TITLE: **Neoadjuvant MRx0518 treatment is associated with significant gene and metagene signature changes in solid tumours**

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**Background**

MRx0518 is an oral live biotherapeutic with potent immunostimulatory activity and anti-tumorigenic efficacy in murine models of lung (LLC1), kidney (Renca) and breast (EMT6) cancer. Previous reports have demonstrated a favourable safety profile in neoadjuvant and metastatic clinical settings, with emerging evidence of immune modulation. We performed a comprehensive analysis of the gene and metagene signature in cancer patients treated with MRx0518 monotherapy.

**Methods**

Treatment-naïve patients with a histologically confirmed diagnosis of cancer scheduled for surgical resection were recruited from April 2019 to February 2020. Patients received 1 capsule of MRx0518 (1x1010 to 1x1011 CFU) twice daily from inclusion until the day preceding surgery. Safety and tolerability (CTCAE v4.03) were the primary endpoints of this study. Comprehensive biomarker analysis was also performed in paired pre-treatment (diagnostic biopsy) and post-treatment (surgical specimen) samples using the NanoString IO 360 panel to explore gene and metagene signatures.

**Results**

31 samples were collected across tumour groups including breast (n=13) prostate (n=8), uterine (n=6), melanoma (n=2) and bladder (n=2). Differential expression analysis showed significant (p<0.05) increases in genes and metagenes associated with anti-tumour activity, including antigen presentation (AXL & CXCL12), innate immune processes (CHUK, RELA, PPARG & HRAS), interferon response (IFNGR1 & IFNGR2), Th1 cells and CD8+ cells following MRx0518 therapy, echoing preclinical findings. Novel changes, not previously detected in murine models, involving endothelial, mast cells, inflammatory myeloid and inflammatory chemokines were also observed, suggesting MRx0518 may have additional *in vivo* anti-tumorigenic effects. These changes were more pronounced in the breast cancer cohort.

**Conclusions**

This analysis, mirrors previous immunostimulatory activity and anti-tumorigenic efficacy observations seen in pre-clinical models following MRx0518 therapy. Furthermore, potentially beneficial novel anti-tumour effects were observed, not previously seen. These results will be further validated in 100 treatment naïve patients, informing future studies.