

Dual inhibition of BCL-xL and MCL-1 Induce Apoptosis in Conditionally Reprogrammed HNSCC tumors



Albert Einstein College of Medicine

Li, Daniel¹; Lopez, Andrea G², Chan, Wesley¹; Shrivastava, Nitisha¹; Thomas, Carlos A¹; Prystowsky Michael B.¹; Gavathiotis, Evripidis^{2,3}; Ow, Thomas J^{1,4}

1. Department of Pathology, Albert Einstein College of Medicine, Bronx NY; 2. Department of Biochemistry, Albert Einstein College of Medicine, Bronx NY; 3. Department of Medicine, Albert Einstein College of Medicine, Bronx NY; 4. Department of Otorhinolaryngology – Head and Neck Surgery, Montefiore Medical Center, Bronx NY



BACKGROUND

- Evasion of apoptosis, a hallmark of cancer, contributes to treatment resistance and tumor survival in head and neck squamous cell carcinoma (HNSCC)
- Intrinsic apoptosis signaling is regulated by BCL-2 family of proteins, and upregulation of pro-survival BCL-2 proteins is a common mechanism for apoptosis evasion
- Pro-survival proteins BCL-xL and MCL-1 are highly expressed in HNSCC, and targeting both with BH3 mimetics may be required to optimally induce apoptosis in these cancers.

OBJECTIVES

- To characterize the native expression of BCL-2 family proteins in a panel of diverse HNSCC lines and a patient-derived ‘conditionally reprogrammed’ primary cell culture
- To measure the effect, measuring both cell viability and apoptosis potential of combination treatment of Navitoclax (ABT-263), a BCL-xL, BCL-2, and BCL-w inhibitor and S63845, an MCL-1 inhibitor in these HNSCC lines and assess for synergy
- To assess BH3 mimetic response in a panel of patient derived, “conditionally reprogrammed” (CR) HNSCC tumor cultures in 2D culture and 3D organoid culture as a more advanced translational model

METHODS

- We used 10 immortalized HNSCC cell lines and 6 primary HNSCC tumor cultures established using a published “conditional reprogramming” method, which uses a ROCK inhibitor (Y-27632) and co-culture in irradiated 3T3 J2 feeders¹
- JC-1 BH3 Profiling, which measures mitochondrial outer membrane permeabilization (MOMP) in response to BH3 peptides, was used to assess apoptotic priming of HNSCC lines and dependence of specific pro-survival BCL-2 proteins²
- Western Blot analysis was used to quantify expression of key BCL-2 family proteins
- HNSCC lines were treated with Navitoclax (ABT-263) and S63845 as single agents and in combination for 72 hours, and cell viability was measured using Cell Titer-Glo (Promega); synergy was assessed through BLISS analysis using Combeneft
- Organoids cultured from 2D CR tumor lines were treated in combination with Navitoclax (ABT-263) and S63845, and viability was measured with 3D Cell Titer-Glo (Promega); synergy was assessed as described above

FIGURE 1. HNSCC LINES EXHIBIT INTACT INTRINSIC APOPTOSIS SIGNALING AND DEMONSTRATE OVERALL CO-DEPENDENCE ON BCL-xL AND MCL-1

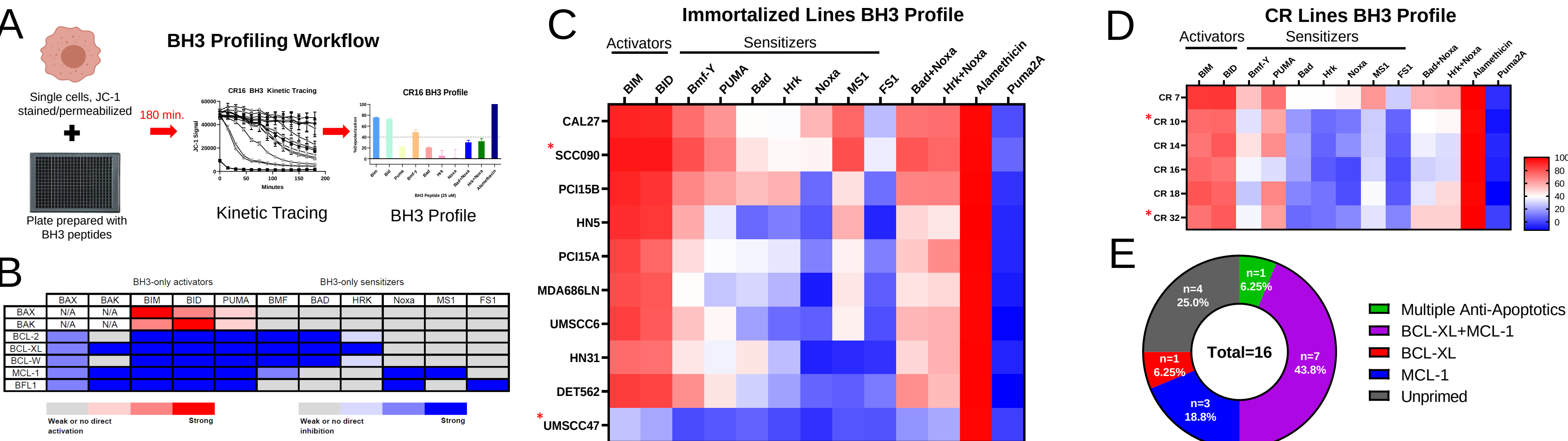


Figure 1: JC-1 plate-based BH3 profiling assay was performed to assess response of HNSCC lines in response to administration of BH3 peptides. A) JC-1 fluorescence was measured over three hours, and area under the curve (AUC) was normalized to positive control CCCP to calculate % depolarization. B) BH3 peptides administered during profiling have various binding specificities to pro-apoptotic BCL-2 family proteins, through which we may assess apoptotic priming and pro-survival protein dependence. A heat map showing depolarization of C) immortalized and D) CR lines in response to BH3 peptides (25 uM, n=2). HNSCC lines are generally competent (functional BAX/BAK) and depolarize in response to coadministration of BH3 peptides with specificity for BCL-xL and MCL-1. * denotes HPV+ lines.

FIGURE 2. RESISTANCE TO NAVITOCLAX IS CORRELATED WITH MCL-1 EXPRESSION

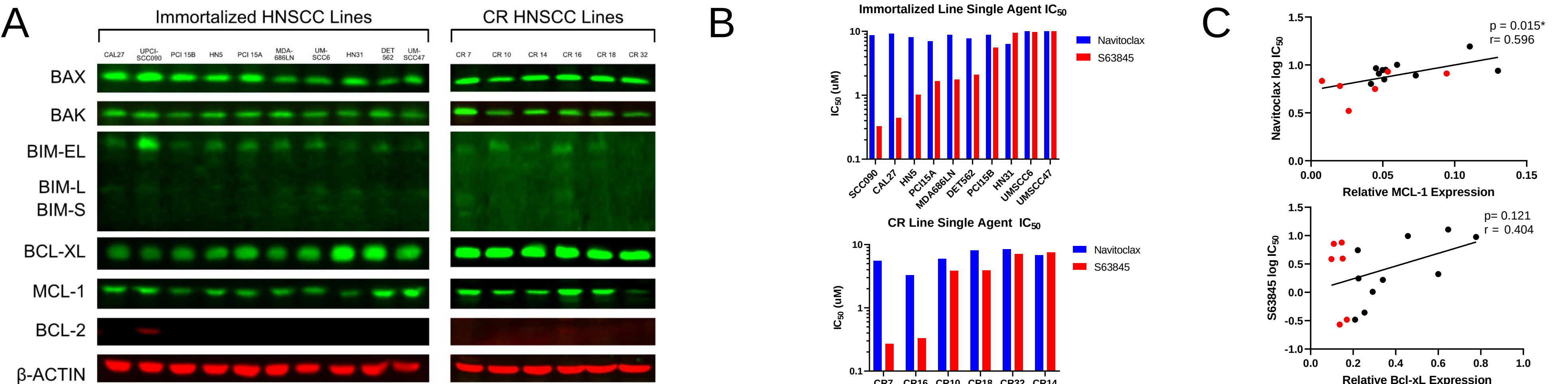


Figure 2: A) Western Blot analysis was used to screen for expression of key BCL-2 family proteins in immortalized and patient derived CR HNSCC lines (n=16). HNSCC lines were treated with Navitoclax and S63845 for 72 hours. B) HNSCC lines show resistance to Navitoclax (IC50 > 3 uM), and a subset are sensitive to S63845 (IC50 < 3 uM, n=7). C) BCL-xL protein expression was correlated with S63845 resistance (black: immortalized lines; red: CR lines). Relative proteins normalized to β-actin loading control, Pearson correlation, p-values calculated using student t-test.

FIGURE 3. NAVITOCLAX AND S63845 SYNERGISTICALLY REDUCE CELL VIABILITY

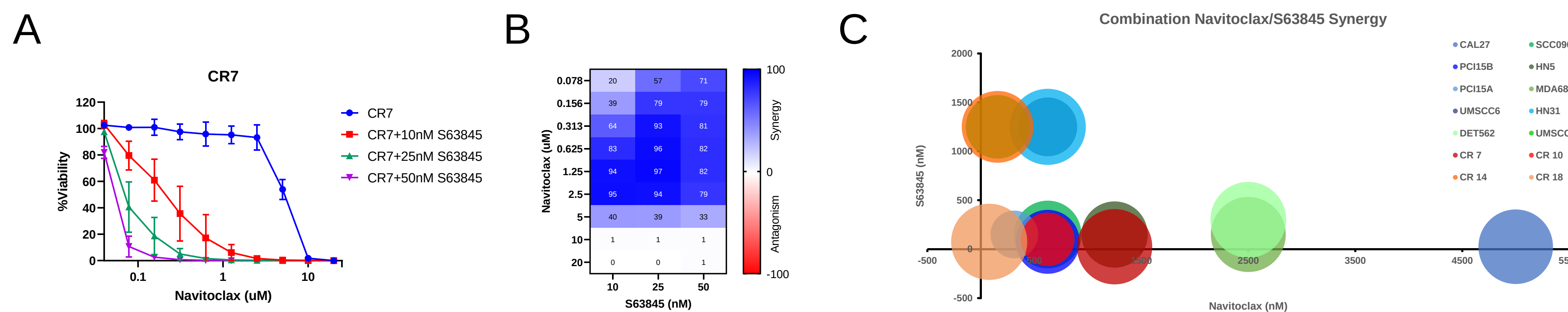


Figure 3: Immortalized and patient derived CR HNSCC lines (n=16) were treated in combination with a range of Navitoclax and S63845 doses for 72 hours. A representative figure is shown for A) cell viability measured using Cell Titer-Glo (Promega) and B) synergy was assessed using BLISS analysis. C) For each line, the highest synergy score (circle size) is plotted against Navitoclax and S63845 doses used to achieve corresponding score. For 9/16 lines (56%), highest synergy was achieved at <0.5 uM for S63845 and <1.5 uM for Navitoclax.

FIGURE 4. NAVITOCLAX AND S63845 SYNERGISTICALLY INHIBIT VIABILITY IN A CR ORGANOID MODEL

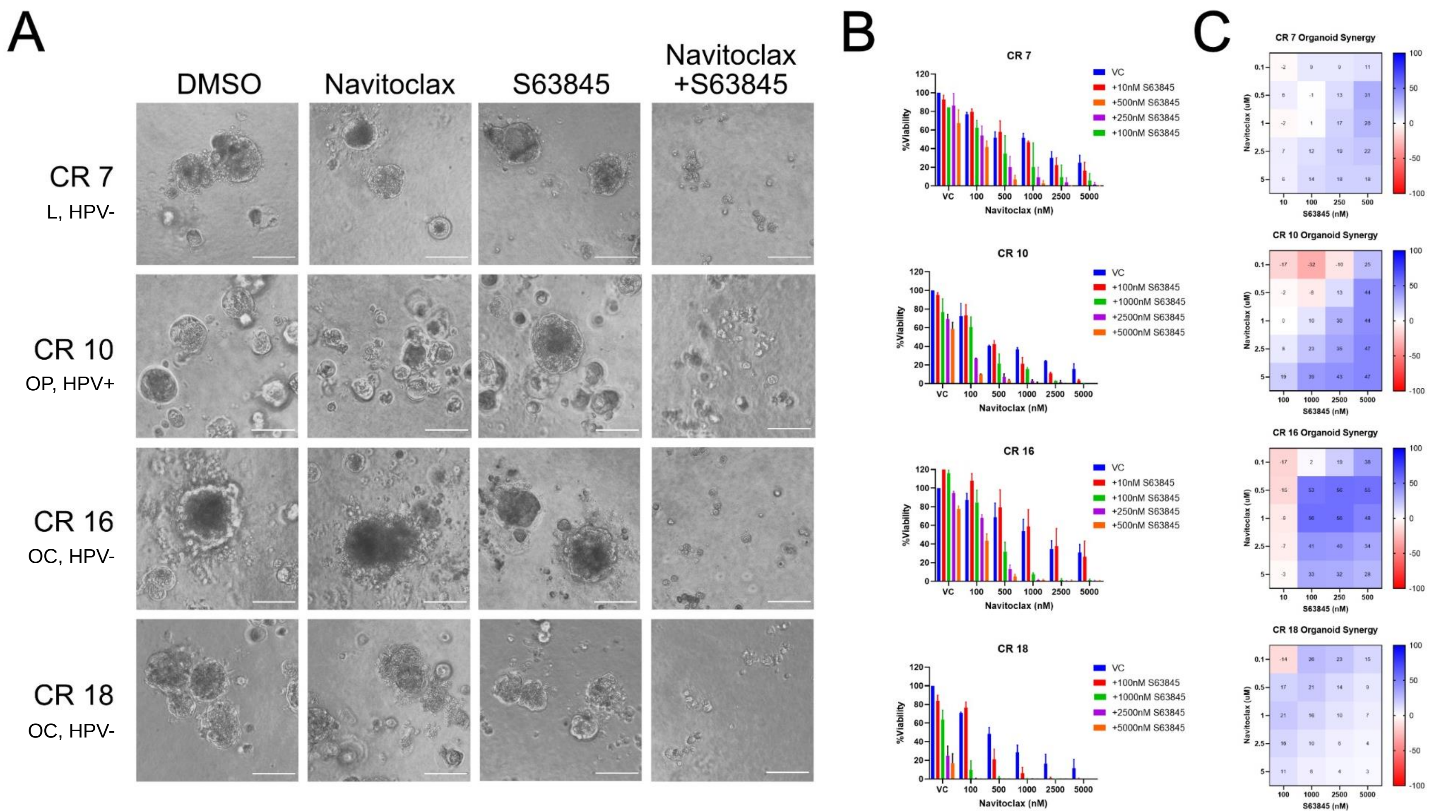


Figure 4: Cells from 2D CR culture (n=4) were seeded into 96-well low attachment plates in Clever's Media³ and 3% Matrigel (Corning) (n=3). A) A range of Navitoclax and S63845 doses were administered to assess organoid viability in response to combination treatment; organoids were treated for 12-14 days (scale bar = 100 um) (L: larynx, OP: oropharynx; OC: oral cavity). B) Viability was measured using 3D Cell Titer Glo (Promega) and C) synergy was assessed using BLISS analysis.

CONCLUSIONS

- BH3 profiling reveals intrinsic apoptosis signaling is generally preserved in HNSCC cell lines
- HNSCC cells are dependent on BCL-xL and MCL-1 for survival, and are resistant to Navitoclax as a single agent *in vitro*. Combination targeting of BCL-xL and MCL-1 synergistically reduce cell viability.
- Patient derived, conditionally reprogrammed HNSCC lines exhibit similar patterns of apoptotic priming and sensitivity to BH3 mimetic therapy in a 2D model, and BCL-xL and MCL-1 inhibition is effective in reducing viability in a 3D organoid model
- Co-inhibition of BCL-xL and MCL1 requires significantly lower dosing, which may reduce off-target toxicities; further in vivo studies will be required

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