The selective ATR inhibitor VX-970 enhances the therapeutic effects of radiation and temozolomide in patient-derived xenografts (PDXs) of glioblastoma (GBM).

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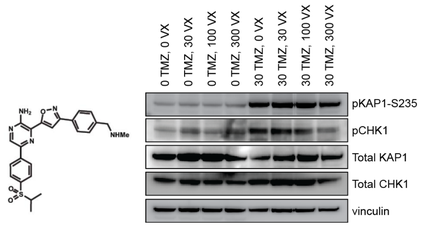
Abstract

GBM represents one of the most aggressive cancer types with the vast majority of patients succumbing to disease within the first five years. This dire prognosis reflects the limited efficacy of our frontline therapies which include radiation therapy and temozolomide (TMZ) chemotherapy. The cellular response to these therapies is critically mediated by DNA damage response signaling networks that are regulated by Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangiectasia and Rad3-related protein (ATR)[1]. Previous work from our laboratory suggested ATR is an important mediator of TMZ sensitivity as both genetic knockdown and chemical inhibition of ATR using VE-821 improved therapeutic responses to TMZ. This was in sharp contrast to ATM knockdown as that demonstrated no such effect on the cytotoxicity of TMZ.

In this study, we evaluated the efficacy of the next generation ATR inhibitor VX-970 as a single agent and in combination with standards of care in both established and PDX culture systems. Target engagement was observed within 24 hours of nanomolar VX-970 exposure in U251 cells that had been pre-treated with TMZ. In addition to its suppression of downstream pCHK1 signaling, VX-970 treatment decreased proliferation in a dose dependent manner within the U251 and T98 cell lines (range of suppression 30%-90%). Investigations into the cell cycle effects of transient VX-970 treatment showed no obvious alterations. When tested alone and in combination with TMZ, decreased viability and neurosphere formation was detected in adherent and stem conditions. Optimal dosing appears to be within the 30-100 nM range for a majority of the cell lines that have been studied. Regression analysis of data from three separate cell lines showed robust synergy between TMZ and VX-970 ($p<0.05$). Early work aimed at understanding the impact of MGMT expression on VX-970 response suggests that more pronounced effects may be achieved if it is silenced.

Apart from its potential value as a chemosensitizer, VX-970 enhanced radiation effects in a colony formation assay to suggest it may also be a valuable radiosensitizer. Despite being a substrate of drug efflux pumps in the brain, initial pharmacokinetics of VX-970 reveal drug concentrations above 100 nM which is well within the therapeutic window we have defined *in vitro* to promote therapeutic benefit. Therefore, this collection of work suggests that ATR inhibition may represent a worthwhile treatment strategy for patients with GBM.

Compound Structure and Target Engagement



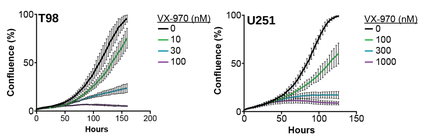
Left. Chemical structure of VX-970[2]. **Right.** U251 cells were treated for 72 hours with TMZ (μM) before treatment with the indicated dose of VX-970 (nM). Lysates were collected 24 hours later at the 96 hour endpoint.

Table 1. Genomic features of culture models used

Cell Line	MGMT status[i]	p53 status[ii]
U251	Methylated	Mutant
T98	Unmethylated	Mutant
U87	Methylated	Wild-type
GBM6	Unmethylated	Mutant
GBM12	Methylated	Wild-type
GBM22	Methylated	Mutant
GBM75	Indeterminate	Wild-type
GBM84	Methylated	Wild-type

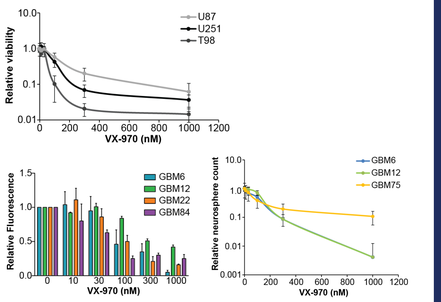
DNA was extracted from flank xenograft samples using the Gentra DNA extraction kit and used for i) the qMS-PCR MGMT assay from ABI and ii) Illumina Tru-Seq.

ATR inhibition reduces cell proliferation



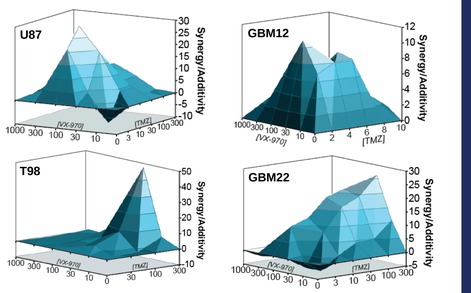
Cells were plated and treated 24 hours later with VX-970. Confluence was determined in real-time by the Incucyte Live Cell Analysis system and plotted as a function of time.

VX-970 treatment demonstrates single agent efficacy in established and primary cell culture models



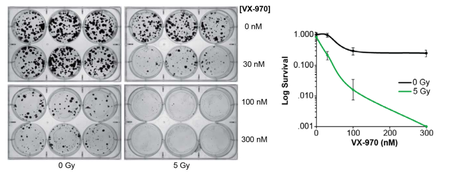
Established cell lines and primary PDX cell lines were plated in serum (**left panels**) and stem (**right panel**) conditions for 24 hours before being treated with VX-970. Once vehicles reached confluence, viability was measured using the CyQUANT proliferation assay in each adherently grown line while neurospheres were counted at days 14 or 21.

Combined treatment with TMZ produces significant synergy across multiple cell lines



Cells were plated and treated 24 hours later with VX-970 (nM) and/or TMZ (μM). At endpoint, cell viability was measured by the CyQUANT proliferation assay (or by neurosphere counts in GBM12) and analyzed using the MacSynergy program.

Colony formation is dramatically reduced in response to radiation when combined with VX-970



U251 cells were plated and treated with VX-970 (at 4 hrs) and irradiation (at 5 hours). Fresh media was applied 24 hours later and colonies were allowed to form for 14 days at which point the plates were fixed, stained, and counted.

Pharmacokinetics of VX-970

Dose	Mouse strain	Route x Doses	Drug levels Brain, Plasma, Tumor
20 mpk	Fvb-WT	PO x 1	118 nM, 1.3 μM, NA
	Fvb-Mdr1a/b$^{-/-}$, Bcrp1$^{-/-}$		3.7 μM, 1.5 μM, NA
30 mpk	Athymic nude	PO x 5	123 nM, 550 nM, 4.2 uM
60 mpk	Athymic nude	PO x 5	268 nM, 684 nM, 8.6 uM

Abbreviations: mpk – mg/kg, po – by mouth

Animals were dosed as indicated until sacrificed. Blood and tissue were collected and stored at -80 until LC-MS/MS analysis.

Conclusions

- Single agent VX-970 treatment inhibits neurosphere formation and decreases viability across multiple cell lines *in vitro*.
- VX-970 potentiates the activity of TMZ and radiation with combination treatments demonstrating marked synergy.
- Preliminary PK experiments suggest that this agent may be brain penetrant.

References

1. Cimprich, K.A. and D. Cortez, *ATR: an essential regulator of genome integrity*. Nature reviews. Molecular cell biology, 2008. 9(8): p. 616-27.
2. Hall, A.B., et al., *Potentiation of tumor responses to DNA damaging therapy by the selective ATR inhibitor VX-970*. Oncotarget, 2014. 5(14): p. 5674-85.

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