Extended treatment with IAP inhibitor xevinapant post radiotherapy improves therapeutic efficacy and promotes antitumor immunity in preclinical models

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SCOPE

- A preclinical study to evaluate the antitumor effect of extended xevinapant treatment post-concurrent radiotherapy (RT) and the modulation of the tumor immune microenvironment in syngeneic tumor-bearing mice and in vitro cell culture models

CONCLUSIONS

- The combination of xevinapant and RT demonstrated improved antitumor effects compared with RT alone in A1419 (squamous cell carcinoma of the head and neck), Lewis lung carcinoma (LLC), and MC38 (colorectal carcinoma) tumor models
- Extended dosing of xevinapant post-concurrent RT showed improved antitumor efficacy and prolonged survival compared with shorter xevinapant dosing durations
- Xevinapant + RT promoted RT-induced cancer cell apoptosis and enhanced antitumor immunity in the tumor microenvironment (TME) of MC38 tumors
- Further mechanistic investigation suggested that xevinapant-conferred therapeutic benefit may be, in part, mediated through the restoration of M1:M2 macrophage ratio reduced by RT as well as the modulation of the secretory profile of fibroblasts, resulting in a TME more amenable to therapy

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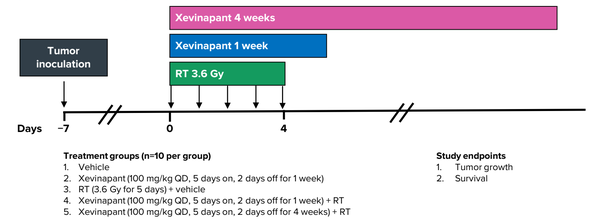
BACKGROUND

- Xevinapant is a potentially first-in-class, potent, oral, small-molecule IAP (inhibitor of apoptosis protein) inhibitor that is thought to restore cancer cell sensitivity to apoptosis (e.g., induced by RT and chemotherapy) and enhance antitumor immunity
- In a randomized phase 2 study of patients with unresected locally advanced squamous cell carcinoma of the head and neck (LA SCCHN), xevinapant + chemoradiotherapy significantly improved locoregional control at 18 months (OR, 2.69 [95% CI, 1.13–6.42]; p=0.026)[1] and improved 3-year progression-free survival (HR, 0.33 [95% CI, 0.17–0.67]; p=0.0019) and 5-year overall survival vs placebo + chemoradiotherapy (HR, 0.47 [95% CI, 0.27–0.84]; p=0.0101)[2]
- A phase 3 confirmatory study (TrilynX; NCT04459715) and a phase 3 study to evaluate xevinapant or placebo + RT in patients with resected, high-risk, cisplatin-ineligible LA SCCHN (XRay Vision; NCT05386550) are ongoing. In these studies, patients receive 6 cycles of xevinapant, in combination with CRT or RT for the first 3 cycles[3]
- Based on the role of IAPs in apoptosis,[4] antitumor immunity,[5,6] and modulation of fibroblastic tumor stroma,[7,8] continual dosing of xevinapant post RT may deliver additional therapeutic benefit through modulation of multiple TME compartments

METHODS

- The impact of xevinapant dosing duration on antitumor efficacy and animal survival was evaluated using the intramuscular A1419, LLC, and MC38 syngeneic tumor models
  - Tumor-bearing mice were treated with vehicle control, xevinapant, RT alone, or xevinapant + RT (**Figure 1**)
- To assess treatment-mediated TME modulation of immune compartments by the combination of xevinapant + RT, in vivo pharmacodynamic studies were performed with the MC38 tumor model
  - Immune compartments of tumors were evaluated by fluorescence-activated cell sorting (FACS)–based immune profiling
  - Tumor-specific T-cell response was investigated by ex vivo enzyme-linked immunosorbent spot (ELISpot) assays
  - Cancer cell apoptosis and CD8+ T-cell infiltration were assessed by immunohistochemistry (IHC) of formalin-fixed, paraffin embedded (FFPE) MC38 tumor tissue samples
- The effect of xevinapant on the TME cell types, including T lymphocytes, macrophages, and fibroblasts, was evaluated using in vitro cell culture models
  - T-cell activation assays as well as macrophage polarization and viability assays were performed to evaluate the effect of xevinapant on immune cell phenotypes
  - To evaluate the effect of xevinapant on RT-induced cancer-associated fibroblast (CAF) activation and CAF-modulated immune phenotype, activation assay and cytokine profiling were performed

Figure 1. Efficacy evaluation in mouse syngeneic tumor models.



Treatment groups (n=10 per group)
1. Vehicle
2. Xevinapant (100 mg/kg QD, 5 days on, 2 days off for 1 week)
3. RT (3.6 Gy for 5 days) + vehicle
4. Xevinapant (100 mg/kg QD, 5 days on, 2 days off for 1 week) + RT
5. Xevinapant (100 mg/kg QD, 5 days on, 2 days off for 4 weeks) + RT

Study endpoints
1. Tumor growth
2. Survival

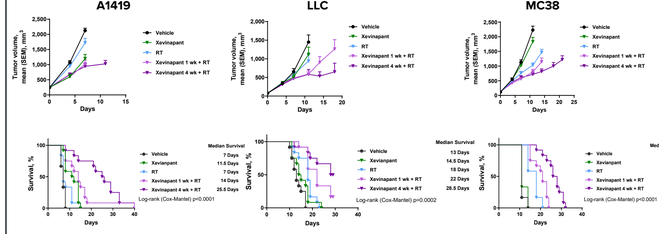
QD, every day; **RT,** radiotherapy.

RESULTS

In vivo efficacy

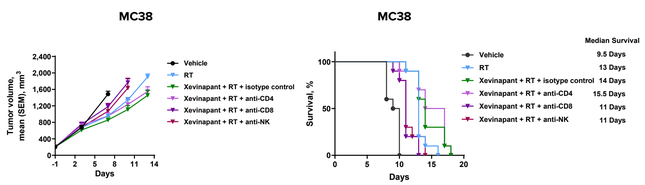
- Xevinapant + RT combination treatment showed increased antitumor efficacy compared with vehicle control and RT alone in the A1419 tumor model. Increased antitumor efficacy compared with vehicle control, xevinapant alone, and RT alone was observed in the LLC and MC38 tumor models (**Figure 2**)
- Extended dosing of xevinapant for 4 weeks vs 1 week further improved antitumor efficacy and animal survival (**Figure 2**)
- Depletion of CD8$^+$ T cells or natural killer (NK) cells, but not CD4$^+$ T cells, abrogated the enhanced efficacy of xevinapant and RT combination vs RT alone (**Figure 3**). These results suggest that the antitumor activity of xevinapant may be, in part, mediated by CD8$^+$ T cells and NK cells

Figure 2. Antitumor efficacy.



RT, radiotherapy.

Figure 3. Immune cell–dependent antitumor efficacy.



NK, natural killer; **RT,** radiotherapy.

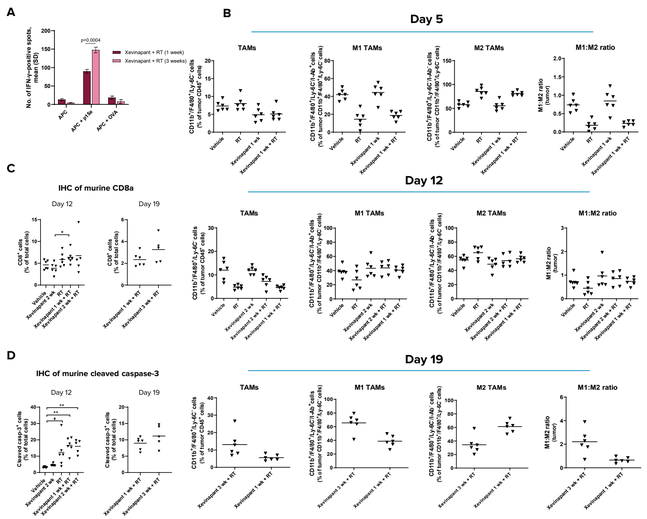
Reprogramming of immune compartments

- In a pharmacodynamic study with immune phenotyping by FACS (**Supplementary Figure 1**), xevinapant + RT modulated infiltration and phenotype of CD8$^+$ T cells. A trend toward increased number of CD8$^+$ TILs and antigen-specific TILs was observed in the combination arm compared with baseline and xevinapant alone (**Supplementary Figure 2A**)
- We observed trends toward increased numbers of dendritic cells, neutrophils, myeloid-derived suppressor cells, and NK cells, and reduced CD4+ cells and regulatory T cells in the combination arm vs baseline; and radiation-induced reduction of M1 macrophages in the RT only arm vs baseline (**Supplementary Figure 2B**)
- Evaluation of splenic immune cells by ELISpot assays confirmed that combination therapy further enhanced tumor-specific T-cell activation compared with vehicle control and monotherapies (**Supplementary Figure 2C**)

Enhanced tumor-specific immune response and cancer cell apoptosis by extended xevinapant dosing

- In a pharmacodynamic time-course study (**Supplementary Figure 3**), ELISpot assays confirmed that a larger number of interferon γ–positive spots were detected in the extended dosing arm, suggesting an enhanced immunologic memory in mice that received continual xevinapant treatment post RT (**Figure 4A**)
- Immune phenotyping by FACS suggested that extended xevinapant treatment post RT may contribute to a more favorable tumor immune microenvironment by restoring the M1:M2 macrophage ratio reduced by radiation (**Figure 4B**)
- IHC results suggested that RT alone or in combination with xevinapant may increase the number of CD8$^+$ T cells in MC38 tumors. Extended dosing of xevinapant showed a trend to further increase the number of tumor-infiltrating CD8$^+$ T cells (**Supplementary Figure 4A, Figure 4C**)
- RT alone or in combination with xevinapant markedly increased the percentage of cleaved caspase-3–positive tumor cells compared with vehicle control (**Supplementary Figure 4B, Figure 4D**)

Figure 4. Assessment of tumor phenotypes modulated by extended xevinapant treatment.*

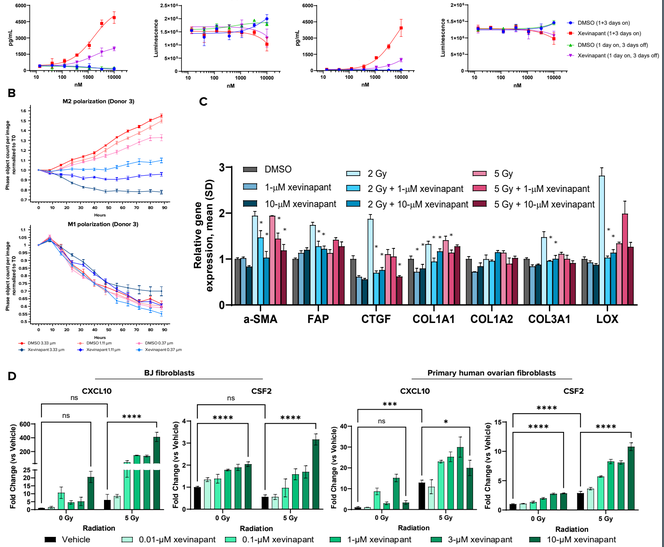


ANOVA, analysis of variance; **APC,** antigen-presenting cell; **casp,** caspase; **CD,** cluster of differentiation; **IFN,** interferon; **IHC,** immunohistochemistry; **OVA,** ovalbumin; **RT,** radiotherapy; **TAM,** tumor-associated macrophage.
*p values from 2-sample *t* test for panel **A** and 1-way ANOVA Kruskal-Wallis tests for panels **C** and **D.**

Impact of xevinapant on TME cell types

- Xevinapant promoted T-cell activation in vitro compared with vehicle control. Extended treatment further enhanced such activation compared with shorter dosing duration as demonstrated by *Staphylococcus aureus* enterotoxin A assays (**Figure 5A, Supplementary Figure 5A**)
- In vitro macrophage polarization assays showed that xevinapant may reduce the viability of M2 but not M1 macrophages compared with vehicle control (**Figure 5B, Supplementary Figure 5B**). Such differential responses to xevinapant by M1 and M2 macrophages may contribute to the restoration of the M1:M2 ratio observed in MC38 tumors
- Xevinapant suppressed RT-mediated fibroblast activation and extracellular matrix protein production, indicated by downregulation of activation markers and CAF-derived secretory protein gene expression (**Figure 5C**)
- Profiling of conditioned medium with cytokine array revealed differentially secreted cytokines induced by xevinapant + RT vs monotherapies and vehicle control (**Supplementary Figure 6**)
- Upregulation of CXCL10 and CSF2 (GM-CSF), which has been shown to modulate immune cell phenotype, was further confirmed in additional fibroblast cultures by qPCR (**Figure 5D**)

Figure 5. Modulation of immune cell and fibroblast phenotype by xevinapant.



ANOVA, analysis of variance; **DMSO,** dimethyl sulfoxide; **IL,** interleukin; **ns,** not significant; **PBMC,** peripheral blood mononuclear cell; **RT,** radiotherapy; **SEA,** *Staphylococcus aureus* enterotoxin A.
*p<0.05 vs corresponding control (2-sample *t* test for panel **C** and ANOVA with Tukey multiple comparison for panel **D**).

REFERENCES: 1. Sun XS, *Int J Radiat Oncol Biol Phys.* 2020;21:1173–87. **2.** TAO Y, *et al. Eur J Cancer.* 2023;183:24–37. **3.** Bourhis J, *et al. Future Oncol.* 2022;18:1669–78. **4.** Ferris RL, *et al. Cancer Treat Rev.* 2022;113:102492. **5.** Roehle K, *et al. Sci Transl Med.* 2021;13:eabf5058. **6.** Hoefsmit EP, *et al. Cancer Immunol Res.* 2023;11:450–65. **7.** Ashley SL, *et al. Am J Respir Cell Mol Biol.* 2016;54:482–92. **8.** Bergmann C, *et al. Ann Rheum Dis.* 2021;80:1048–56.
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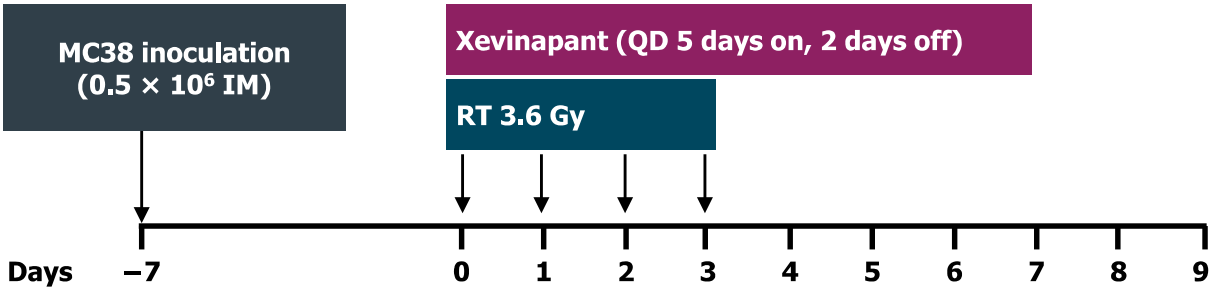
Supplementary Figure 1. Immune modulation by xevinapant + RT



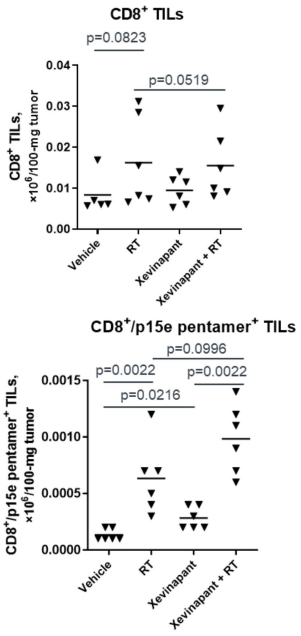
Treatment Groups: N=6 per group

1. Vehicle
2. RT (3.6 Gy QD for 4 days) + vehicle
3. Xevinapant (100 mg/kg QD 5 days on, 2 days off)
4. Xevinapant (100 mg/kg, QD 5 days on, 2 days off) + RT

IM, intramuscularly; **QD**, every day; **RT**, radiotherapy.

Supplementary Figure 2. Immune modulation by xevinapant + RT*

A

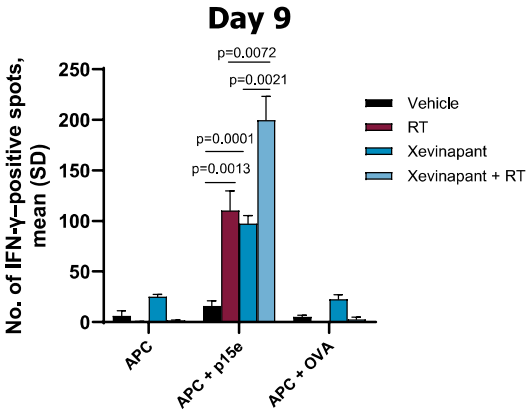


B

Immune cell type	Treatment		
	RT	Xevinapant	Xevinapant + RT
CD8⁺ cell	↑	—	↑
CD8⁺/p15e pentamer⁺ T cell	↑	↑	↑↑
CD4⁺ T cell	↓	↓	↓
Treg	—	↓	↓
NK cell	—	↑	↑
NKT cell	—	↓	—
TAM	↓ M1 TAM	—	—
Dendritic cell	↑	↑	↑
Neutrophil	↑	↑	↑
MDSC	↑	↑	↑

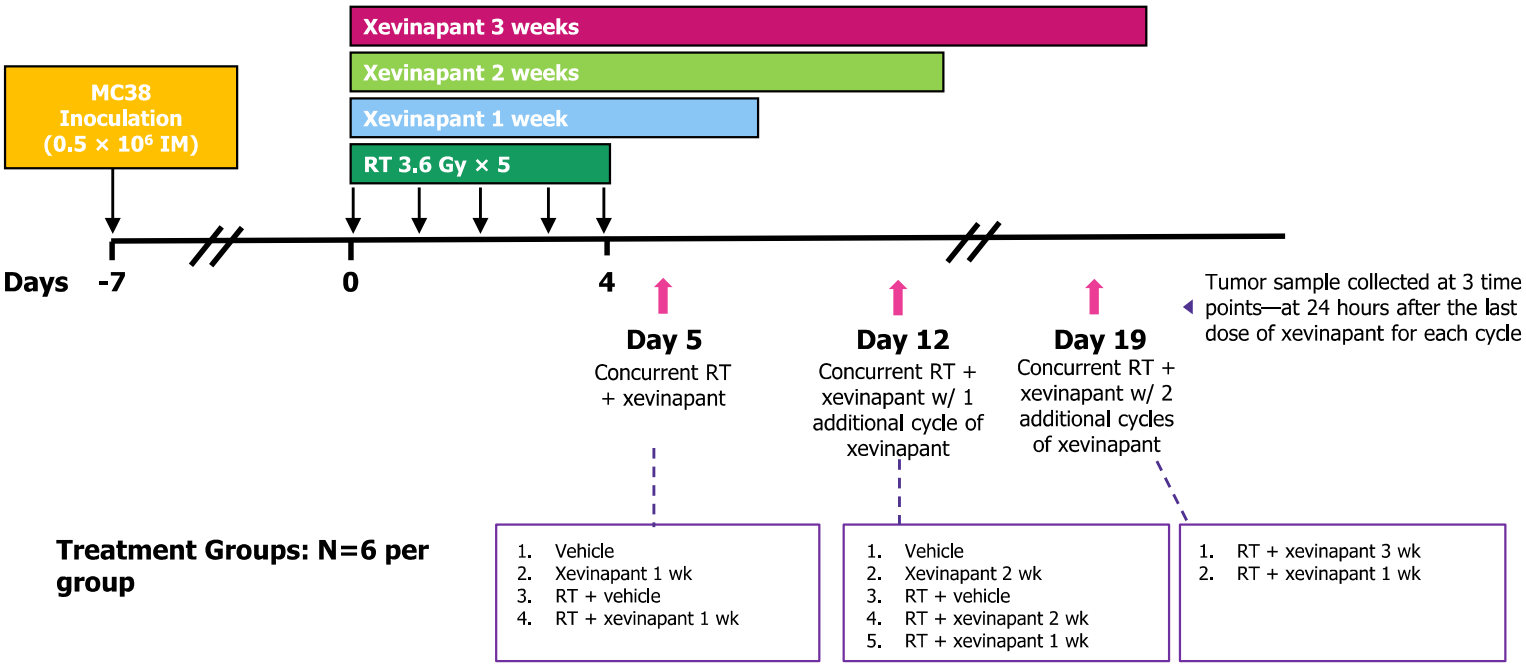
Note: Size and number of arrows indicate magnitude of change vs vehicle control.

C



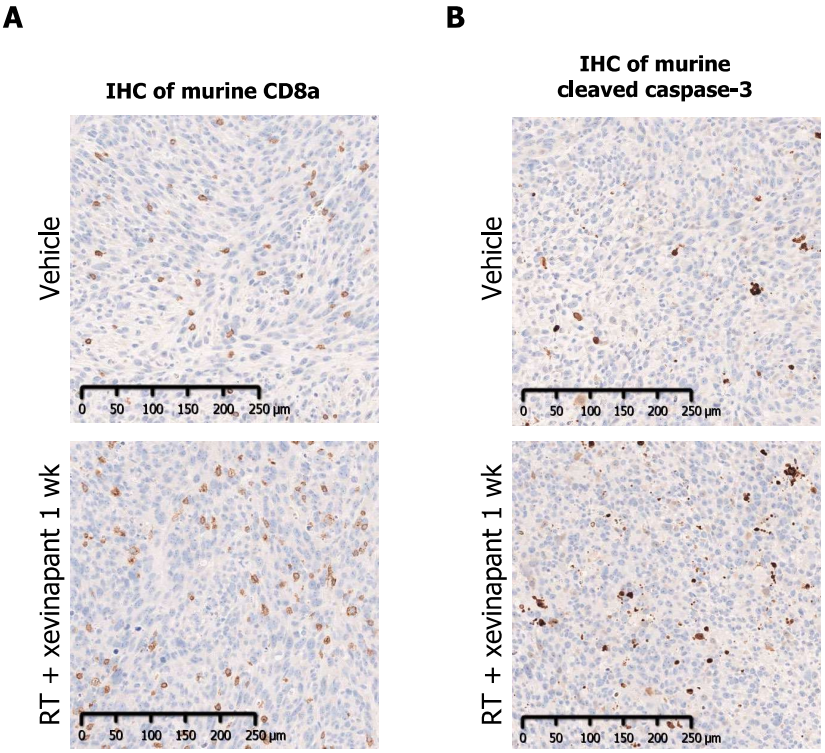
APC, antigen-presenting cell; **CD,** cluster of differentiation; **IFN,** interferon; **MDSC,** myeloid-derived suppressor cell; **NK,** natural killer; **NKT,** natural killer T; **OVA,** ovalbumin; **PBMC,** peripheral blood mononuclear cell; **RT,** radiotherapy; **TAM,** tumor-associated macrophage; **TIL,** tumor-infiltrating lymphocyte; **Treg,** regulatory T cell.
*p values from Mann-Whitney tests (panel **A**) and 2-sample *t*-test (panel **C**).

Supplementary Figure 3. Time-course study of tumor phenotype modulation by xevinapant + RT



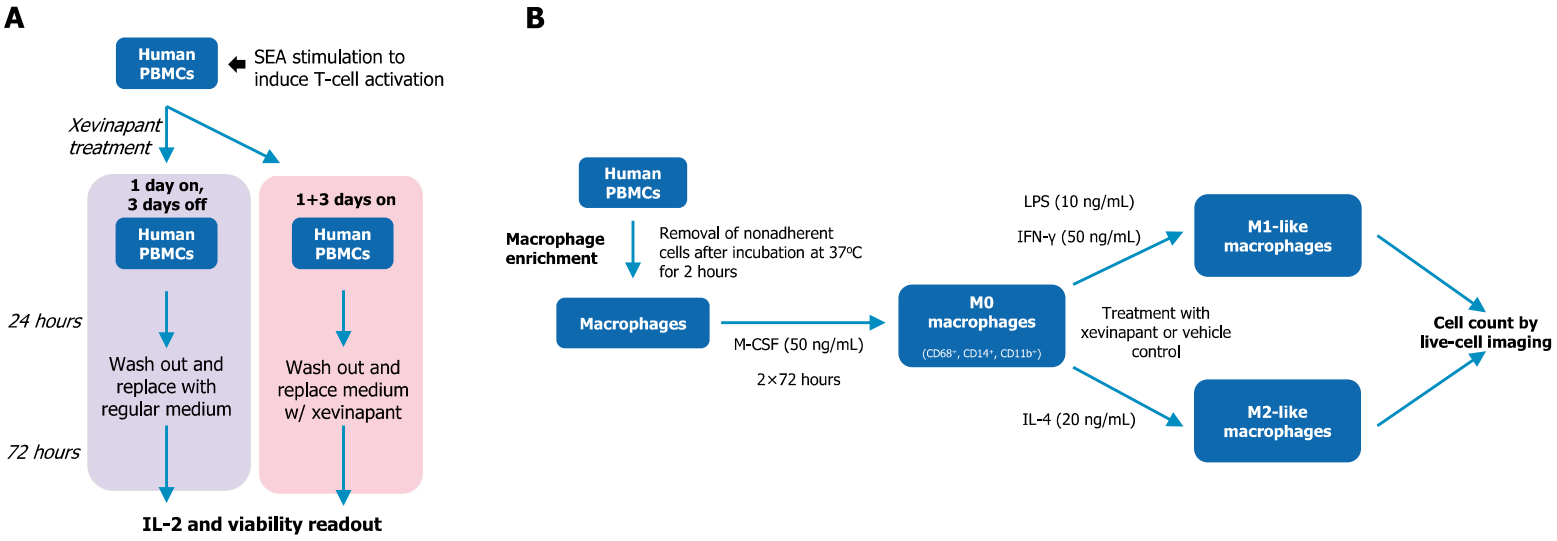
IM, intramuscularly; **RT**, radiotherapy.

Supplementary Figure 4. Assessment of tumor phenotypes modulated by extended xevinapant treatment



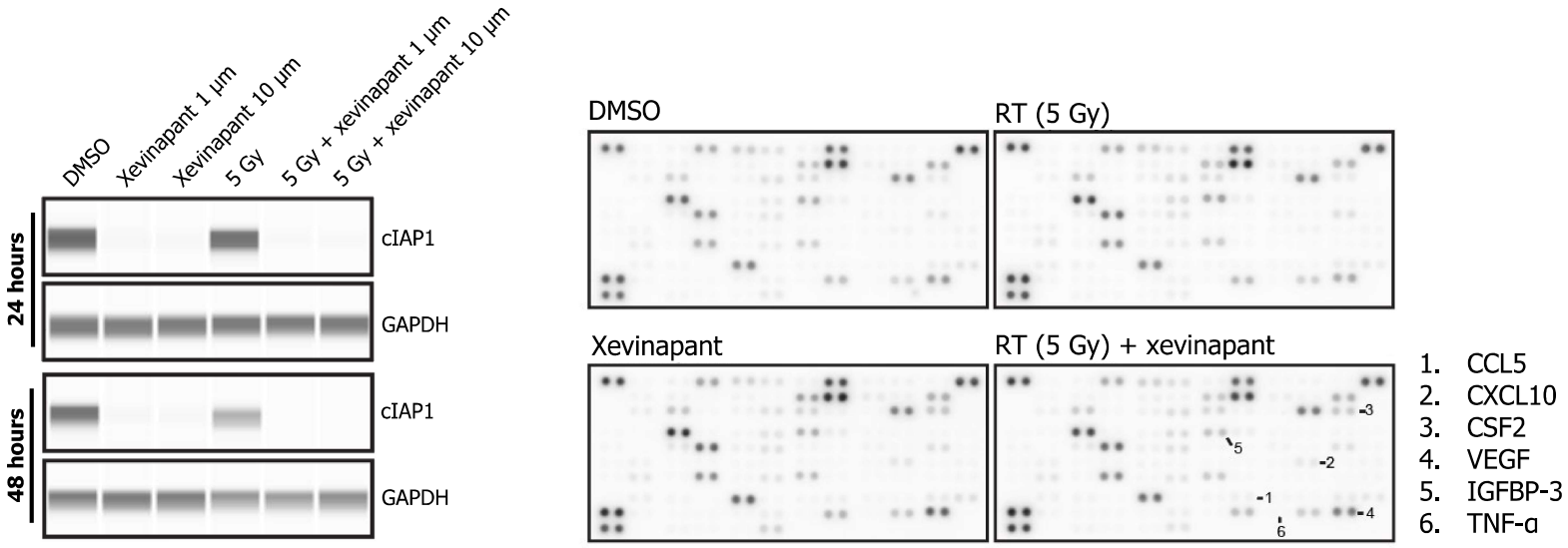
CD, cluster of differentiation; **IHC,** immunohistochemistry; **RT,** radiotherapy.

Supplementary Figure 5. Modulation of immune cell and fibroblast phenotype by xevinapant (A) IL-2 and viability readout and (B) cell count assay designs



IFN, interferon; **IL,** interleukin; **LPS,** lipopolysaccharide; **M-CSF,** macrophage colony-stimulating factor; **PBMC,** peripheral blood mononuclear cell.

Supplementary Figure 6. Western blot and cytokine array images showing modulation of immune cell and fibroblast phenotype by xevinapant



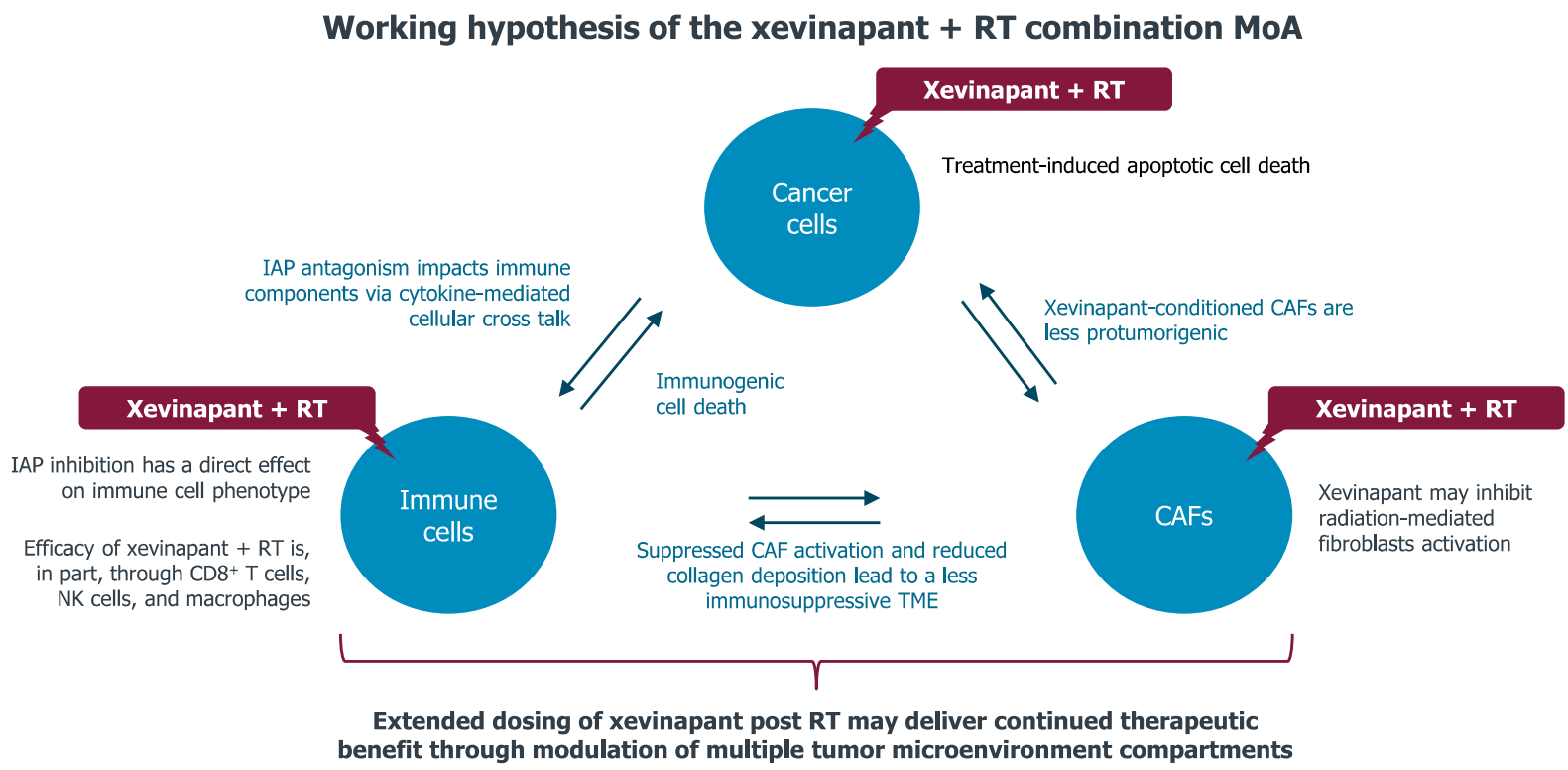
DMSO, dimethyl sulfoxide; **RT,** radiotherapy.

Supplementary Results

Proposed mechanism of action (MoA)

- Evidence suggests that IAP inhibitors could enhance the efficacy of anticancer treatments by resensitizing cancer cells to apoptosis and modulate immune cell types; however, some mechanistic details remain unexplored, including the effect of IAP inhibitors in combination with RT on the tumor microenvironment and how continual administration of IAP inhibitors post RT may confer additional therapeutic benefit
- To address this gap in knowledge, we used preclinical models to delineate the MoA underlying the therapeutic benefit mediated by (1) combination therapy vs monotherapy and (2) extended xevinapant dosing post RT
- Based on the current findings, we believe that the combination of xevinapant + RT may modulate several key tumor microenvironment components (**Supplementary Figure 7**):
  - Combination treatment directly promotes cell death via apoptotic pathways
  - For stromal fibroblasts, a highly relevant compartment in the RT setting, xevinapant may inhibit radiation-mediated fibroblast activation and reduce collagen production, resulting in fibroblasts that are less tumor promoting and immunosuppressive
  - Xevinapant + RT promotes a tumor-specific T-cell response, and continual dosing of xevinapant post RT may encourage the development of a stronger immunologic memory
- Extended dosing of xevinapant post concurrent RT improved antitumor efficacy, which may be, in part, mediated by xevinapant-enhanced antitumor immunity and immunologic memory

Supplementary Figure 7. Overall MoA of xevinapant + RT combination therapy



CAF, cancer-associated fibroblast; **IAP,** inhibitor of apoptosis protein; **MoA,** mechanism of action; **RT,** radiotherapy; **TME,** tumor microenvironment.