Destabilizing EBNA1 Through USP7 Inhibition Preferentially Targets EBV+ Cancer Cells

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Abstract

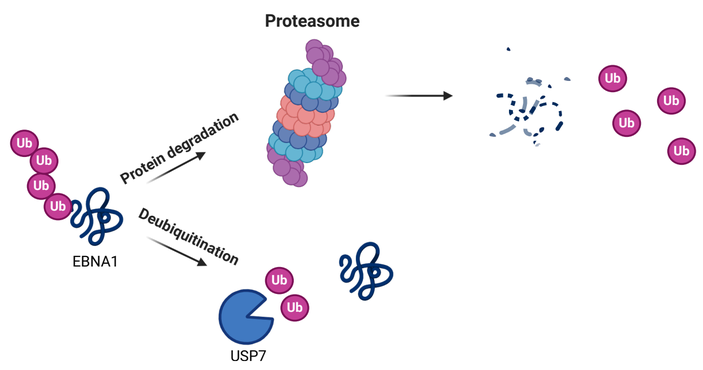
Various cancers have been associated with Epstein-Barr Virus (EBV), a herpesvirus infecting over 95% of the global population. All EBV-related malignancies feature Epstein-Barr nuclear antigen 1 (EBNA1), an essential viral protein facilitating viral replication and cell immortalization by binding to host cell DNA. Despite lacking enzymatic functions, EBNA1 interacts with other proteins, notably ubiquitin-specific protease 7 (USP7), a deubiquitinating enzyme that can protect proteins from degradation. It is unclear whether USP7 acts as a proviral factor. We hypothesized that inhibiting USP7 would adversely affect EBV+ cancer cell growth. Our data demonstrate that pharmacologically inhibiting USP7's enzymatic activity reduces EBNA1 levels in EBV+ cells. Consequently, EBV+ cancer cells show increased sensitivity to USP7 inhibition compared to EBV- cells, suggesting selective targeting potential through USP7. Initial findings also indicate that USP7 inhibition in EBV+ cells impedes cell cycle progression and lowers viral DNA levels. Combining USP7 inhibitors with an established EBNA1 inhibitor shows promise for enhanced selectivity and efficacy. Overall, USP7 inhibition presents a promising opportunity as a novel intervention to disrupt EBV+ cancer cell function.

Introduction

Epstein-Barr Virus (EBV) and EBNA1

- DNA tumor virus that has infected over 90% of the global population
- Linked to various cancers
  - Gastric carcinoma
  - Burkitt's Lymphoma
- All EBV-linked tumors express EBNA1
  - Binds to host cell DNA
  - Enables viral replication
  - Necessary for cell immortalization

Ubiquitin-Proteasome Pathway



The ubiquitin system maintains protein turnover via the conjugation of ubiquitin. This is reversible by deubiquitinating enzymes such as ubiquitin-specific protease 7 (USP7).

Previously we have determined that siRNA inhibition of USP7 results in reduced EBNA1 levels, and this effect was replicated via multiple pharmacological USP7 inhibitors:
1) GNE6776
2) XL177A
3) (R)-FT671

Primary Research Question

- Is there a therapeutic potential in targeting EBV+ cancers via USP7 inhibition?

Results

What is the effect of USP7 inhibition on EBNA1 levels?

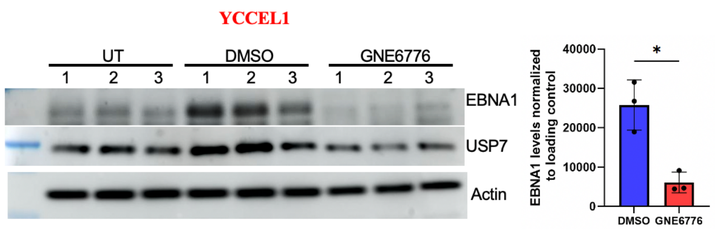


Figure 1: Reduction in EBNA1 levels following GNE6776 treatment
Western Blot and Densitometry following 48 hours 20uM GNE6776 treatment in YCCEL1 (EBV+)

Results

Can USP7 inhibition target viral replication?

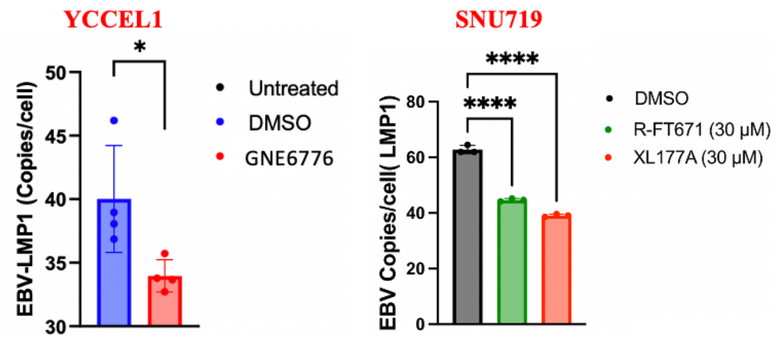


Figure 2: Reduction in viral DNA following USP7 inhibition
EBNA1 and Viral DNA levels in YCCEL1 and SNU719 (EBV+) following GNE6776, (R)-FT671, XL177A treatment

How does USP7 inhibition impact EBV+ cancer cell metabolism?

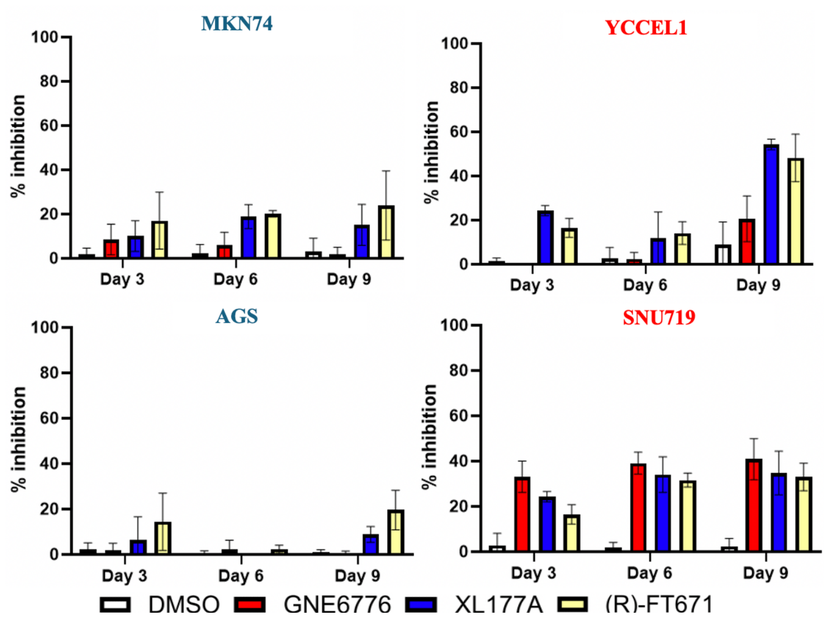


Figure 3: Selective metabolic inhibition of EBV+ cancer cells via three unique USP7 inhibitors
Resazurin assay of MKN74/AGS (EBV-) vs. YCCEL1/SNU719 (EBV+) following GNE6776, (R)-FT671, and XL-177A timecourse

Do USP7 inhibitors slow EBV+ cell growth?

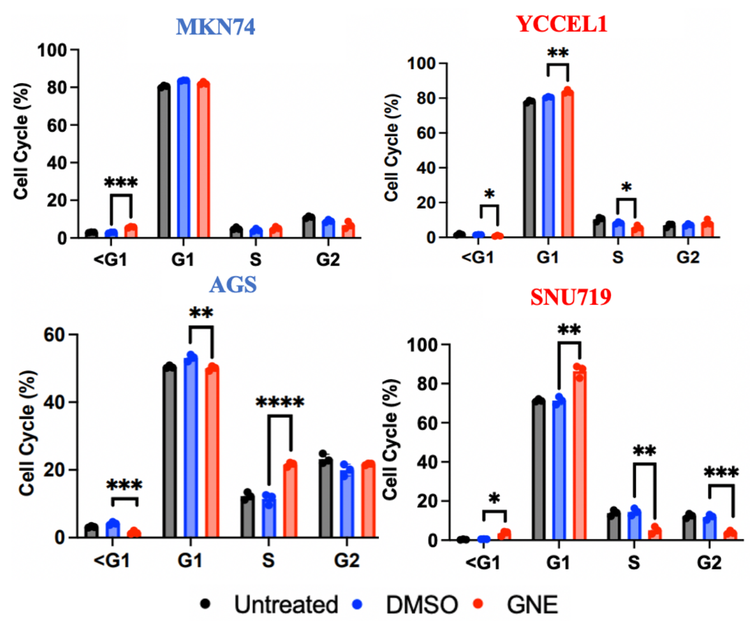


Figure 4: GNE6776 induces G1 arrest in EBV+ cancer cells
Cell cycle analysis via propidium iodide staining of MKN74/AGS (EBV-) vs. SNU719/YCCEL1 (EBV+) following 6 days GNE6776 treatment

Can an EBNA1 inhibitor enhance a USP7 inhibitor's impact on EBV+ cancer cells?

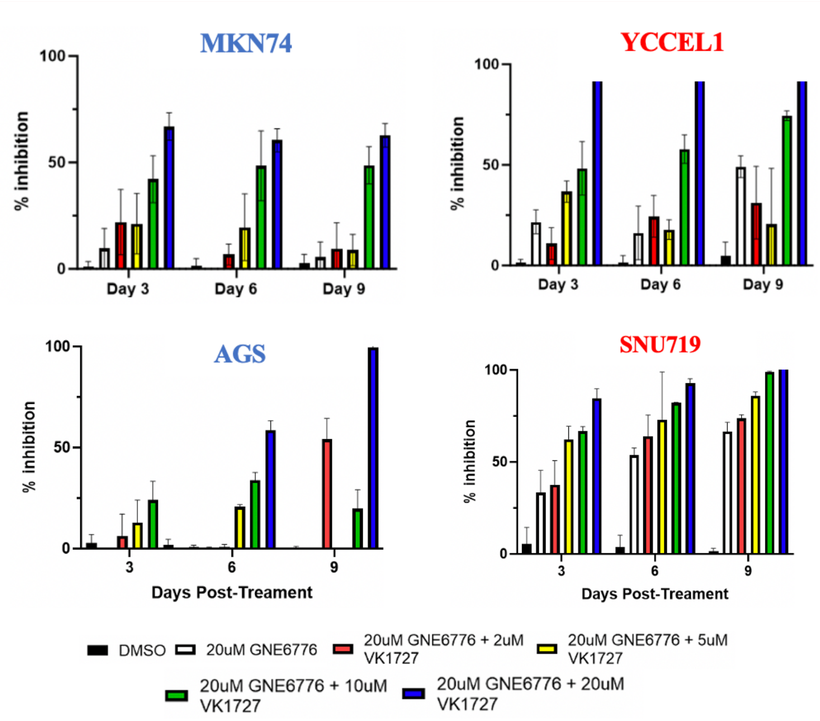


Figure 5: Potential for increased selectivity and efficacy when using USP7 and EBNA1 inhibitors in combination
Resazurin assay of MKN74/AGS (EBV-) vs. YCCEL1/SNU719 (EBV+) following GNE-6776 and VK1727 timecourse

Results

Is this potentiation replicated when an alternate USP7 inhibitor is used?

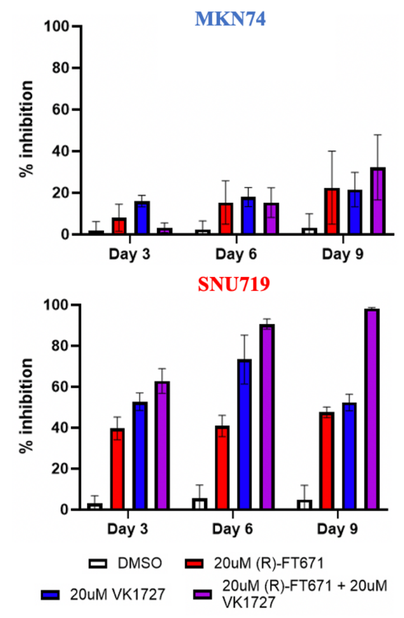


Figure 6: Increase in selectivity and efficacy replicated via alternate USP7 inhibitor and EBNA1 inhibitor combination
Resazurin assay of MKN74 (EBV-) and SNU719 (EBV+) following (R)-FT761 and VK1727 timecourse

Can a new bifunctional molecule enhance selectivity and efficacy?

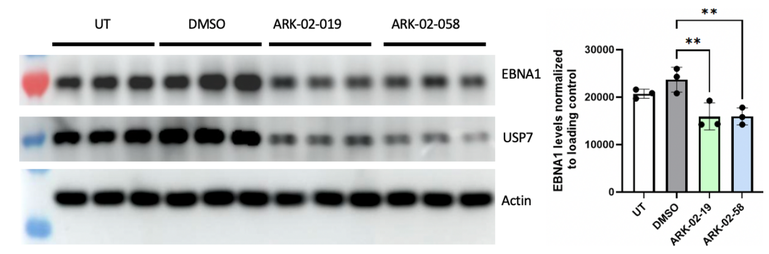


Figure 7: Reduction in EBNA1 levels following ARK-02-19 and ARK-02-58 treatments
Western Blot and Densitometry following 6 days treatment with ARK inhibitors in SNU719 (EBV+)

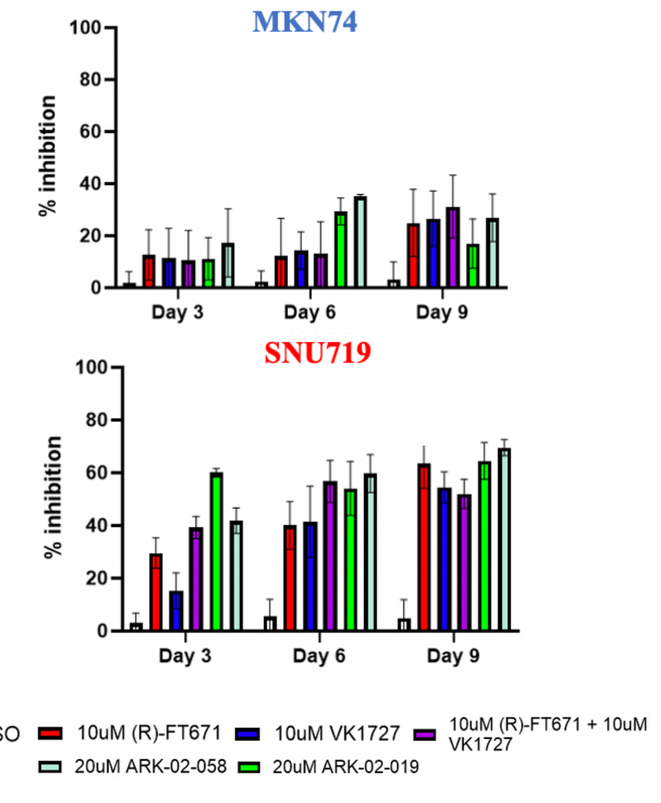


Figure 8: Novel bifunctional molecule demonstrates preferential targeting of EBV+ cancer cells
Resazurin assay of MKN74 (EBV-) and SNU719 (EBV+) following (R)-FT761, VK1727, ARK-02-058, and ARK-02-019 timecourse

Conclusions

- Pharmacological inhibition of USP7 selectively targets EBV+ cancer cells via a reduction in EBNA1 levels
  - Decrease in EBV+ cell metabolism
  - Increased G1 arrest
  - Reduction in viral DNA
- Addition of an EBNA1 inhibitor enhances the impact of USP7 inhibition on EBV+ cancer cells

Future Directions

- Evaluating efficacy of novel bifunctional drugs in selectively targeting EBV+ cancer cells
- Currently developing an orthotopic mouse model to test efficacy of USP7 inhibition *in vivo*

Acknowledgements

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