



Targeting hypoxic prostate tumours using the novel hypoxia-activated prodrug OCT1002 inhibits expression of genes associated with malignant progression

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intervention arm and three from the placebo arm of the trial. We performed MeDIP (Methylated DNA Immunoprecipitation) on all 12 samples and hybridized to a Roche Nimblegen Delux 2.1M promoter array. While we observed DNA methylation changes pre- and post- folic acid intervention in each individual, *the actual DNA methylation sites were not consistent across all three individuals*. Of course, it is possible that a more in-depth Next Generation Sequencing approach might yield our elusive FS-DMRs. However, the published literature to date does not appear to support such a promise.

P23. A Novel Approach to Promoter Identification – Development of a Ganglion Cell-specific Promoter for AAV-mediated Gene Therapy

KS Hanlon¹, S Millington-Ward¹, N Chadderton¹, A Palfi¹, P Humphries¹, PF Kenna^{1,2}, GJ Farrar¹

¹Ocular Genetics Unit, Smurfit Institute of Genetics, Trinity College Dublin, Dublin 2, Ireland, ²Royal Victoria Eye and Ear Hospital, Adelaide Road, Dublin 2, Ireland

The loss of retinal ganglion cells (RGCs) is a hallmark of a number of retinopathies. There are a number of gene therapies being developed that have shown efficacy in preserving RGCs when administered using an AAV vector. Localising expression of any therapeutic to the target cell type (ganglion cell layer, GCL) would represent a significant optimisation of the approach. The packaging capacity of AAV (4.7kb) imposes a limit on the size of promoters and genes relevant for AAV-mediated gene delivery. Few GCL-specific promoter sequences have been defined of a size suitable for use in AAV-guided gene expression.

Exploring this, a panel of genes was chosen with GCL-limited expression profiles. A pipeline program was developed that analysed regions upstream of these genes for sequence conservation across placental mammals (as a proxy for putative promoter function), weighted by enriched GCL expression levels. Adopting this strategy, ganglion cell promoter 1 (GCP1), demonstrating the key features outlined above, was identified. To test its function, GCP1 (2.2kb in size) was engineered into an AAV2 virus expressing EGFP.

Here we demonstrate the effectiveness of GCP1 in localising EGFP expression to the GCL when administered via intravitreal injection. Furthermore, absence of EGFP expression was demonstrated when targeted towards photoreceptors via subretinal injection, verifying GCP1 tissue-specificity. Expression of AAV2.GCP1-EGFP was compared to expression from a non-specific promoter construct, AAV2.CMV-EGFP. GCP1-EGFP was shown to provide equivalent expression to CMV-EGFP in the GCL. GCP1 thus offers a tissue-specific promoter option, suitable for deployment within AAV vectors without compromising functionality.

P24. Targeting hypoxic prostate tumours using the novel hypoxia-activated prodrug OCT1002 inhibits expression of genes associated with malignant progression.

H Nesbitt¹, NM Byrne^{1,5}, L Ming¹, J Worthington^{1,2}, RJ Errington³, LH Patterson⁴, PJ Smith⁴, SR McKeown^{1,4}, DJ McKenna¹

¹Biomedical Sciences Research Institute, University of Ulster, Cromore Road, Coleraine, Northern Ireland, BT52 1SA, UK, ²Axis Bioservices Ltd, Coleraine, Northern Ireland, BT51 3RP, UK, ³BioStatus Ltd, Shepshed, Leicestershire LE12 9NP, UK, ⁴OncoTherics Ltd, Shepshed, Leicestershire LE12 9NP, UK, ⁵Garvan Institute of Medical Research, 384 Victoria Street, Sydney, New South Wales 2010, Australia.

Purpose: Hypoxia is a common hallmark of the tumour microenvironment. Recently we have shown the anti-androgen bicalutamide induces profound hypoxia in prostate tumours *in vivo*. This resulted in the promotion of epithelial to mesenchymal transition. Here we target tumour hypoxia using a novel unidirectional hypoxia-activated prodrug OCT1002 to enhance the anti-tumour effect of bicalutamide.

Experimental Design: The effect of OCT1002 treatment on LNCaP-luc cells was measured in normoxia and hypoxia *in vitro*. *In vivo*, tumour growth and lung metastases were measured in mice treated with bicalutamide, OCT1002 or a combination. Dorsal skin fold chambers were used to image tumour vasculature *in vivo*. Longitudinal genetic changes in tumours were analysed using PCR.

Results: Reduction of OCT1002 to its active form (OCT1001) decreased LNCaP-luc cell viability. In LNCaP-luc spheroids, OCT1002 caused increased apoptosis and decreased clonogenicity. *In vivo*, treatment with OCT1002 alone or with bicalutamide, showed significantly greater tumour growth control and reduced lung metastases compared to controls. Re-establishment of the tumour vasculature following bicalutamide-induced vascular collapse is inhibited by OCT1002. Significantly, the up-regulation of *RUNX2* and its targets caused by bicalutamide alone were also blocked by OCT1002.

Conclusions: OCT1002 selectively targets hypoxic tumour cells and enhances the anti-tumour efficacy of bicalutamide. Furthermore, bicalutamide causes changing genetic profiles during treatment, with development of a more malignant genotype; OCT1002 can block this effect. This study indicates that more attention should be attached to understanding genetic changes that may occur during treatment. Early targeting of hypoxic cells with OCT1002 can provide a means of inhibiting prostate tumour growth and malignant progression.

P25. Regulation of miR-200c and miR-141 by methylation in prostate cancer

SM Lynch¹, KM O'Neill^{1,2}, MM McKenna³, CP Walsh¹, DJ McKenna¹

¹Biomedical Sciences Research Institute, University of Ulster, Cromore Road, Coleraine, BT52 1SA, UK, ²School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, UK, ³Department of Cellular Pathology, Western Health & Social Care Trust, Altnagelvin Area Hospital, Glenshane Road, Co. Derry, BT47 6SB, UK.

Background: In prostate cancer (PCa), abnormal expression of several microRNAs (miRNAs) has been previously reported. Increasing evidence shows that aberrant epigenetic regulation is a contributing factor to their altered expression in cancer. In this study we investigate whether expression of miR-200c and miR-141 in PCa is related to the DNA methylation status of their promoter.

Methods: PCR analysis of miR-200c and miR-141, and CpG methylation analysis of their common promoter, was performed in PCa cell-lines and in FFPE prostate biopsy specimens. The functionality of miR-200c and miR-141 expression in prostate cancer cells was assessed by a series of *in vitro* bioassays.

Results: miR-200c and miR-141 expression correlates inversely with the methylation status of the miR-200c/miR-141 promoter in PCa cells. In PC3 cells, miR-200c and miR-141 expression is elevated by treatment with the demethylating agents suggesting their expression is linked to methylation. Expression of miR-200c and miR-141 in prostate biopsy tissue was inversely correlated

