Characterising the function and interaction of our novel radiosensitiser with the tumour microenvironment in Oesophageal Adenocarcinoma

Amy Buckley [1] , Niamh Lynam-Lennon [1] , Susan Kennedy [1], Aoife Cannon [1] , Róisín Byrne [1] , Alison Reynolds [2] , Stephen Maher [1],David Gomez-Matallanas [3], Narayanasamy Ravi [1], Dermot O'Toole [1], John V Reynolds [1], Breandán Kennedy [2] , Jacintha O'Sullivan [1]

[1]Trinity Translational Medicine Institute, Department Of Surgery, St. James's Hospital, Trinity College Dublin
[2]UCD Conway Institute & UCD School of Biomolecular and Biomedical Science, University College Dublin
[3] Systems Biology Ireland

Introduction

Oesophageal Cancer (OAC) is an aggressive disease with a dismal cure rate of approximately 15-20%. Current therapeutic regimes focus on neo-adjuvant treatment with chemo-radiation therapy prior to surgery. Unfortunately, only 20-30% of patients show a beneficial response, with 70-80% of patients receiving a toxic treatment with no benefit and a delay to surgery. An upregulation of angiogenesis, metabolism and DNA repair has been correlated with treatment resistance to radiation therapy in OAC. This major clinical challenge of treatment resistance reinforces the need for the discovery and validation of novel targeted therapies that can act as neo-adjuvant radio-sensitisers.

Methods

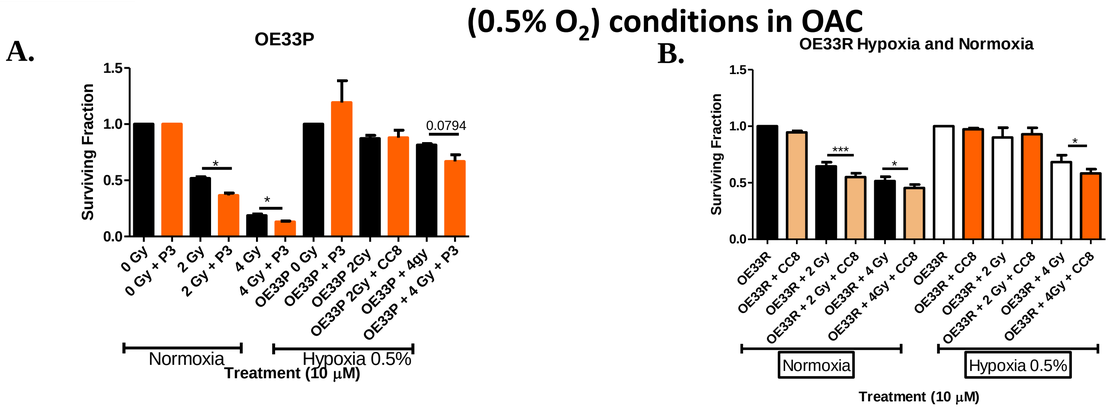
We have previously identified a novel anti-angiogenic and anti-metabolic compound *in-vivo* in zebrafish and *in-vitro* in OAC cells. The ability of our lead compound to act as an anti-metabolic agent under hypoxia was evaluated using the XFe24 Seahorse analyser and the whitley i2 workstation. In addition the ability of 11B_CC8 to radiosensitise our isogenic OAC cells under hypoxic conditions was evaluated by clonogenic assay using the Whitley H35 Hypoxystation. The effect of 11B_CC8 on inflammatory, metabolic and angiogenic protein secretions from OAC treatment naïve tumour conditioned media (TCM) was evaluated by multiplex ELISA. Fresh treatment naïve patient biopsies were screened for their metabolic activity in the XFe24 seahorse analyser at baseline and following treatment with our novel radiosensitiser 11B_CC8. The elucidation of the possible mechanism of action of our novel radiosensitiser was evaluated by Mass Spectrometry.

Results

Figure 1.

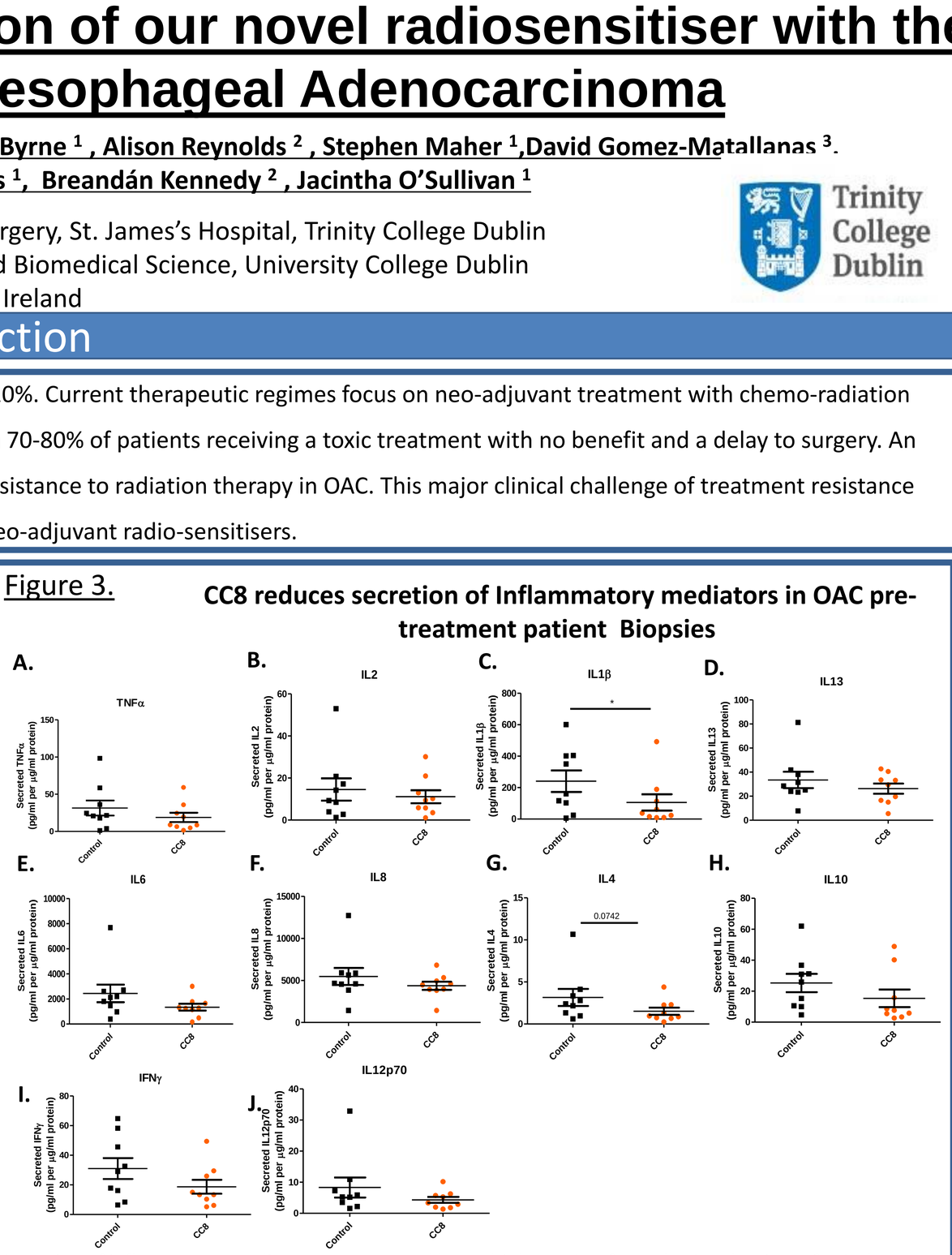
The metabolic rate of OE33P and OE33R cells under normoxic and hypoic conditions was evaluated using the XFe24 seahorse analyser within the i2Whitley chamber and using the H35 hypoxic workstation. (A) Oxygen consumption rate (OCR) in OE33P and OE33R cells cultured under nomixic and hypoxic conditions(0.5%) usi (n=5) (B) CC8 significantly inhibits OCR in OAC biopsies, (n=10), Mann Whitney t-test, **p<0.01 (C) Percentage change ECAR following treatment with CC8 and Oligomycin (n=10)

Figure 2. **CC8 can enhances radiosensitivity under both normoxic and hypoxic (0.5% O_2) conditions in OAC**



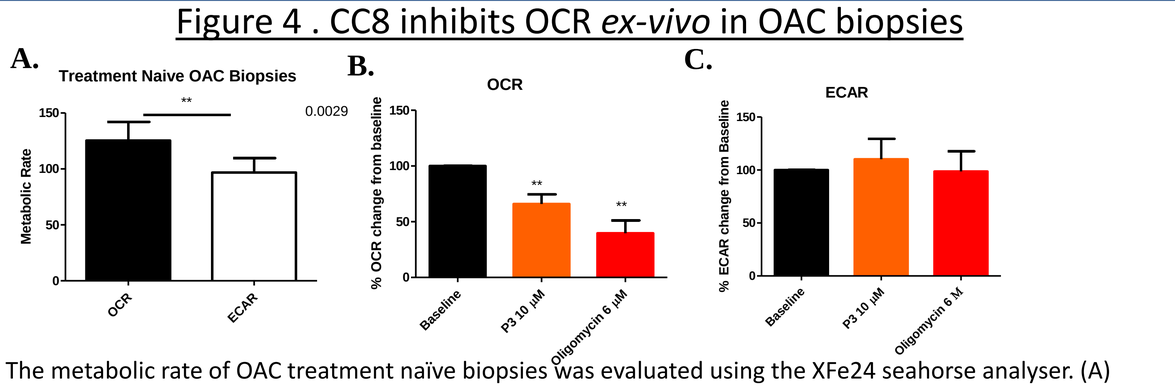
.The ability of CC8 to enhance radiosensitivity was evaluated by clonogenic assay under normoxia and hypoxia using the Whitley h35 hypoxia chamber **(A)** Surviving Fraction of OE33P cells following treatment with 11B_CC8 + 0,2 and 4 Gy irradiation. paired t-test, *p<0.05 (n=4). **(B)** Surviving Fraction of OE33R cells following treatment with 11B_CC8 and 0, 2, and 4 Gy irradiation. Paired t-test, *p<0.05, **p<0.01. (n=4).

Figure 3. **CC8 reduces secretion of Inflammatory mediators in OAC pre-treatment patient Biopsies**



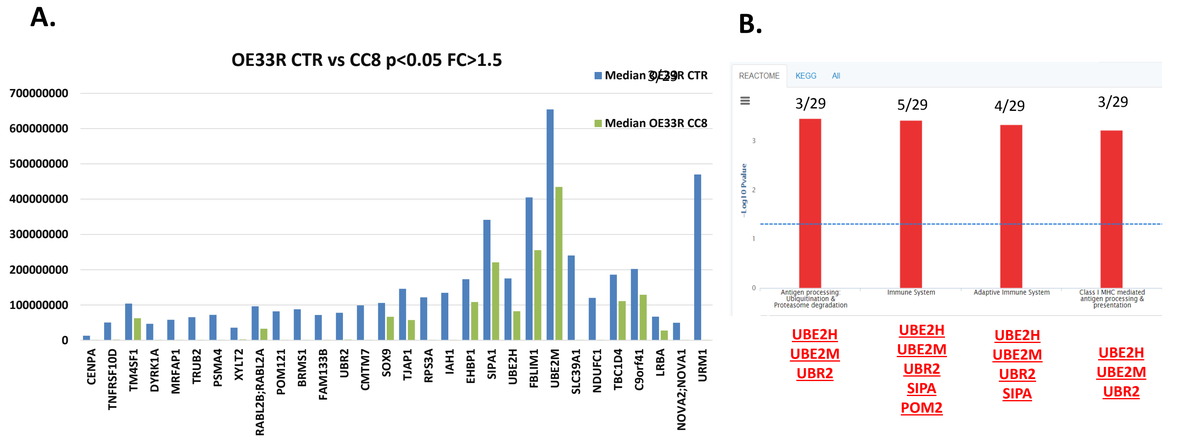
Secretion of inflammatory mediators was assessed by multiplex ELISA after culturing the tissue for 24 hours with DMSO control or 10 μM CC8. **(A)** TNFα **(B)** IL-2 **(C)** IL-1β **(D)** IL-13 **(E)** IL-6 **(F)** IL-8 **(G)** IL-4 **(H)** IL-10 **(I)** IFNγ and **(J)** IL12p70 secretions from OAC treatment naïve biopsies; Wilcoxon paired t-tests. *p<0.05. Secretions were normalised to protein content.

Figure 4 . CC8 inhibits OCR *ex-vivo* in OAC biopsies



The metabolic rate of OAC treatment naïve biopsies was evaluated using the XFe24 seahorse analyser. (A) Oxygen consumption rate (OCR), is significantly higher than extracellular acidification rate (ECAR) in OAC treatment naïve patient biopsies, (n=11), Wilcoxon paired t-test. **p<0.01 (B) CC8 significantly inhibits OCR in OAC biopsies, (n=10), Mann Whitney t-test, **p<0.01 (C) Percentage change ECAR following treatment with CC8 and Oligomycin (n=10)

Figure 5. **Identification of potential targets of CC8 by Mass Spectrometry**



(A) LFQ intensity levels of genes whose expression level was significantly reduced (p<0.05, FC>1.5) following 11B_CC8 10 μ,M treatment compared to control in the OE33R cell line. (B) Pathway analysis using InnateDB of the significant genes identified in (A) revealed 11B_CC8 is acting on genes involved in the immune system.

Conclusions

Our novel anti-angiogenic and anti-metabolic agent can enhance radiosensitivity *in-vitro* under both normoxic and hypoxic conditions. In treatment naïve OAC patient samples, 11B_CC8 can significantly reduce the secretion of IL1β in TCM and reduce the rate of oxidative phosphorylation.

References

(1) Alison L. Reynolds, Yolanda Alvarez, Temitope Sasore, Nora Waghorne, Clare T. Butler, Claire Kilty, Andrew J. Smith, Carmel McVicar, Vickie H. Y. Wong, Orla Galvin, Stephanie Merrigan, Janina Osman, Gleb Grebnev, Anita Sjölander, Alan W. Stitt and Breandán N. Kennedy. Phenotype-Based Discovery of 2-[(E)-2-(Quinolin-2-yl)vinyl]phenol as a Novel Regulator of Ocular Angiogenesis. Journal of biological chemistry. Vol. 291, 7242-7255. 2016 **(2)** Orla Galvin, Akshay Srivastava, Oliver Carroll, Rajiv Kulkarni, Steve Dykes, Steven Vickers, Keith Dickinson, Alison L. Reynolds, Claire Kilty, Gareth Redmond, Rob Jones, Sharon Cheetham, Abhay Pandit and Breadán N Kennedy. A sustained release formulation of novel quininib-hyaluronan microneedles inhibits angiogenesis and retinal vascular permeability in vivo. Journal of Controlled Release, Vol. *233*, 198-207. 2016 **(3)** Murphy, Adrian G., et al. "Preclinical validation of the small molecule drug quininib as a novel therapeutic for colorectal cancer." *Scientific Reports* 6 (2016). (4) Butler, Clare T., et al. "Phenotype-based discovery and development of novel therapies for the treatment of colorectal cancer." (2016): 3058-3058.

Irish Cancer Society Research