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Digital image based quantification of signalling responses of the IGF pathway in Ewing's Sarcoma of Bone.

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Despite advances in surgical techniques and dose-intense chemotherapy, overall survival from Ewing's sarcoma remains unchanged. Ewing's sarcoma is characterised by the chromosomal translocation t(11,22) resulting in the generation of chimeric forms of ETS family of transcription factors, such as EWS-FLI1. The IGF pathway is activated as a consequence, as EWS-FLI1 reduces IGFBP3 expression, a negative regulator of ligand activation of the IGF1 receptor (IGF1R). Phase I/II trials have demonstrated clinical responses to IGF1R antibody monotherapy, indicating that targeting the IGF pathway may be a new therapeutic modality which requires further testing in randomised trials. In order to assess the extent of activation of the IGF pathway, we developed an unbiased quantitative multi-fluorescence based assay following multi-spectral and confocal imaging of Ewing's sarcoma tissue biopsy cores, sections, and cell lines.

We first analysed signalling reponses based on marker localisation in cell lines, and developed an image segmentation method to quantify signalling at the single cell level. We treated sections with DAPI and phalloidin to identify the nuclear and cytoplasmic boundaries before applying fluorescent signals using an image segmentation method based on level sets. We then localised and assessed a set of antibodies in cells with differing dose and time-dependent exposure to IGF2, the IGF1R ligand. Combined markers which were the most reliable readouts of pathway activation were applied to a Ewing's sarcoma tissue microarray. This enabled us to take measurements of pathway activation in formalin fixed tissue prior to and subsequent to chemotherapy.

Our method of unbiased quantification of signalling pathway activation in Ewing's sarcoma provides an approach for patient stratification and selection, as well as a pharmacodynamic assessment of pathway inhibition.