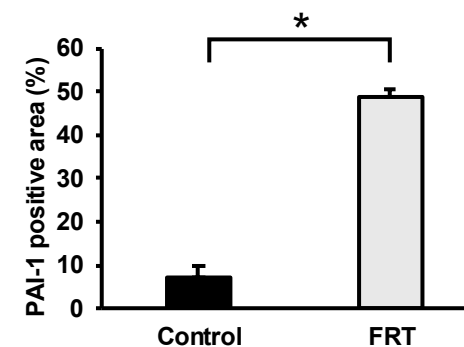
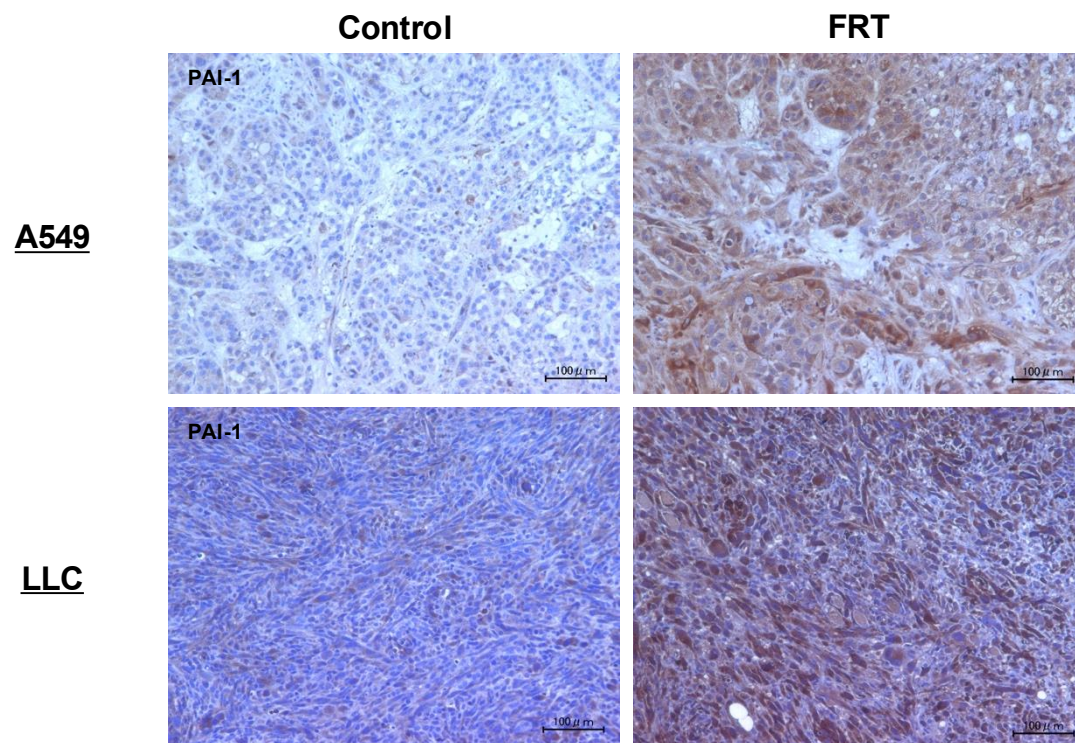


PAI-1 mRNA expression in tumor tissues was significantly higher in the FRT group than in the control group.

Result- *in vivo* analysis

PAI-1 inhibition enhances FRT antitumor efficacy *in vivo*

PAI-1 expression of protein in tumors

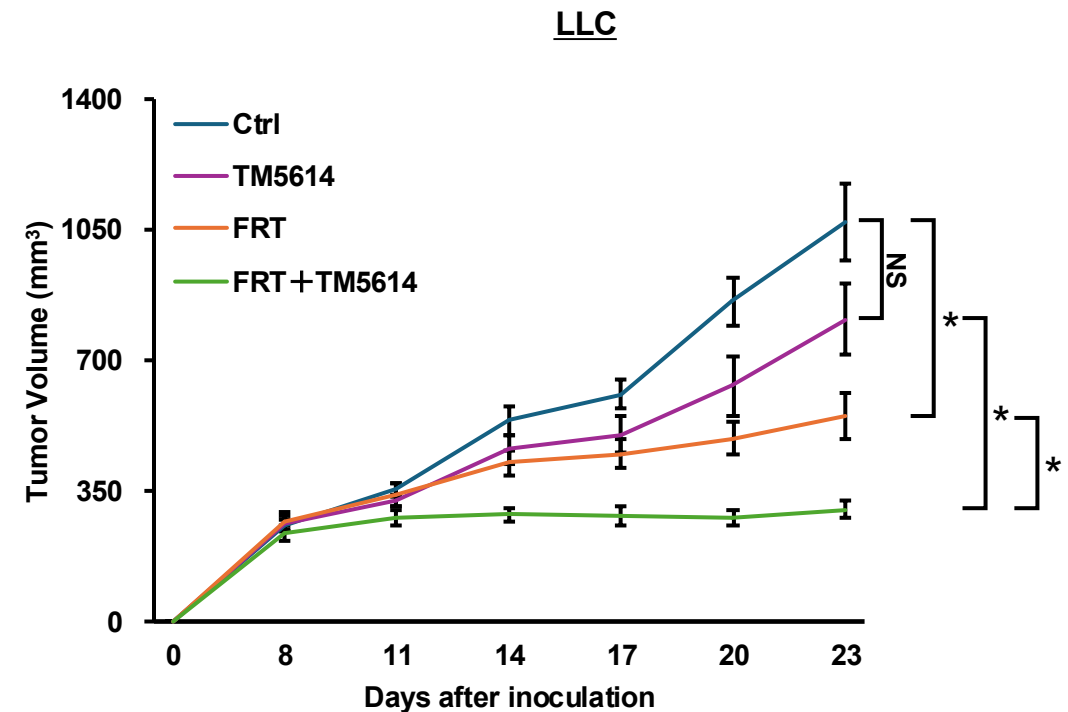
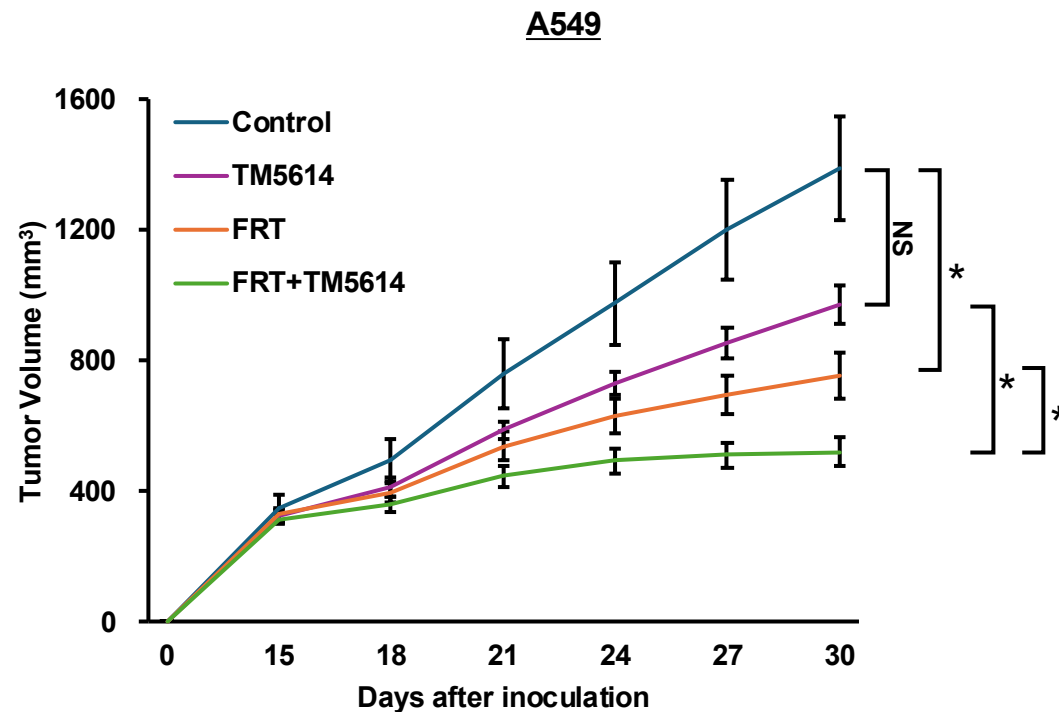


The PAI-1-positive area was significantly greater in the FRT group than in the control group.

Result- *in vivo* analysis

PAI-1 inhibition enhances FRT antitumor efficacy *in vivo*

Tumor volume

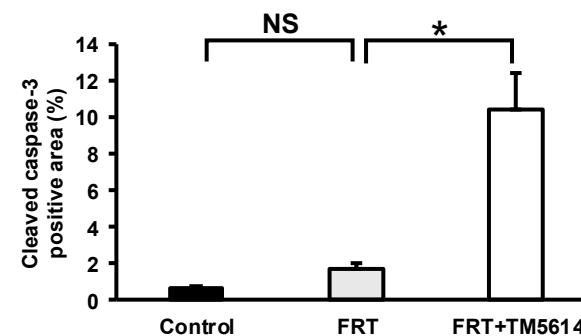
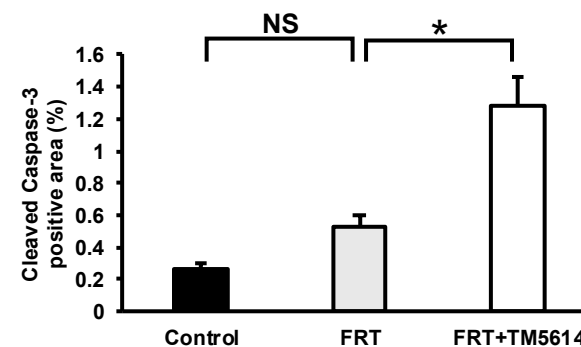
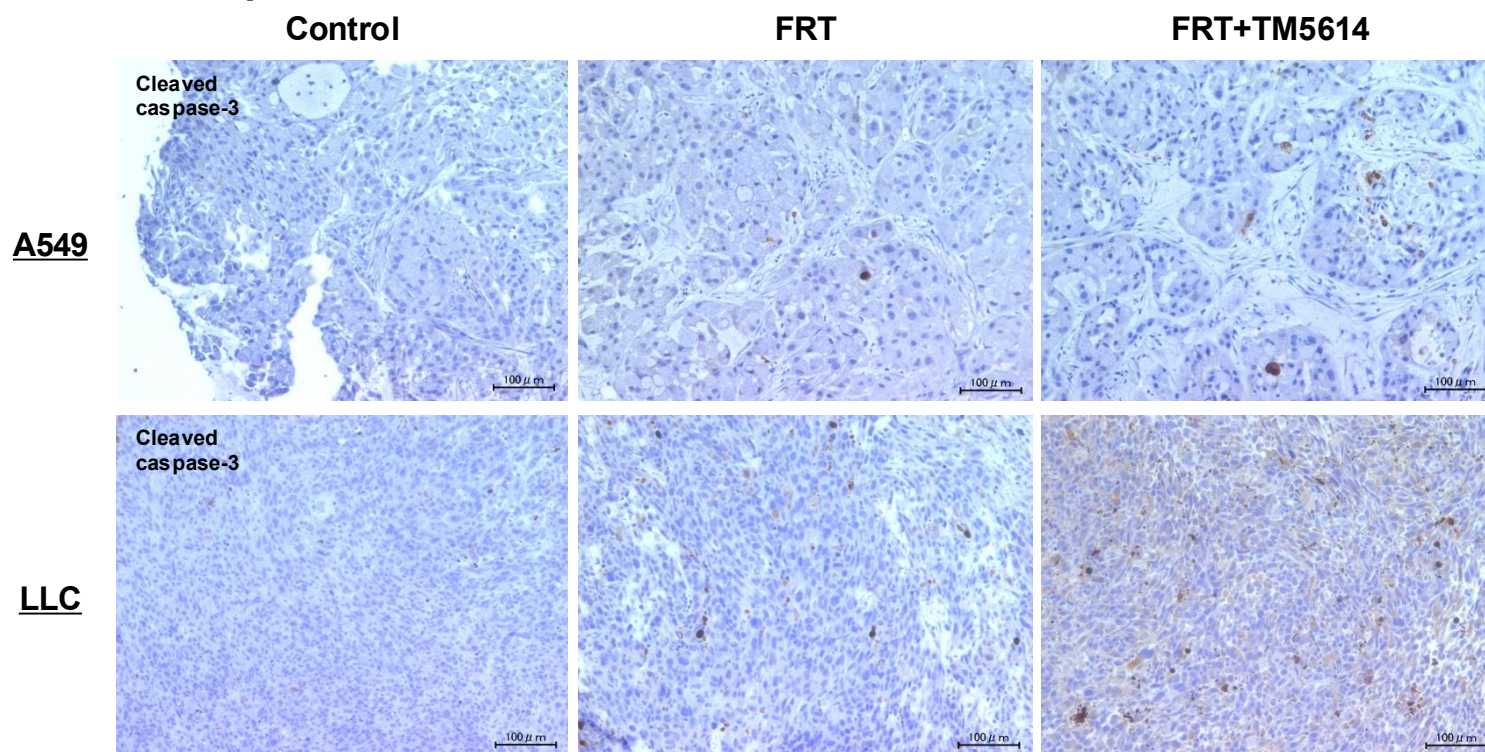


Tumor growth was significantly suppressed in the FRT+TM5614 group compared with FRT alone.

Result- *in vivo* analysis

PAI-1 inhibition enhances FRT antitumor efficacy in vivo

Cleaved caspase-3 IHC

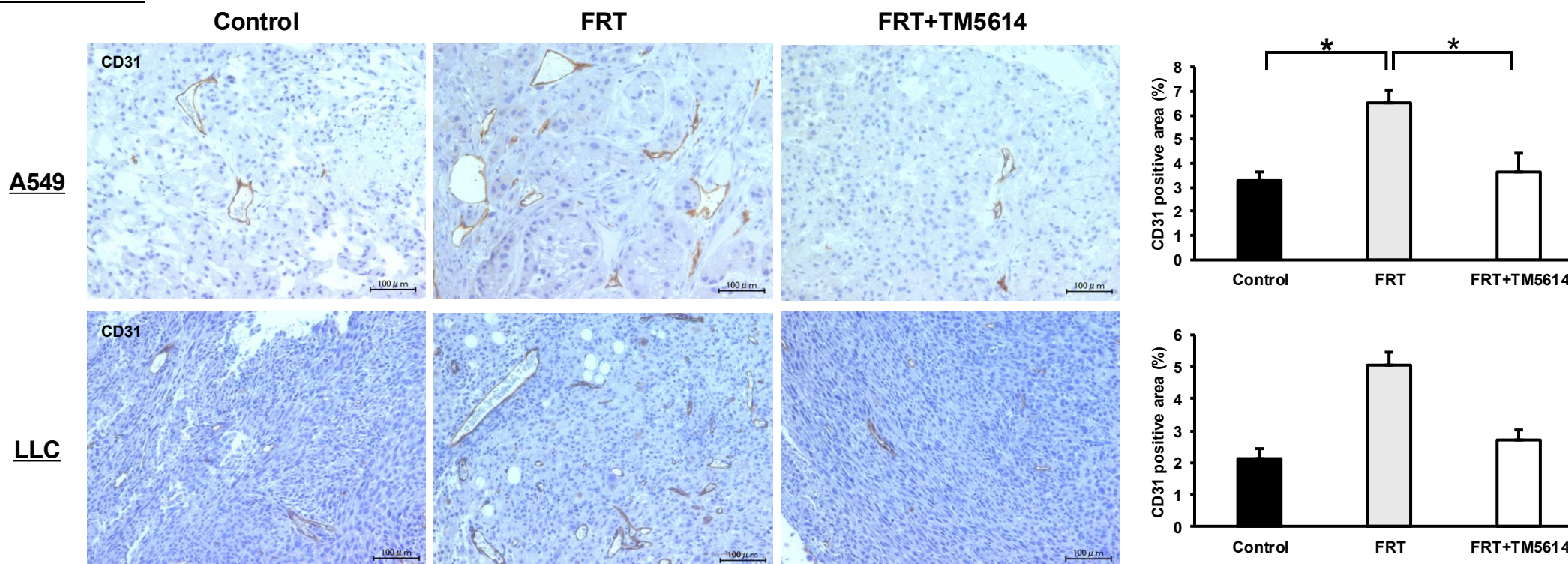


The cleaved caspase-3-positive area was greater in the FRT+TM5614 group compared with FRT alone.

Result- *in vivo* analysis

PAI-1 contributes to radiation tolerance through tumor angiogenesis

CD31 IHC



The CD31-positive area was significantly reduced by FRT+TM5614 compared with FRT alone.

Result- *in vivo* analysis

Summary

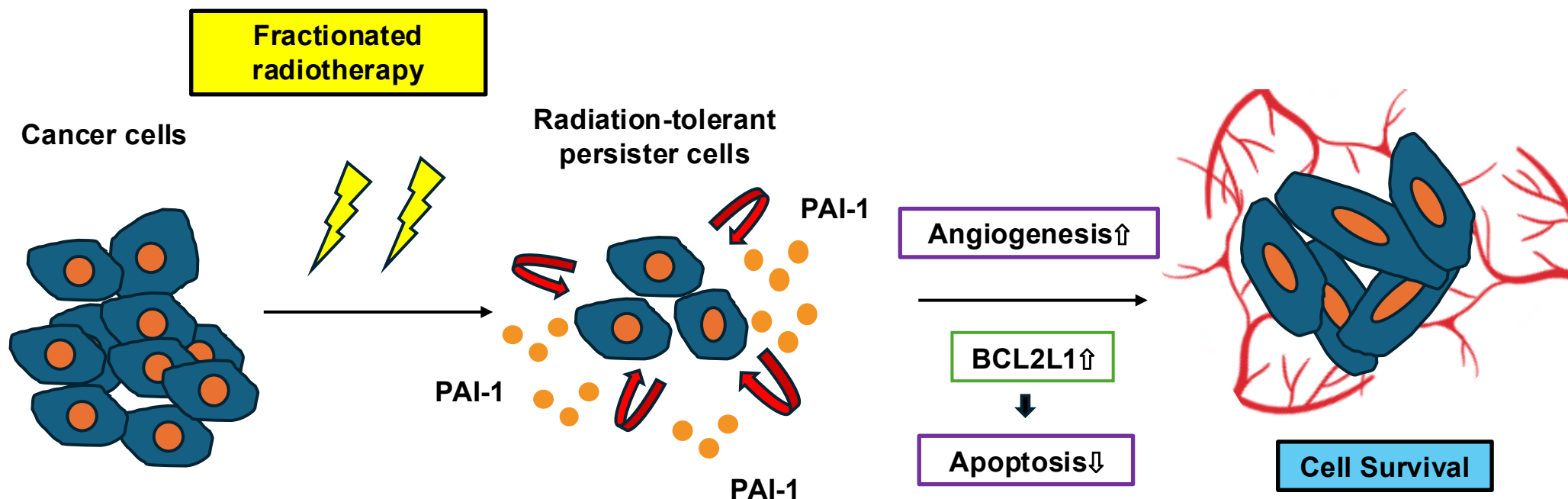
- PAI-1 is associated with radiation tolerance by suppressing apoptosis.
- PAI-1 Inhibition with TM5614 enhances the antitumor efficacy of FRT by inducing apoptosis and suppressing tumor angiogenesis.

Discussion

- This is the first study to demonstrate increased PAI-1 expression in FRT-generated RTPs and to show that **targeting PAI-1** may enhance the therapeutic efficacy of FRT.
- PAI-1 contributes to RTP survival by suppressing apoptosis and promoting tumor angiogenesis.
- The combination of the **PAI-1 inhibitor, TM5614, with radiotherapy may represent a promising therapeutic strategy** for overcoming radiation tolerance in locally advanced NSCLC.

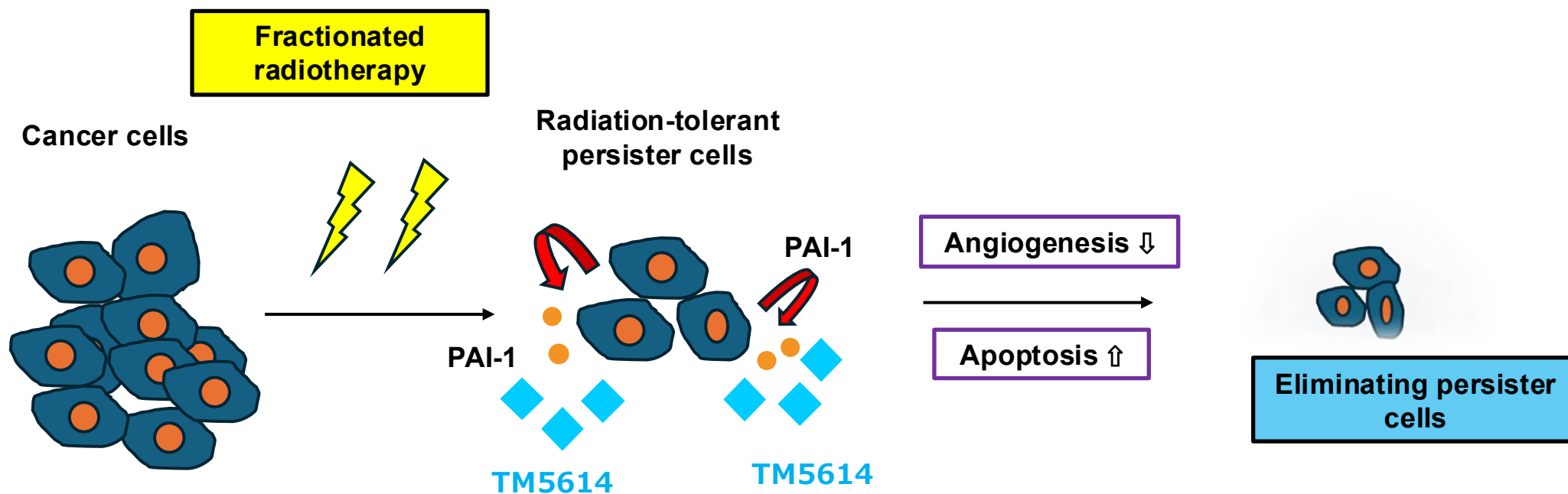
Discussion- Graphical abstract

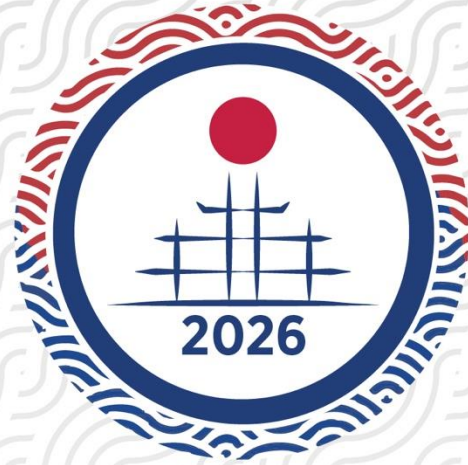
Fractionated radiotherapy



Discussion- Graphical abstract

Fractionated radiotherapy + PAI-1 inhibitor





Thank You.