Mechanisms of therapeutic synergy between pattern recognition response agonists and CDK4 inhibitors


Palbociclib enhances the anti-cancer efficacy of the oncolytic virus Ri3D

Reovirus type-3 Dearing (Ri3D) is an oncolytic wild-type, double-stranded RNA virus studied in multiple clinical trials. We performed a high-throughput drug screen to employ a non-biased approach to determine any synergistic drug-virus interactions. A range of different cancer drugs (\(10^{-5}\)) was screened against Ri3D in the BAPY6000-mutant A375 cell line (a). Palbociclib enhanced Ri3D cell death and was a top hit in the screen. Palbociclib induces a G1 arrest with palbociclib (as expected), and an increase in the subG1 population with combination treatment (c). Western analysis shows the appearance of cleaved caspase 3 and PARP due to Ri3D that was enhanced by palbociclib, indicating that combination therapy increases death through an apoptotic process (d).

Palbociclib causes extensive DNA modification

Proteomic analysis revealed that palbociclib markedly altered proteins under the GO term DNA modification (a). This includes inhibition of DNA methyltransferase 1 (DNMT1) and EZH2, regardless of the presence of Ri3D. To test direct DNMT inhibition combined with Ri3D, we used the Ri3D inhibitor decitabine. Decitabine sensitized melanoma cells to Ri3D-induced cell kill (b). DNMT inhibitors have been shown to upregulate ER chaperones, indicating demethylation by palbociclib as a potential cause of the ER stress profile observed in combination with Ri3D.

Palbociclib potentiates interferon signalling in A375

In addition to the ER stress and demethylation signatures described, proteomics (a) and RNA sequencing (b) data indicated a pronounced virus response and interferon signatures due to combination treatment.

Validation of Ri3D in combination with palbociclib

Cell kill was enhanced between Ri3D (multiplicity of infection dose, MOI 0.1) plus palbociclib (1 µM) in A375 melanoma cells (a). Ri3D plus palbociclib reduced A375 tumour burden in CD1 nude mice in vivo (b). Cell cycle stage revealed a G1 arrest with palbociclib (as expected), and an increase in the subG1 population with combination treatment (c). Western analysis shows the appearance of cleaved caspase 3 and PARP due to Ri3D that was enhanced by palbociclib, indicating that combination therapy increases death through an apoptotic process (d).

Palbociclib potentiates innate immune activity

We hypothesized that enhanced dsRNA sensing and ER stress may also enhance the immunogenicity of therapy. Rs3D-palbociclib increased HLA class I proteins (a), and surface expression of calreticulin (CRT, green), (b) CRT is pro-phagocytic and is associated with immunogenic cell death. Labeled PBMC-derived human macrophages were co-cultured with tumour cells stained with phalloidin, a dye that fluoresces red in acidic conditions such as engulfment. Microscope pictures show macrophages (CD11b+ F4/80+, white arrows), and engulfed tumour cells within macrophages (blue arrows) (c). Phagocytosis was significantly enhanced by combination treatment, measured by dual positive staining (d).

Summary

Oncolytic viruses are an attractive treatment option because they are self-amplifying, kill through multiple mechanisms and have good potential to promote anti-tumour immune responses.

• Combinatorial therapy of Ri3D plus palbociclib was identified by a high-throughput drug screen.
  • Palbociclib altered cell cycle dynamics and DNA methylation, while Ri3D activated interferon signaling via IFN-\(\gamma\). In addition, both agents appeared to have non-overlapping ER stress profiles.
  • The combination, however, enhanced ER stress, potentiated ISG-induction, ERVlibration and phagocytosis in vitro.

Our pre-clinical data show a strong rationale for the combination of Ri3D with novel pharmacological inhibitors, such as the CDK4/6 inhibitor palbociclib.