Molecular medicine, Royal College of Surgeons Ireland

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INTRODUCTION
Multiple activating genetic mutations in the phosphatidylinositol-3 kinase (PI3K) and the mitogen activated protein kinase (MAPK) pathways have been implicated in the development of resistance to anti-cancer therapies. Ribociclib has limited activity as a single agent in CRC. However, combining Ribociclib with targeted therapies of the MAPK and PI3K pathways may be a promising treatment strategy in CRC.

AIM
Determination of the molecular basis for the synergy of the combination of the CDK4/6 inhibitor Ribociclib and the PI3K inhibitor Alpelisib in vitro in colorectal cancer cell line models with various mutational backgrounds.

METHOD
We explored the in vitro efficacy of drug combinations (Ribociclib and Alpelisib (R+A)) in four CRC cell line models with different mutational status; CACO-2 (PIK3CA/KRAS wildtype), LS-1034 (PIK3CA/KRAS mutated), SNJU-C4 (PIK3CA mutated) and DLD-1 (PIK3CA/KRAS mutated). Drug combination index (CI) was calculated by using CalcuSyn Biosoft software. A western immunoblotting method was used for protein analysis.

RESULTS

- The LS1034, SNJU-C4 cells were sensitive to both alpelisib and ribociclib.
- The Caco-2 wild-type cell line was relatively resistant to both drugs, in particular ribociclib (IC50 >15µM).
- Drug combination analysis showed that the combination of R+A has a synergistic anti-proliferative effect in all CRC cell lines tested.
- The combination of R+A is highly synergistic in LS1034 cells which harbour a KRAS mutation (CI=0.16).
- The combination of R+A is also synergistic in DLD-1 cells which have co-occurring KRAS and PIK3CA mutations (CI=0.78), as well as in SNJU-C4 (PIK3CA-mutated; CI=0.49) and CACO-2 (wild-type; CI=0.28) cell lines.

Fig 1 Representative dose-response curves showing the cytotoxicity effects of alpelisib, ribociclib and their combination in all four cell lines

- We demonstrated that combined inhibition of CDK4/6 and PI3Kα caused a simultaneous reduction of p-akt and p-S6 and a more complete inhibition of the PI3K/AKT/mTOR pathway
- Reduction in levels of E2F-1 and pRb in combination group confirms cell-cycle inhibition
- Cyclin D1 results were inconsistent across cell lines and their repeats, possibly due to effect of failed serum starvation and that not all cells were in same phase of cell cycle
- Marginal increment in pBCL-2 in drug treatment groups, possibly due to shorter duration of treatment i.e., 24 hour,
- Aim to repeat apoptotic markers after treating cells for 72 hours and by collecting dead cells from media

CONCLUSIONS
A synergistic response to treatment with the combination of R+A is seen in all cell lines. We are currently investigating this combination in CRC xenograft models (chick chorioallantoic membrane (CAM) assay).

REFERENCES
1. Combination therapy with BYL719 and LEE011 is synergistic and causes a greater suppression of p-S6 in triple negative breast cancer (Yuan, Wen et al. 2019)
2. Combined CDK4/6 and PI3K(β) Inhibition Is Synergistic and Immunogenic in Triple-Negative Breast Cancer(Teo, Versaci et al. 2017)
4. Targeting activated PI3K/mTOR signaling overcomes acquired resistance to CDK4/6-based therapies in preclinical models of hormone receptor-positive breast cancer(O’Brien, McDermott et al. 2020)
5. PI3K and mTOR inhibitors Enhance Anti-Tumor Efficacy of Abemaciclib in Human Colorectal Cancer Cells(Lee)