Assessing replication stress as an actionable therapeutic opportunity in chordoma

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ABSTRACT #6195

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- Tumor genomic profiling and functional genomics studies have identified replication stress as a potential therapeutic vulnerability in chordoma, which is a rare type of bone cancer with no approved systemic therapies.
- We demonstrate that preclinical models of chordoma exhibit a replication stress phenotype and dependence on ATR activity – particularly when replication stress is exacerbated pharmacologically by ATR inhibition or gemcitabine.
- A panel of 14 chordoma cell lines were treated with the ATR inhibitor (ATRi) elimusertib (BAY1895344) or gemcitabine. Chordoma cells displayed differential sensitivity to elimusertib, with 9 of 14 cell lines having Absolute EC50 values below 100 nM. Chordoma cells are highly sensitive to gemcitabine, with Absolute EC50s below 100 nM in all 14 cell lines and less than 10 nM in 9 of 14 lines.
- Untreated ATRi- and gemcitabine-sensitive cells are characterized by an ongoing replication stress response and double-stranded DNA breaks at baseline.
- DNA fiber assays indicate that ATRi causes a mild decrease in replication fork speed and a significant increase in fork asymmetry in ATRi-sensitive cells, suggesting possible fork stalling or collapse. This is supported by flow cytometry, which indicates that ATRi or gemcitabine promote replication catastrophe.
- ATRi and gemcitabine are highly active in several chordoma PDX models, in some cases producing tumor regressions and complete responses.
- ATRi and gemcitabine synergistically reduce viability of chordoma cell lines.

BACKGROUND

- Chordoma is a rare bone cancer of the skull base and spine that arises from remnants of the embryonic notochord.
- Disease incidence is 1 per million, with median survival from diagnosis of 8 years.
- Standard care is maximal surgical resection +/- radiation, which cures ~30% of patients.
- Chordoma is a relentless disease with a high rate of recurrence; most patients experience serial recurrences with progressively shorter disease-free intervals.
- There are no approved systemic therapies for the treatment of chordoma, motivating the search for effective strategies.
- Chordomas exhibit frequent alterations in DNA damage repair or SWI/SNF chromatin remodeling genes, which have been associated with replication stress.
- Many chordomas are characterized by genomic signatures indicative of defective homologous recombination repair (S. Groschel et al., *Nat Commun*, 2019), which may create a vulnerability to replication stress.
- Replication stress response genes DSCC1 and FANCM are selectively essential in chordoma cell lines (T. Sharifnia et al., *Nat Commun*, 2023).

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

RESULTS

Proliferation assay optimization

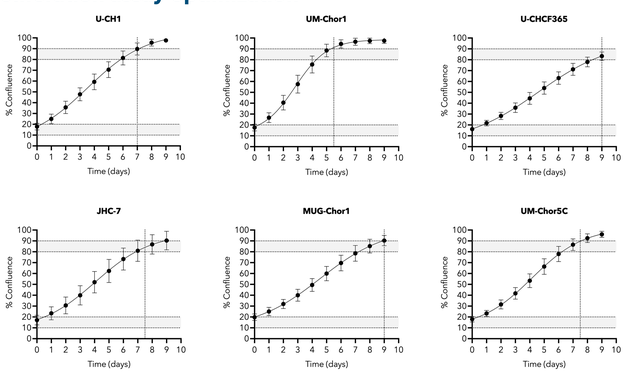
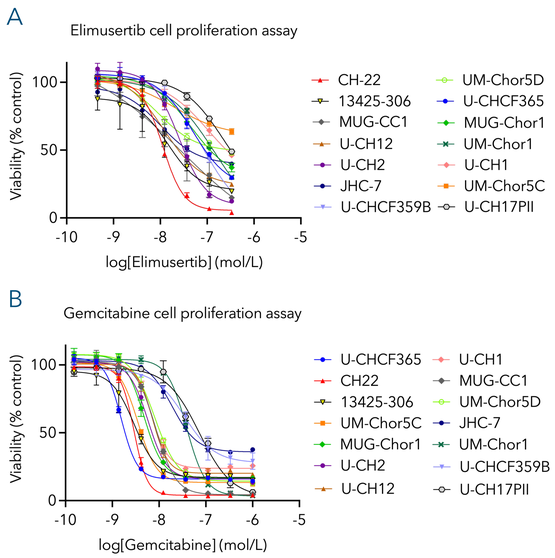


Figure 2. Optimization of proliferation assay parameters using Incucyte Live-Cell Analysis. Representative data is shown for six chordoma cell lines. Accurate interpretation of drug activity in proliferation assays requires optimization of seeding densities (10-20% confluence) and assay durations (endpoint 80-90% confluence). This is particularly important for drugs that may exert their effects through cytostatic mechanisms; untreated control cells must undergo 2-3 population doublings for an accurate determination of EC50.

Chordoma cells are sensitive to therapies that promote replication stress



Cell line	Gemcitabine Abs EC50 (nM)	Elimusertib Abs EC50 (nM)
U-CHCF365	1.8	88
CH22	2.8	13
13425-306	3.5	19
UM-Chor5C	4.2	> 1000
MUG-Chor1	5.6	86
U-CH2	6.6	40
U-CH12	8.9	26
U-CH1	9.2	250
MUG-CC1	9.2	26
UM-Chor5D	14	> 1000
UM-Chor1	42	113
JHC-7	42	43
U-CHCF359B	63	85
U-CH17PII	72	316

Table 1. Absolute EC50 values plotted for chordoma cell lines. This parameter is defined as the drug concentration that inhibits proliferation 50% compared to control cells treated with DMSO vehicle.

Figure 3. Cell proliferation assays in chordoma cell lines treated with ATR inhibitor elimusertib (BAY1895344) **(A)** or gemcitabine **(B)**. Assay endpoint for each cell line was time to 90% confluence for untreated cells. Experiments were repeated for each cell line at least once (N = 2-3 biological replicates) to ensure reproducibility; representative curves are shown.

ATR inhibitors and Gemcitabine induce replication stress and DNA damage in chordoma cells

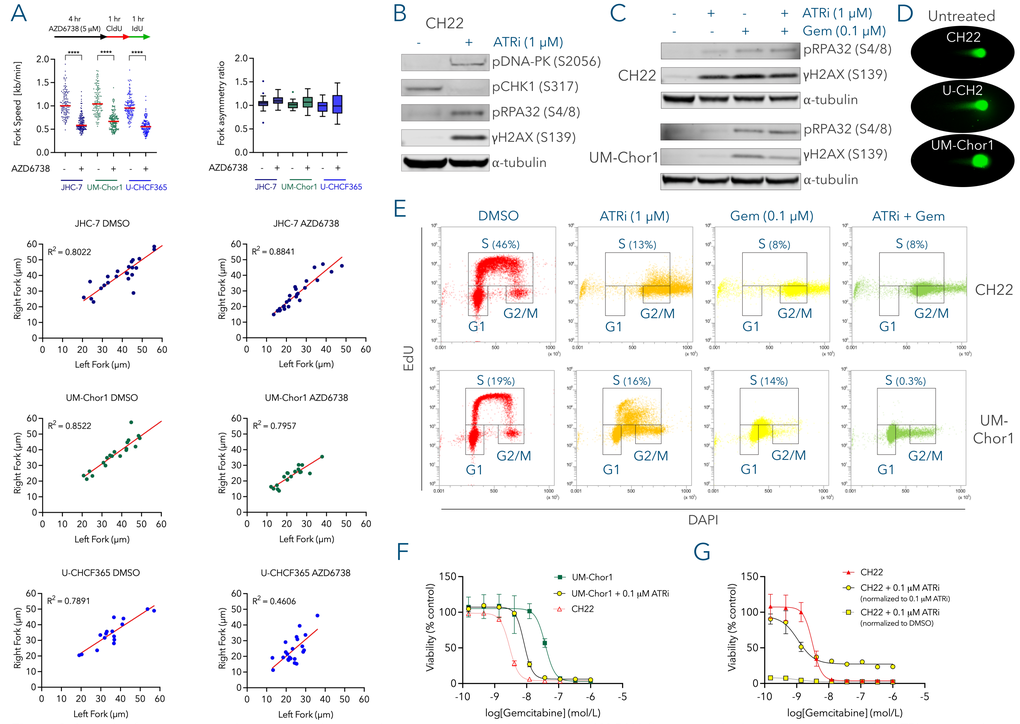


Figure 4. Analysis of chordoma cell lines treated with therapies that promote replication stress. **(A)** DNA fiber assays indicate that treatment with ATRi ceralasertib (AZD6738) at 5 μM causes a mild decrease in replication fork speed and a significant increase in fork asymmetry in ATRi-sensitive cells, suggesting possible fork stalling or collapse. **(B)** CH22 cells exhibit baseline phosphorylation of CHK1, suggesting an ongoing replication stress response which is exacerbated following 48 hrs of treatment with 1 μM ATRi elimusertib, as indicated by an increase in phospho-RPA and γH2AX. **(C)** ATRi elimusertib and gemcitabine induce higher replication stress in sensitive cells. **(D)** Comet assays reveal double-stranded DNA breaks in CH22 and U-CH2 cells at baseline, but not UM-Chor1 cells. Representative nuclei are shown at 20X magnification. **(E)** Flow cytometry shows ATRi elimusertib and/or gemcitabine induce replication catastrophe following 48 hrs of treatment. **(F, G)** Combination treatment with 0.1 μM of ATRi elimusertib enhances gemcitabine potency 2-4 fold in proliferation assays in UM-Chor1 cells (F) and CH22 cells (G). In UM-Chor1 cells, the enhancement in potency with combination treatment approaches that of CH22 cells treated with gemcitabine alone.

Efficacy of Gemcitabine and ATR inhibitors in chordoma PDX models

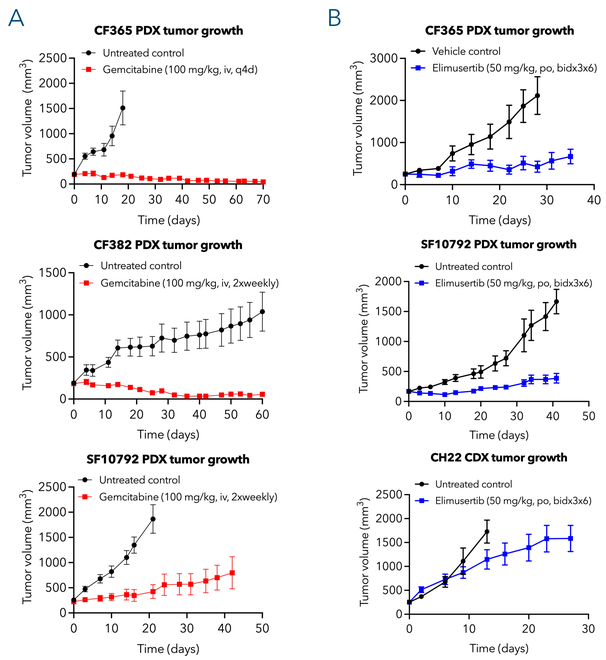


Figure 5. In vivo efficacy studies in chordoma mouse models. **(A)** Antitumor activity of gemcitabine in three patient-derived xenograft (PDX) models (N=6-7 mice/arm). For CF365, there were 3 complete responses (CRs), 3 partial responses (PRs), and 1 stable disease (SD). For CF382, there were 1 CR, 5 PRs, and 1 SD, and for SF10792 there were 1 PR, 2 SD, and 3 tumors exhibited progressive disease. We treated three additional PDX models with gemcitabine (SF8894, CF538, and CF555) and observed minimal tumor growth inhibition (data not shown), indicating that not all chordomas are sensitive. **(B)** Three chordoma xenograft models were treated with ATRi elimusertib (N=4-7 mice/arm), which induced significant tumor growth inhibition in each model.

Potential replication stress drivers in chordoma models

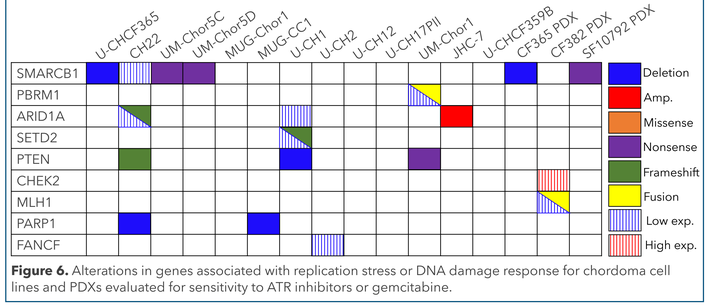


Figure 6. Alterations in genes associated with replication stress or DNA damage response for chordoma cell lines and PDXs evaluated for sensitivity to ATR inhibitors or gemcitabine.

KEY FINDINGS AND NEXT STEPS

- Our data reveal that a subset of chordomas are sensitive to therapeutic agents that promote replication stress, including ATR inhibitors and gemcitabine.
- Chordoma cells appear to be highly sensitive to gemcitabine, which induces complete tumor responses in a subset of chordoma PDX models.
- ATR inhibitors or gemcitabine promote replication catastrophe and lethal DNA damage, particularly in cells with pre-existing replication stress or unresolved double-stranded DNA breaks.
- ATRi and gemcitabine synergistically reduce cell viability in chordoma cell lines. Continuing efforts will focus on identifying synergistic combination therapies that further enhance the magnitude and/or duration of antitumor response.
- Bioinformatics analysis is ongoing to identify molecular features that predict response to ATR inhibitors or gemcitabine.

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