Abstract #8753

Targeting replication stress promotes immunogenic cell death in chordoma

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BACKGROUND

- Chordoma is a rare (incidence 1 per million) bone cancer of the skull base and spine that arises from remnants of the embryonic notochord.
- Standard care is maximal surgical resection +/- radiation.
- Disease recurrence is common (>50% cases), and most patients experience serial relapses with progressively shorter disease-free intervals.
- There are currently no approved drug therapies for advanced chordoma, highlighting a serious unmet need.
- Chordoma is characterized by strong etiological links to replication stress, including recurring alterations in DNA damage repair and SWI/SNF chromatin-remodeling genes, complex genomic rearrangements, and chromosomal instability.
- Immune checkpoint inhibitors have shown clinical activity in recurrent chordoma, supporting the potential relevance of antitumor immunity in this disease (Bishop et al., J Immunother., 2022; JY Blay et al., Lancet Oncol, 2023).
- These findings provide a rationale for therapeutic strategies that promote DNA damage and tumor immunogenicity, potentially enhancing response to immunotherapy.

Figure 1. Chordoma is a bone cancer that forms in the skull base or spine.

RESULTS

Chordoma exhibits a high tumorigenic replication stress signature

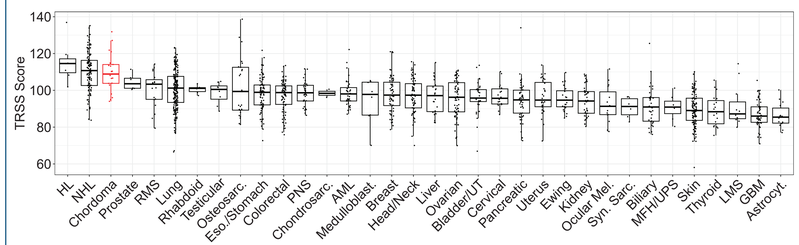
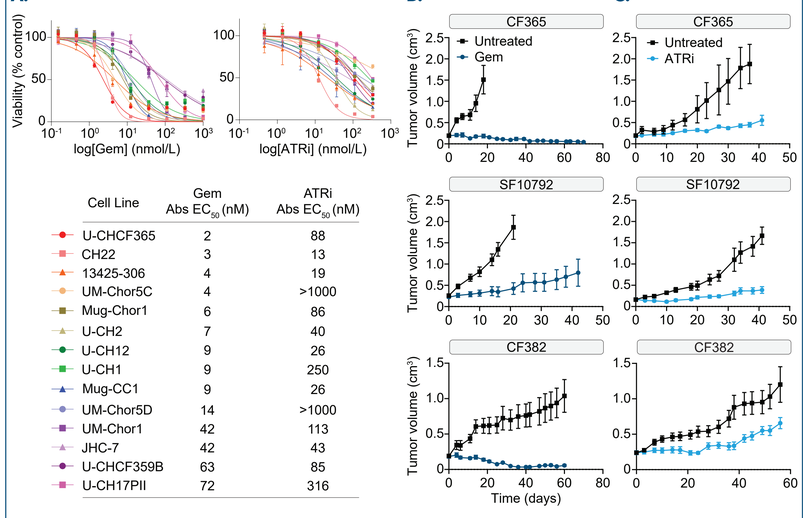


Figure 2. RNA-seq data from 21 chordoma cell lines were combined with DepMap OmicsExpressionGenesExpectedCountProfile (1,330 cancer models; downloaded Feb 2025). The combined count matrix was normalized using DESeq2 variance-stabilizing transformation (VST). For chordoma models profiled in quadruplicate, median VST values were used for downstream analysis. Tumorigenic replication stress signature (TRSS) scores were then calculated using the published TRSS model (Jungk and Kschischo, Cell Stress, 2025). Boxplots show TRSS score distributions across cancer types, with chordoma highlighted in red.

Chordoma models are sensitive to replication-stress targeting agents



Cell Line	Gem Abs EC_{50} (nM)	ATRi Abs EC_{50} (nM)
U-CHCF365	2	88
CH22	3	13
13425-306	4	19
UM-Chor5C	4	>1000
Mug-Chor1	6	86
U-CH2	7	40
U-CH12	9	26
U-CH1	9	250
Mug-CC1	9	26
UM-Chor5D	14	>1000
UM-Chor1	42	113
JHC-7	42	43
U-CHCF359B	63	85
U-CH17PII	72	316

Figure 3. **(A)** Cell proliferation assays in chordoma cell lines treated with gemcitabine (Gem) or ATR inhibitor (ATRi) elimusertib. Assay endpoint for each cell line was time to 90% confluence for untreated cells. Experiments were repeated for each cell line at least once to ensure reproducibility; representative curves are shown. Absolute EC50 values for Gem and ATRi treatments are plotted in the table. **(B)** Antitumor activity of Gem in three PDX models (N=6-7 mice/arm). CF365 was treated with gemcitabine at 100 mg/kg, i.v., q4d; CF382 and SF10792 were treated at 100 mg/kg, i.v., biw. Three additional PDX models were treated with gemcitabine (SF8894, CF538, and CF555) and minimal tumor growth inhibition was observed. **(C)** Three chordoma PDX models were treated with elimusertib at 50 mg/kg, p.o., bidx3x6 (N=4–7 mice/arm), resulting in significant tumor growth inhibition.

ATRi and Gem induce replication fork problems, DNA damage, and extranuclear dsDNA

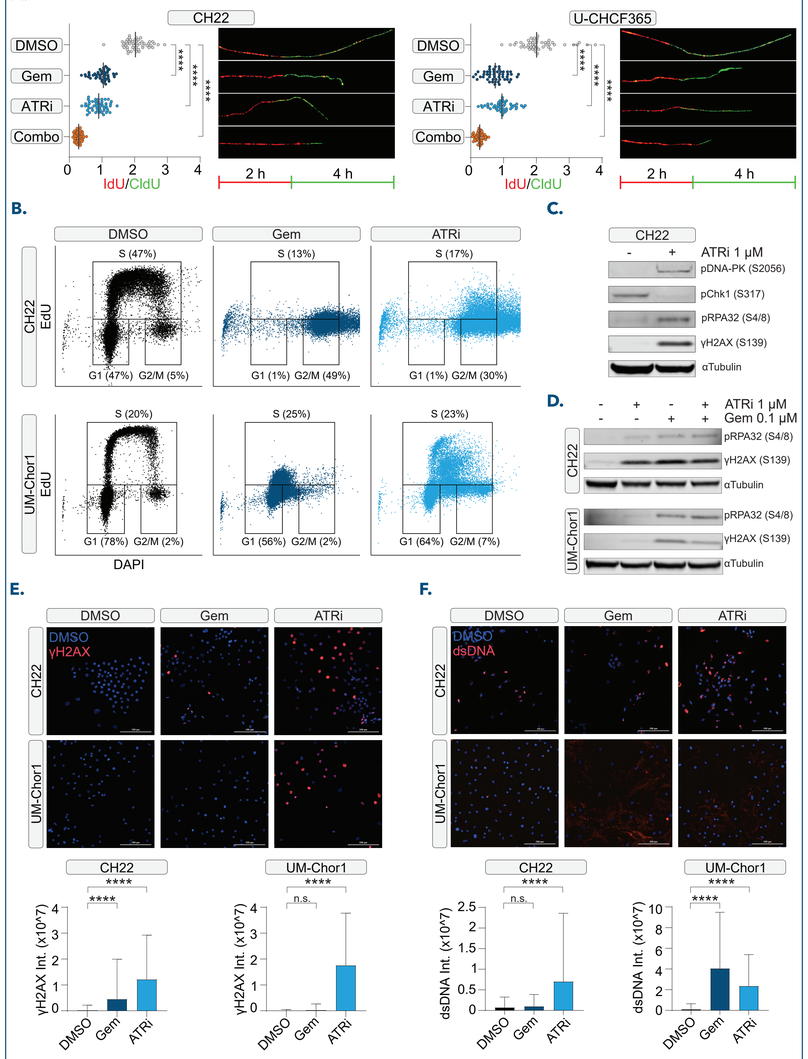


Figure 4. CH22 and UM-Chor1 cells were treated with 100 nM Gem and/or 300 nM ATRi (elimusertib), unless otherwise stated. **(A)** Cells were sequentially labeled with CldU for 2 h followed by IdU for 4 h in the presence or absence of treatment. ATRi and Gem reduced fork progression and promoted fork problems in both cell lines. **(B)** Flow cytometry shows that 1 μM ATRi and/or 100 nM Gem induce replication catastrophe following 48 h of treatment. **(C)** CH22 cells exhibit baseline phosphorylation of Chk1, suggesting an ongoing replication stress response which is exacerbated following 48 h of treatment with 1 μM ATRi, as indicated by an increase in pRPA32 and γH2AX. **(D)** ATRi and Gem promote phosphorylation of H2AX and RPA32. **(E)** IF analysis of γH2AX in cells treated for 24 h. γH2AX signal was quantified by nuclear segmentation and measurement of integrated intensity per nucleus, showing increased DNA damage following treatment. **(F)** IF analysis of extranuclear double-stranded DNA (dsDNA) in CH22 and UM-Chor1. CH22 cells were treated for 24 h and UM-Chor1 cells for 72 h to allow DNA accumulation. Statistical analyses were performed using one-way ANOVA with Dunnett's multiple comparisons test.

ATRi and Gem upregulate IFNβ1, chemokines, and PD-L1

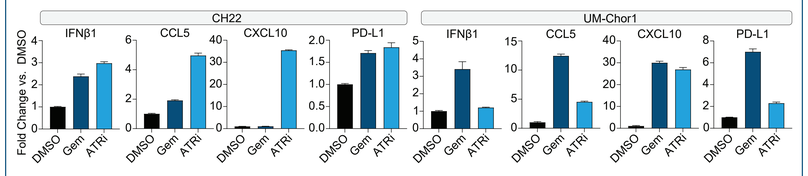


Figure 5. Both lines were treated with 100 nM Gem and 300 nM ATRi. CH22 cells were treated for 24 h, whereas UM-Chor1 cells were treated for 72 h. Gene expression was normalized to GAPDH and expressed as fold change relative to DMSO control.

ATRi and Gem promote immunogenic cell injury/death in chordoma

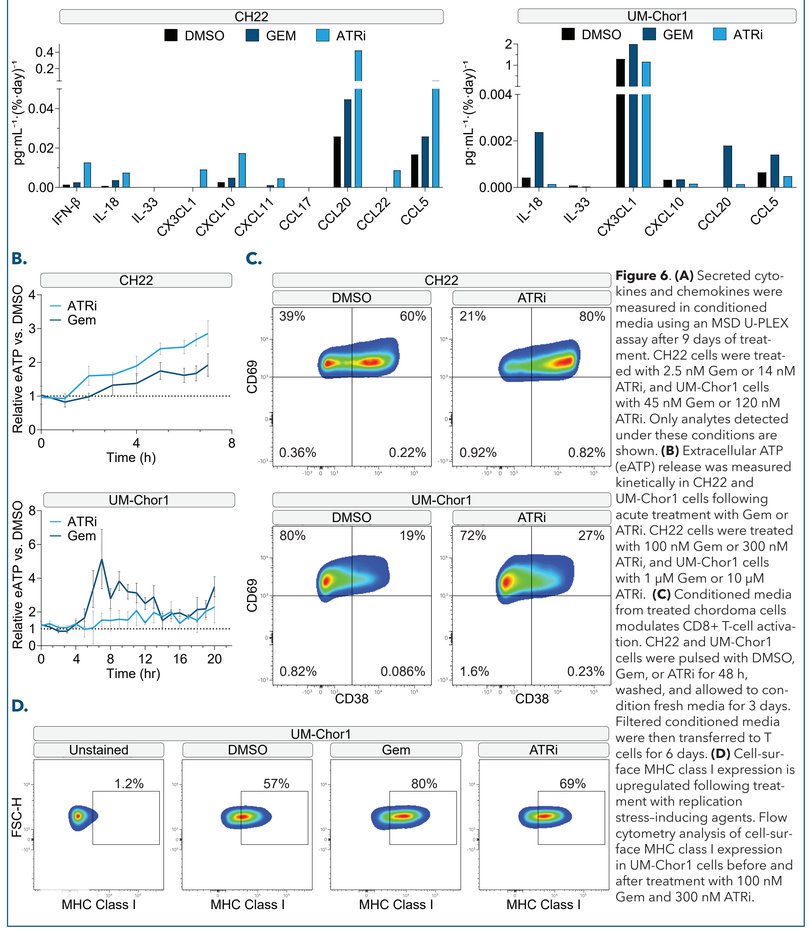


Figure 6. **(A)** Secreted cytokines and chemokines were measured in conditioned media using an MSD U-PLEX assay after 9 days of treatment. CH22 cells were treated with 2.5 nM Gem or 14 nM ATRi, and UM-Chor1 cells with 45 nM Gem or 120 nM ATRi. Only analytes detected under these conditions are shown. **(B)** Extracellular ATP (eATP) release was measured kinetically in CH22 and UM-Chor1 cells following acute treatment with Gem or ATRi. CH22 cells were treated with 100 nM Gem or 300 nM ATRi, and UM-Chor1 cells with 1 μM Gem or 10 μM ATRi. **(C)** Conditioned media from treated chordoma cells modulates CD8+ T-cell activation. CH22 and UM-Chor1 cells were pulsed with DMSO, Gem, or ATRi for 48 h, washed, and allowed to condition fresh media for 3 days. Filtered conditioned media were then transferred to T cells for 6 days. **(D)** Cell-surface MHC class I expression is upregulated following treatment with replication stress–inducing agents. Flow cytometry analysis of cell-surface MHC class I expression in UM-Chor1 cells before and after treatment with 100 nM Gem and 300 nM ATRi.

WORKING MODEL

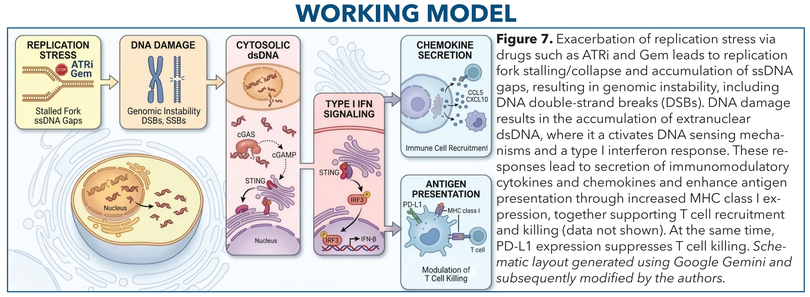


Figure 7. Exacerbation of replication stress via drugs such as ATRi and Gem leads to replication fork stalling/collapse and accumulation of ssDNA gaps, resulting in genomic instability, including DNA double-strand breaks (DSBs). DNA damage results in the accumulation of extranuclear dsDNA, where it a ctivates DNA sensing mechanisms and a type I interferon response. These responses lead to secretion of immunomodulatory cytokines and chemokines and enhance antigen presentation through increased MHC class I expression, together supporting T cell recruitment and killing (data not shown). At the same time, PD-L1 expression suppresses T cell killing. *Schematic layout generated using Google Gemini and subsequently modified by the authors.*

KEY FINDINGS

- Chordoma cell lines and xenograft tumors are highly sensitive to ATRi and Gem, which induce replication catastrophe, S-phase collapse, and lethal DNA damage.
- ATRi and Gem treatment results in accumulation of cytoplasmic dsDNA, activation of type I interferon signaling, and eATP release – consistent with an immunogenic cell death mechanism.
- This immunogenic cell death is associated with secretion of immunomodulatory factors that may enhance T cell activation and immune surveillance.
- Together, these results provide compelling rationale for combining drugs that target DNA replication with PD-1 blockade in chordoma.

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For more information, visit:
www.chordoma.org