

Investigation of TRAIL resistance in lung cancer cell lines

DOWLING, Joshua, FERRIERA DE MATOS, Cristiana, LEYLAND, Rebecca and CROSS, Neil <<http://orcid.org/0000-0003-2055-5815>>

Available from Sheffield Hallam University Research Archive (SHURA) at:

<https://shura.shu.ac.uk/23047/>

This document is the Accepted Version [AM]

Citation:

DOWLING, Joshua, FERRIERA DE MATOS, Cristiana, LEYLAND, Rebecca and CROSS, Neil (2018). Investigation of TRAIL resistance in lung cancer cell lines. In: British Society for Immunology: Yorkshire Immunology Group Symposium 2018, University of York, UK, 15th June 2018. (Unpublished) [Conference or Workshop Item]

Copyright and re-use policy

See <http://shura.shu.ac.uk/information.html>

Investigation of TRAIL Resistance in Lung Cancer Cell Lines

Joshua Dowling, Cristiana de Matos, Rebecca Leyland, Neil Cross.

Biomedical Research centre, City Campus, Sheffield Hallam University, Howard Street, Sheffield, S1 1WB

Introduction

TNF-related apoptosis-inducing ligand (TRAIL) is an important protein expressed by Natural Killer cells within the immune system.

It induces apoptosis preferentially in cancer cells and is a potential therapeutic target. Many tumours are TRAIL-resistant, and TRAIL-resistance emerges readily during therapy. TRAIL-sensitizers may overcome both existing, and emerging TRAIL insensitivity (see Fig 1). Non-proliferative quiescent cancer stem cell-like cells are TRAIL-resistant in some tumour models (Cross, NA. Unpublished observations). Translational reprogramming agents such as EIF2 inhibitors may overcome the quiescent phenotype and sensitize cells to (Schewe & Aguirre-Ghiso, 2009).

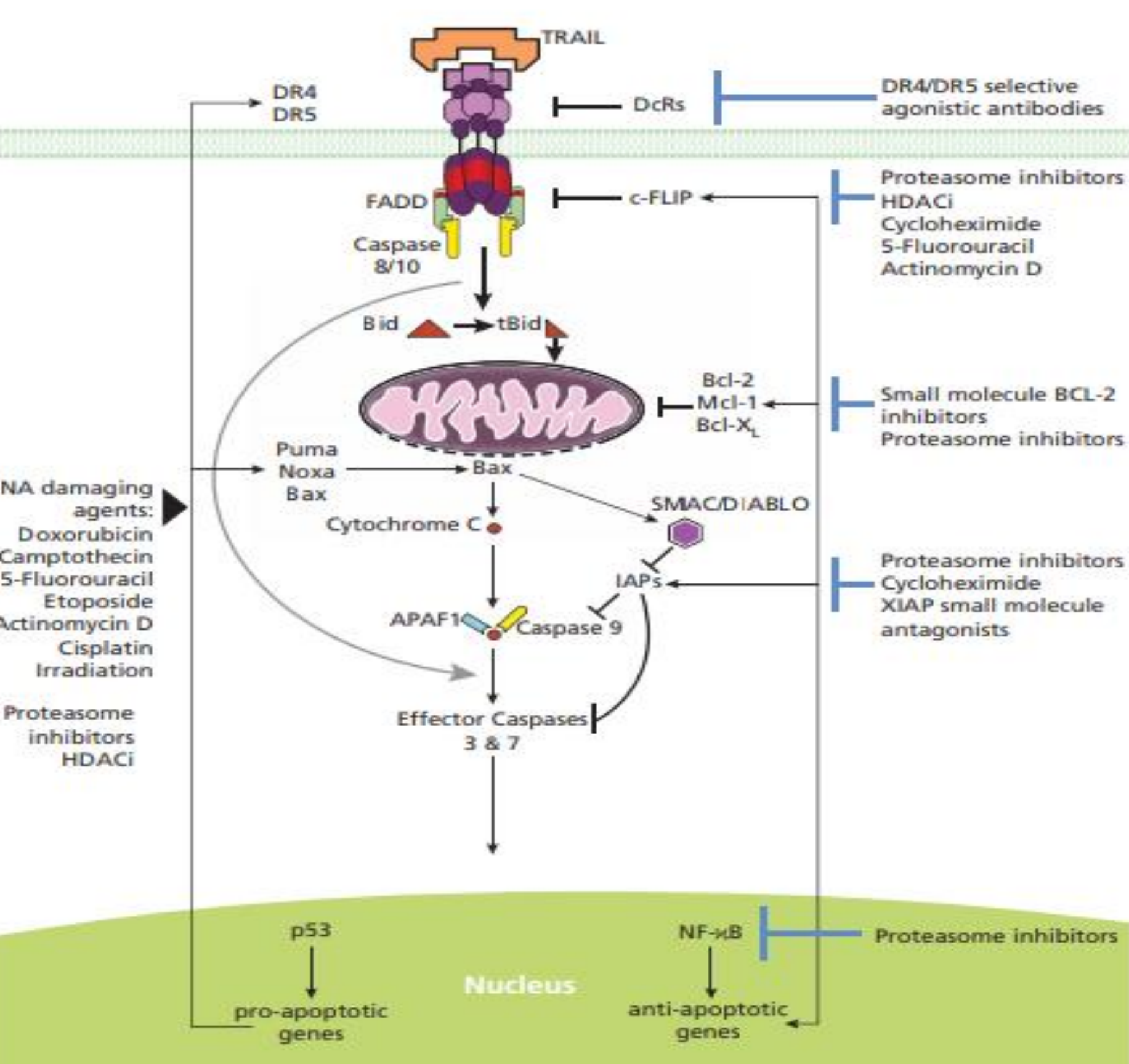


Figure 1. Known TRAIL-sensitizers and mechanisms of action (Cross and Sayers, 2014)

Aims and Hypothesis

The hypothesis of this study was that quiescent cells isolated from lung cancer could be translationally reprogrammed to bring them out of a resistant state.

Aims:

- 1) Isolate a quiescent phenotype within lung cancer cell lines.
- 2) Induce translational reprogramming using drug Salubrinal and re-sensitize the population.

Methods

Non-proliferating cells from lung cancer cells were distinguished using lipophilic membrane PKH67 dye which is lost on cell proliferation and flow cytometry to identify PKH67^{Hi} non-proliferating cells.

Apoptosis was assessed in response to TRAIL at 24 and 72 hours using Propidium Iodide and Hoechst 33342 staining.

Cells were re-challenged with to establish a TRAIL resistant (TRAIL^R) population vs. the parent line

•TRAIL^R cells were treated with Salubrinal (10/25µM) in combination with TRAIL cells to assess the effects of translational reprogramming on acquired TRAIL^R

References Schewe, D & Aguirre-Ghiso (2009). Inhibition of eIF2 Dephosphorylation Maximizes Bortezomib Efficiency and Eliminates Quiescent Multiple Myeloma Cells Surviving Proteasome Inhibitor Therapy. *Cancer Research*, 69(4), 1545-1552. -

Results

A549 does not contain a quiescent population, and all PKH67^{Hi} cells were associated with spontaneous cell death (Fig 2)

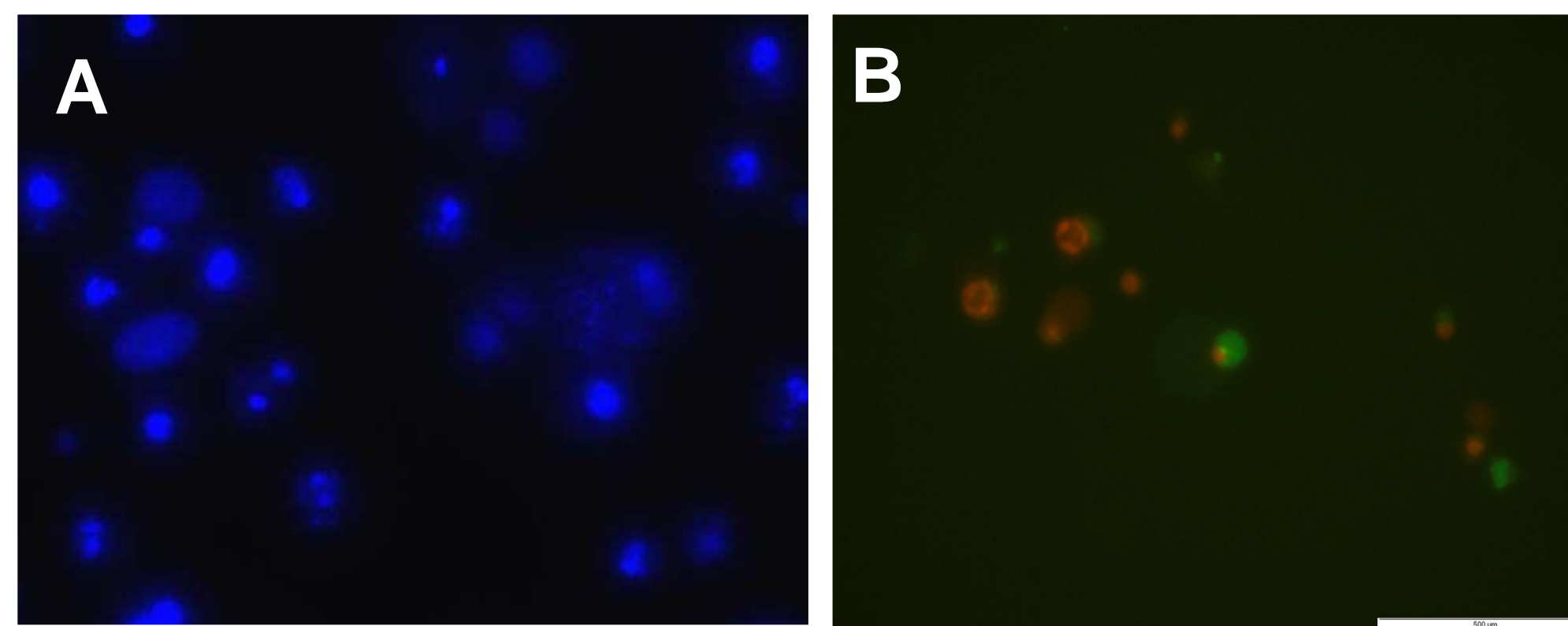


Figure 2. (A) A549 cells stained with Hoechst 33342 and stimulated with 25ng/mL of TRAIL at 24 hours imaged using fluorescent microscopy to show apoptosis. (B) Images show PKH67^{Hi} cells undergoing apoptosis when exposed to 25ng/mL of TRAIL at 72 hours in cell line A549 at 20x magnification.

Identification of quiescent cells

Flow cytometry was used to identify a population of cells which retained PKH67 over a 12 day period. In the A549 cell lines, there was no high presence of PKH67 high cells.

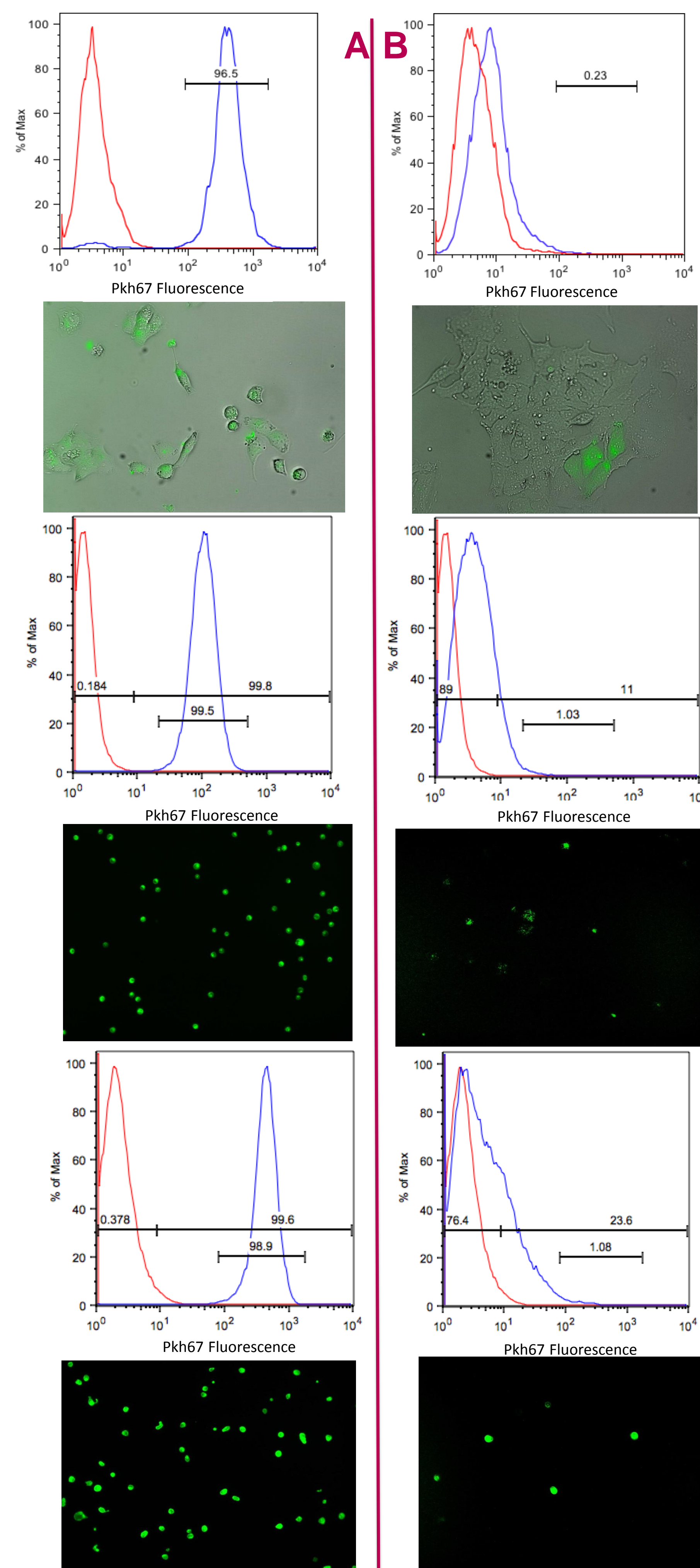


Figure 3. Flow cytometry and fluorescent microscopy data of NCI-H2170 (TOP), NCI-H838 (MIDDLE), and TWIT-Q (BOTTOM) cells. Column (A) represents PKH67 stain populations compared to the parent strain taken at day 1 and column (B) represents the same cells taken at day 12. Microscopy images taken using the FITC camera of the cells at the same time as flow cytometry are displayed underneath the corresponding flow cytometry data.

Investigation of Translational reprogramming agent Salubrinal on TRAIL^R Populations

The TRAIL sensitivity of 4 cell lines was determined (fig 4) and subsequently, TRAIL^R populations isolated after persistent TRAIL-treatment by culture of surviving cells after TRAIL treatment. TRAIL^R populations were confirmed to be less sensitive to TRAIL than parental populations (fig 5). TRAIL did not synergistically enhance TRAIL responses in TRAIL^R cells (Fig 6).

Effects of TRAIL on Lung Cancer Cell lines.

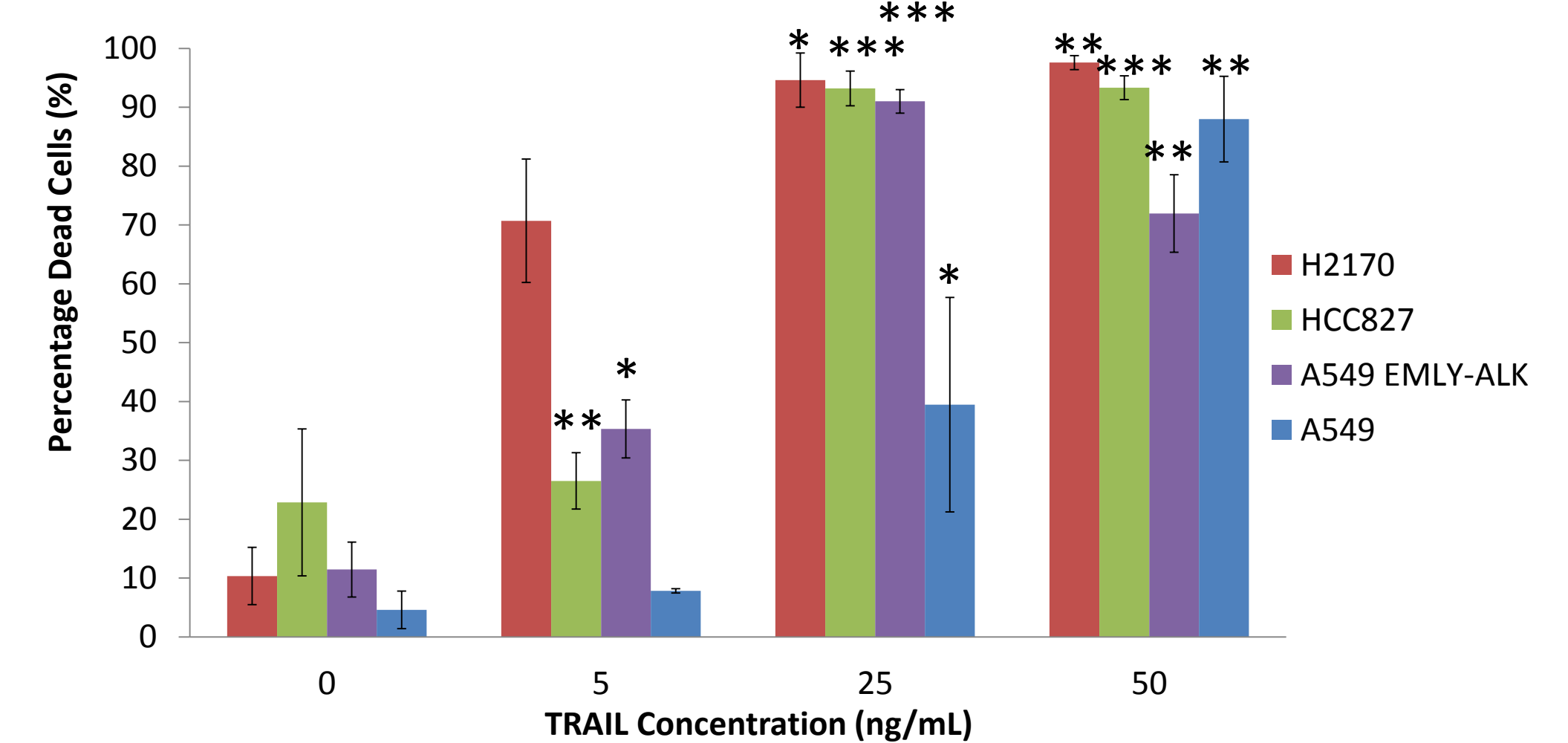


Figure 4. The percent apoptosis in lung cancer cell lines when exposed to TRAIL at 0/150ng/ml for 24hours. Comparison of treated vs. untreated for each cell line is shown and assessed by Kruskal-Wallis multiple comparisons test (p<0.05=* P<0.01=** P<0.001).

Effect of TRAIL on surviving fraction of TRAIL treated cells

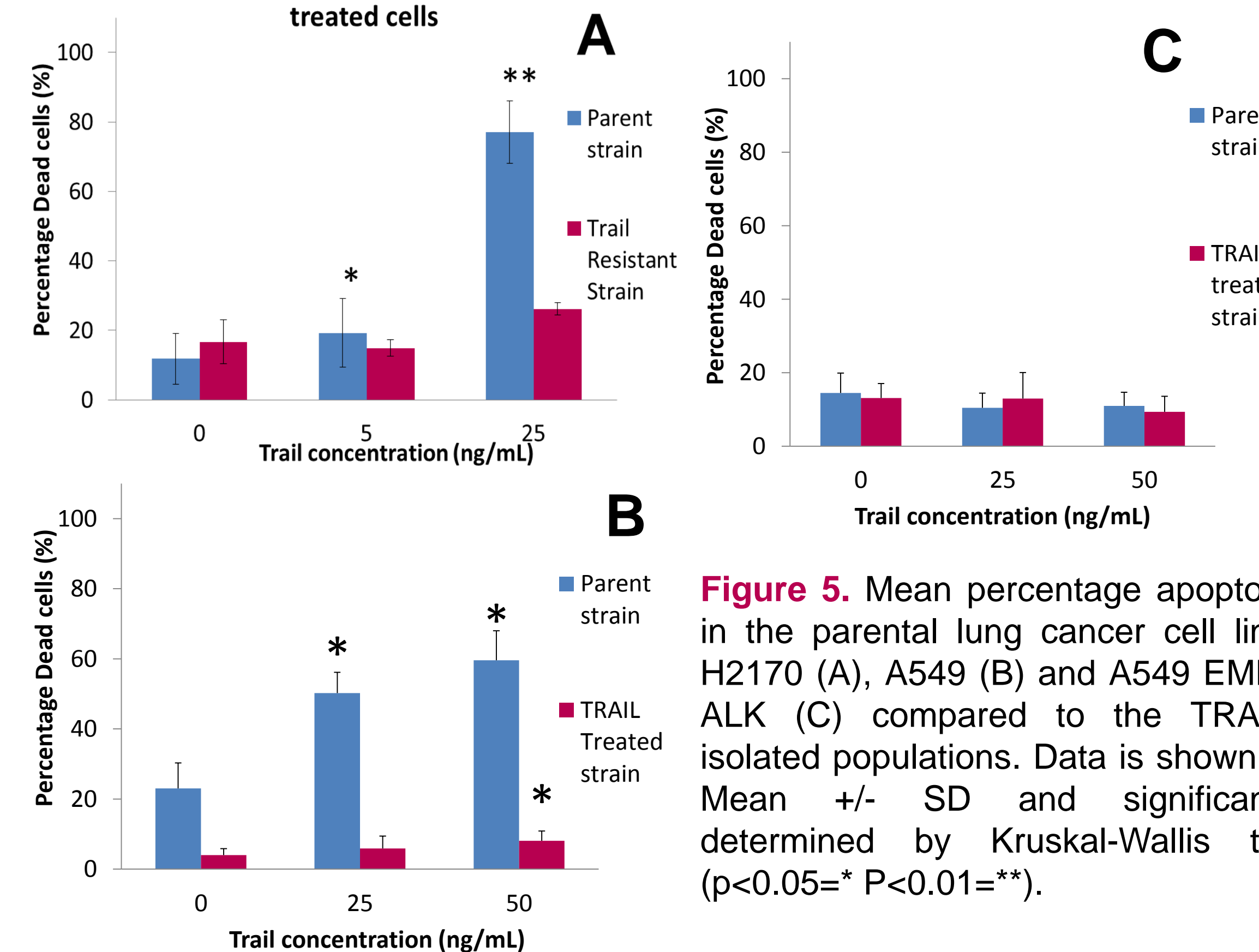


Figure 5. Mean percentage apoptosis in the parental lung cancer cell lines H2170 (A), A549 (B) and A549 EML4-ALK (C) compared to the TRAIL^R isolated populations. Data is shown as Mean +/- SD and significance determined by Kruskal-Wallis test (p<0.05=* P<0.01=**).

Challenge of TRAIL^R cells with the translational reprogramming agent Salubrinal

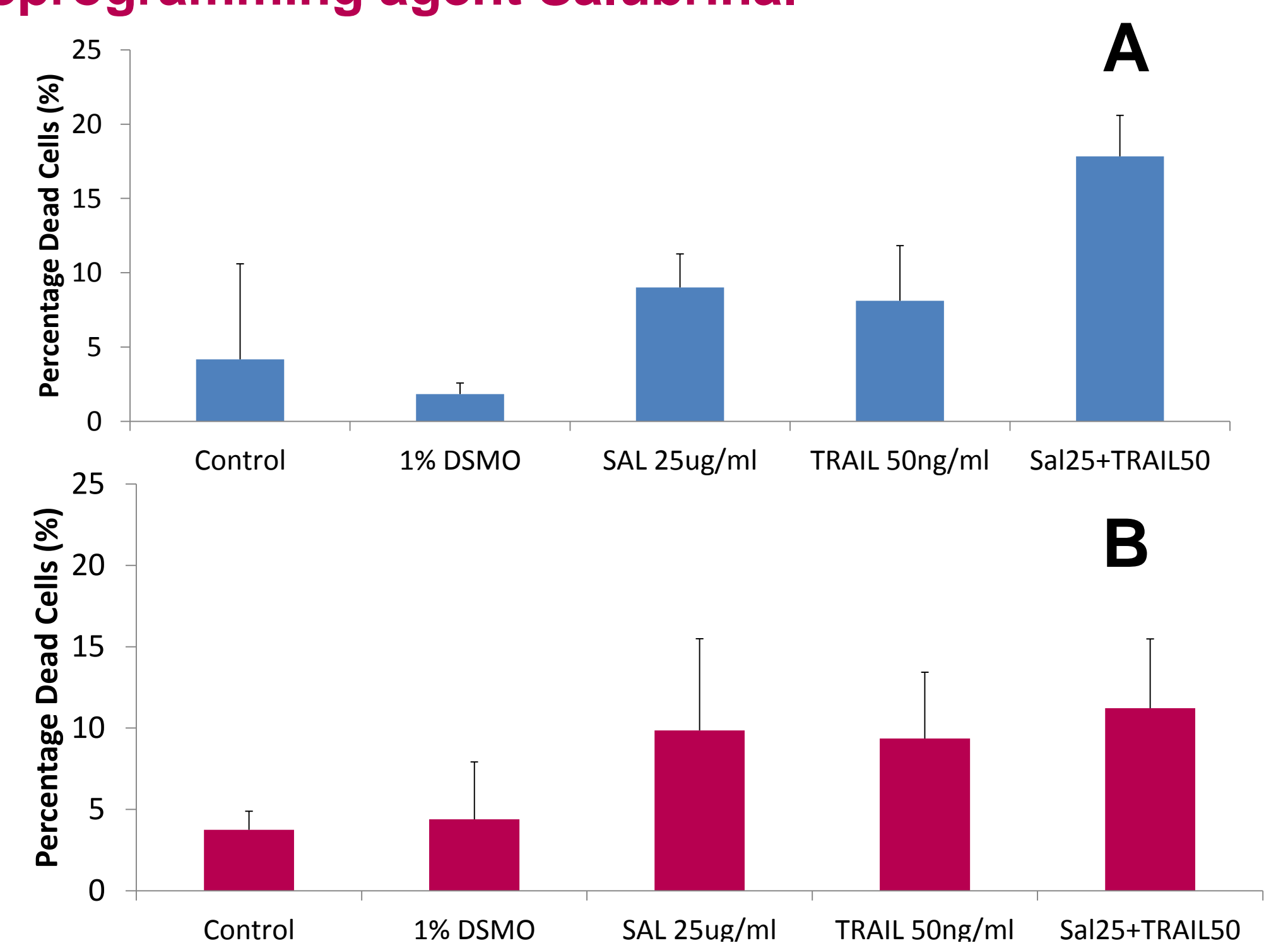


Fig 6 Mean percent apoptosis in cell lines A549 (A) and A549 EML4-ALK (B) (ALK translocation) with combination treatments of Salubrinal (25µg/ml) and TRAIL (50ng/ml).

Conclusions and future work.

The cell lines A549, and NCI-H2170 do not contain a quiescent population. However more recent work has successfully isolated quiescent cells from other lung cancer cell lines (Fig 3). TRAIL-sensitive cell lines can readily be made TRAIL^R by persistent treatment with sub-toxic doses of TRAIL, mirroring clinical findings. TRAIL^R cells could not be sensitized to TRAIL by Salubrinal. Ongoing work is aimed at assessing gene expression changes in TRAIL^R cells and assessing TRAIL resistance in quiescent cells in the new TWIT-Q cell line.