

Quantitative analysis of IGF-1R expression in FFPE human rhabdomyosarcoma tumor tissue by mass spectrometry

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Introduction

Measuring protein biomarker levels in patient tissue has promising application to the field of personalized medicine. We have developed a mass spectrometry based tissue proteomics platform capable of measuring protein expression in standard formalin-fixed paraffin-embedded (FFPE) tissue using Selected Reaction Monitoring (SRM) methodology.

The IGF-1R Signaling Pathway Potential Biomarkers of IGF-1R Inhibitor Activity

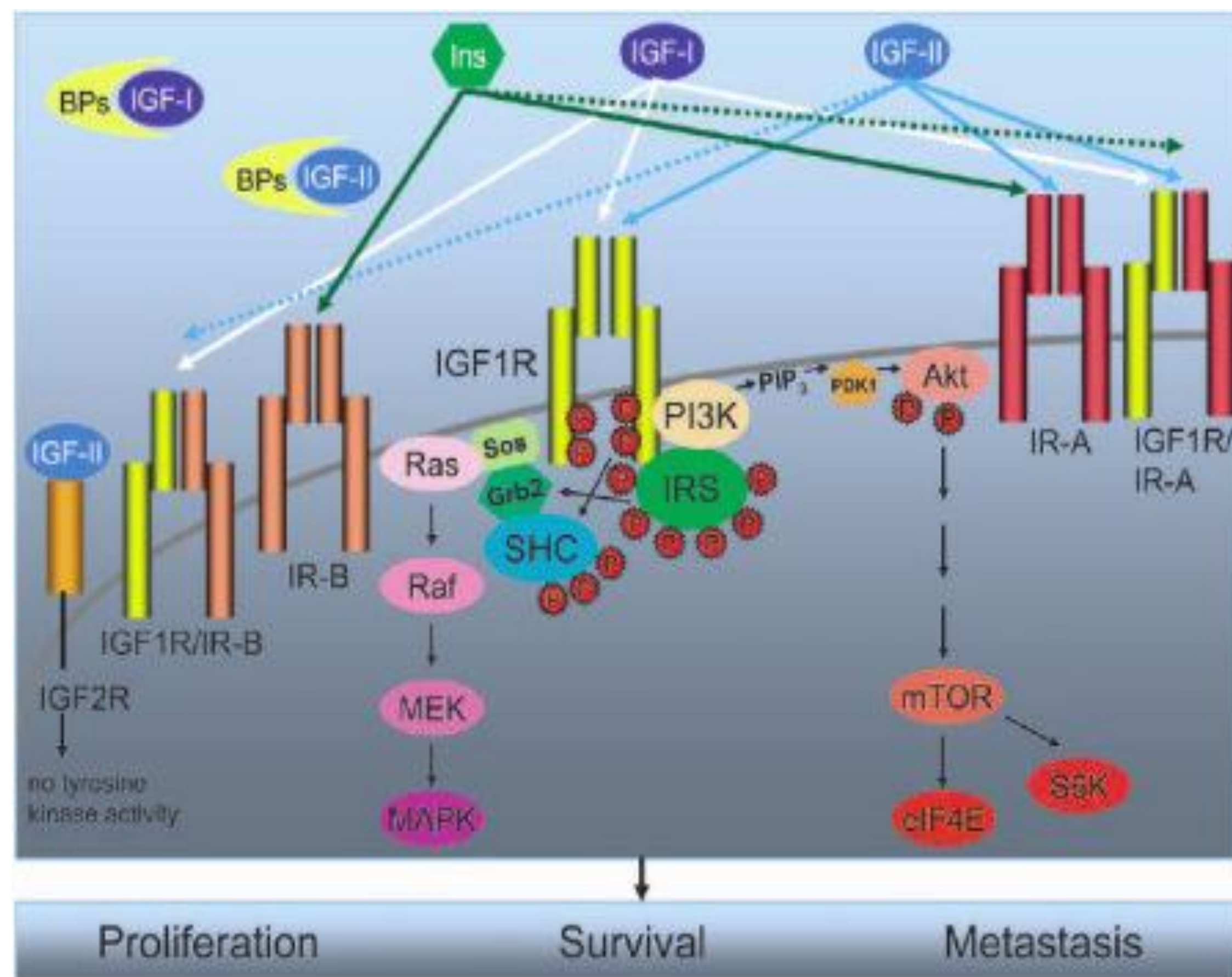


Figure 1. The IGF-1R Signaling Pathway. Illustration from Sachdev and Yee (2007) in *Molecular Cancer Therapeutics* 6(1): 1-12.

Liquid Tissue Mass Spectrometry Method

Formalin fixed paraffin embedded tissue mounted on DIRECTOR slides is laser microdissected and then processed using Expression Pathology's Liquid Tissue protocol. The resulting soluble tryptic digest is analyzed by SRM on a Thermo Vantage triple quadrupole mass spectrometer. Quantitative analysis is performed by spiking a heavy isotope labeled peptide into samples and comparing the ratio of AUC between endogenous analyte and control analyte.

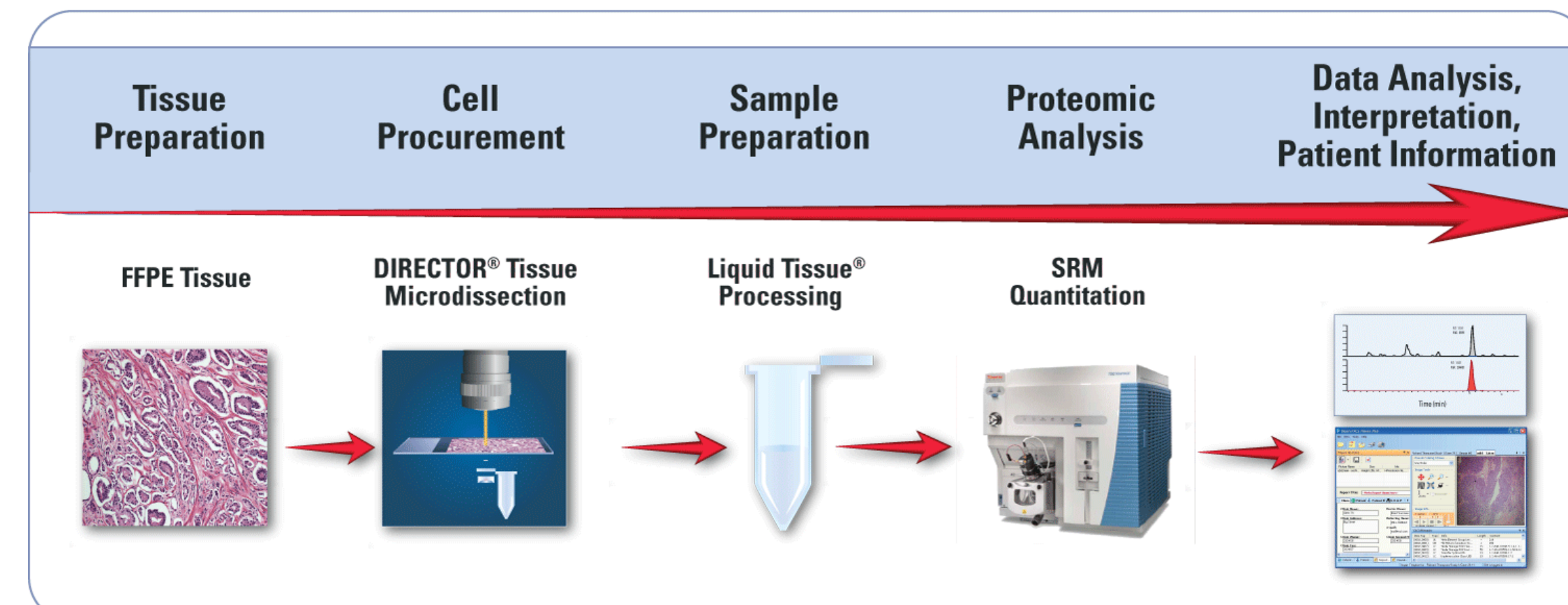


Figure 2. Experimental method for absolute SRM quantitation of Liquid Tissue processed FFPE human RMS tumor samples.

Detection of IGF-1R in Rhabdomyosarcoma Cell Lines by SRM Mass Spectrometry

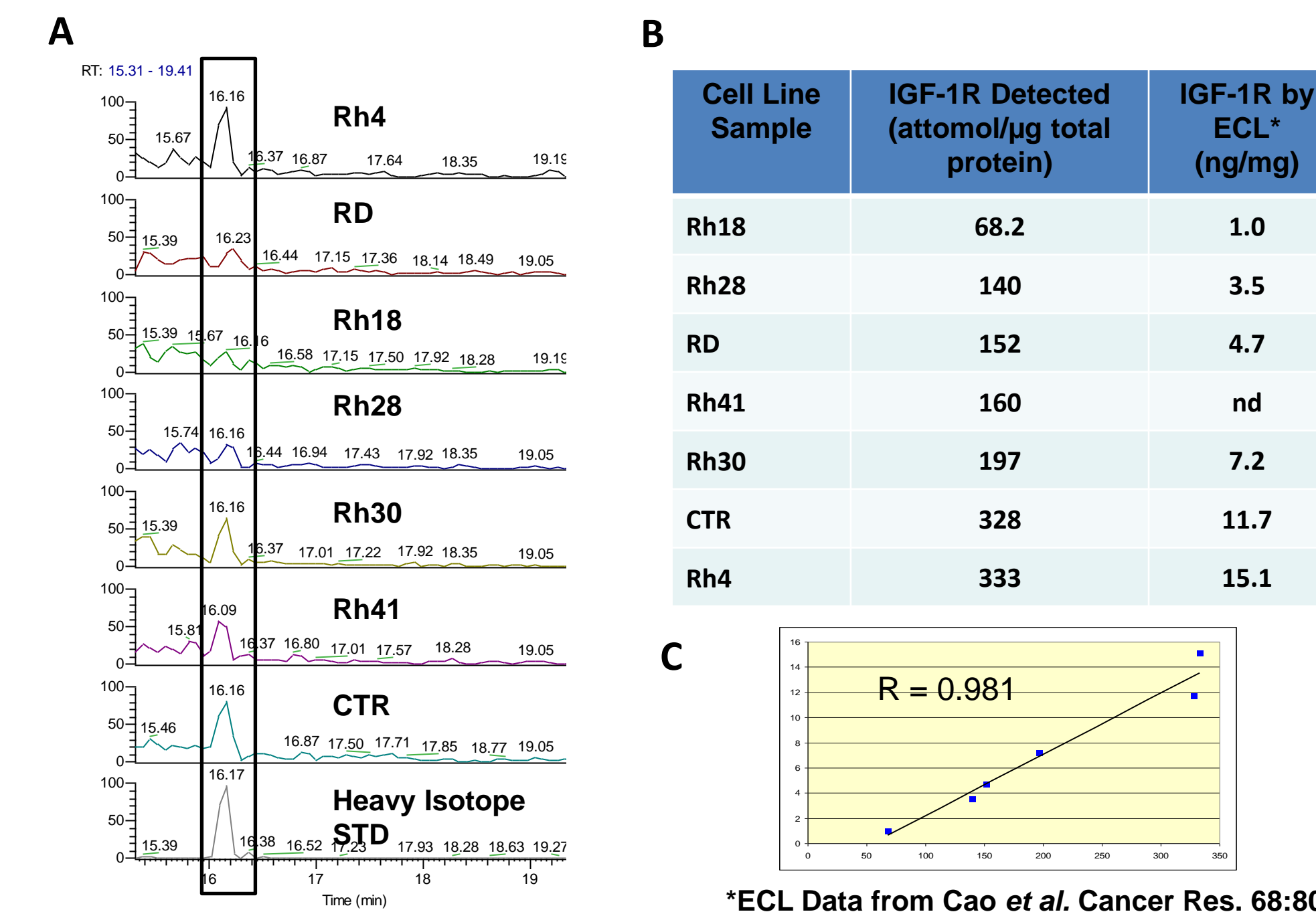


Figure 3. IGF-1R Expression in Formalin Fixed Cells. A. Ion chromatograms demonstrating quantitation of IGF-1R in seven formalin fixed rhabdomyosarcoma cell lines. The bottom trace corresponds to the isotopically labeled peptide used for quantitation. IGF-1R analyzed injecting 0.5 μg of total protein. B. Quantitated data from A) compared with electrochemiluminescence (ECL) generated quantitation of IGF-1R from solubilized cell lines. C. Correlation curve comparing SRM quantitation with ECL quantitation.

Detection of IGF-1R in Rhabdomyosarcoma Xenograft Samples by SRM Mass Spectrometry

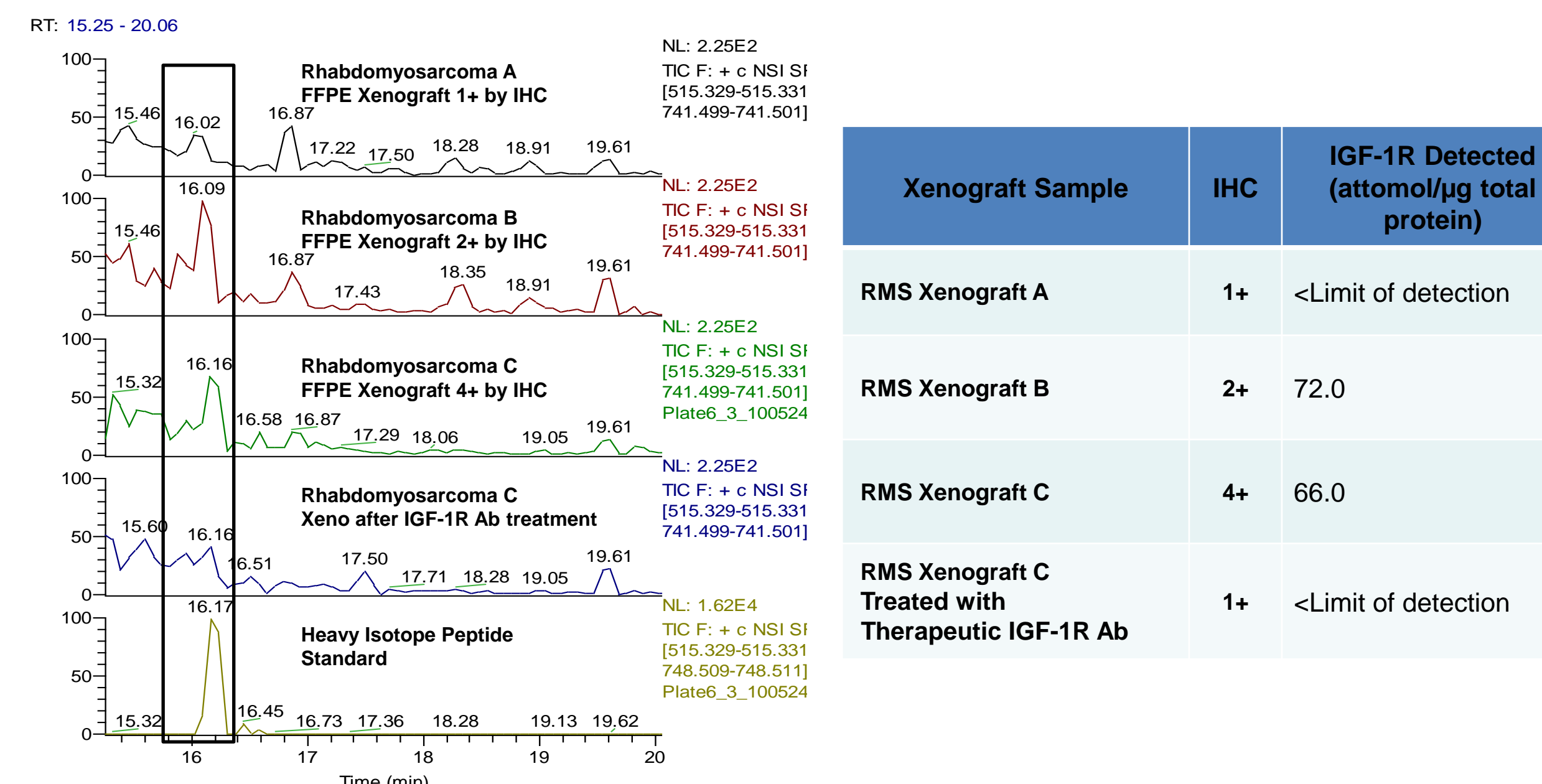


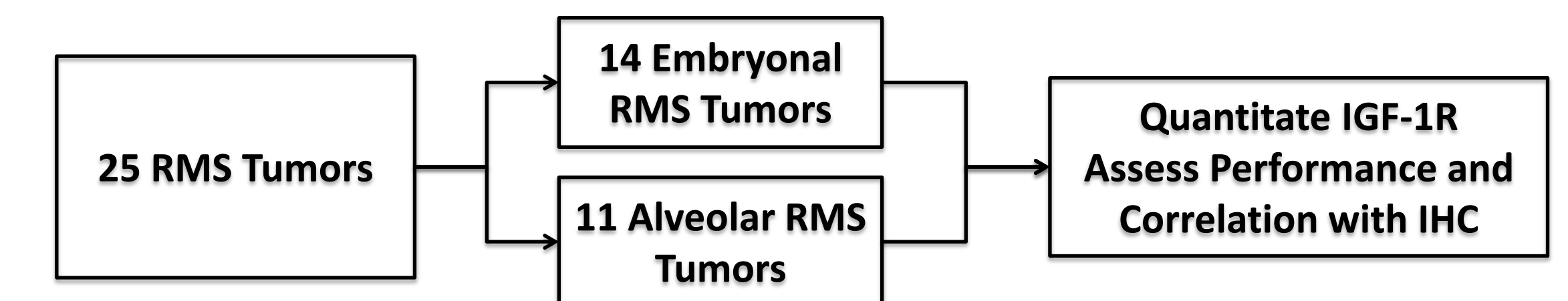
Figure 4. Quantitation of IGF-1R Expression in Formalin-Fixed Human Tumor Xenografts.

Left. Ion chromatograms demonstrating quantitation of IGF-1R in four formalin-fixed rhabdomyosarcoma xenografts with variable levels of IGF-1R expression by IHC (1+, 2+, 4+ and 4+/after treatment with IGF-1R antibodies). The bottom trace corresponds to the isotopically labeled peptide used for quantitation. IGF-1R analyzed using injecting 0.5 μg of total protein.

Right. Quantitated data from the analytical runs on the left. It is not possible to use the ECL analysis from Figure 3 with the tryptic peptides recovered by the Liquid Tissue® process.

Clinical Validation of IGF-1R Assay in RMS Tumor Samples

FFPE rhabdomyosarcoma (RMS) tumor samples (embryonal and alveolar subtypes) were assessed for levels of IGF-1R expression. After Liquid Tissue processing, the samples were run in triplicate to assess assay reproducibility and variation. Correlation of SRM mass spectrometry and immunohistochemistry (IHC) in determining IGF-1R expression will also be evaluated.



Quantitation of IGF-1R Levels in Alveolar (ARMS) and Embryonal (ERMS) Rhabdomyosarcoma Tumors

RMS tumor samples were acquired from the Cooperative Human Tissue Network. Tumor tissue was laser microdissected, and then subjected to Liquid Tissue processing followed by SRM mass spectrometric analysis. 24/25 tumors showed detectable levels of IGF-1R, and one tumor expressed 45-fold higher levels than the median expression level, suggesting it may harbor an amplification or other genetic alteration. Comparable amounts of protein per cell were seen in clinical samples vs. cell lines.

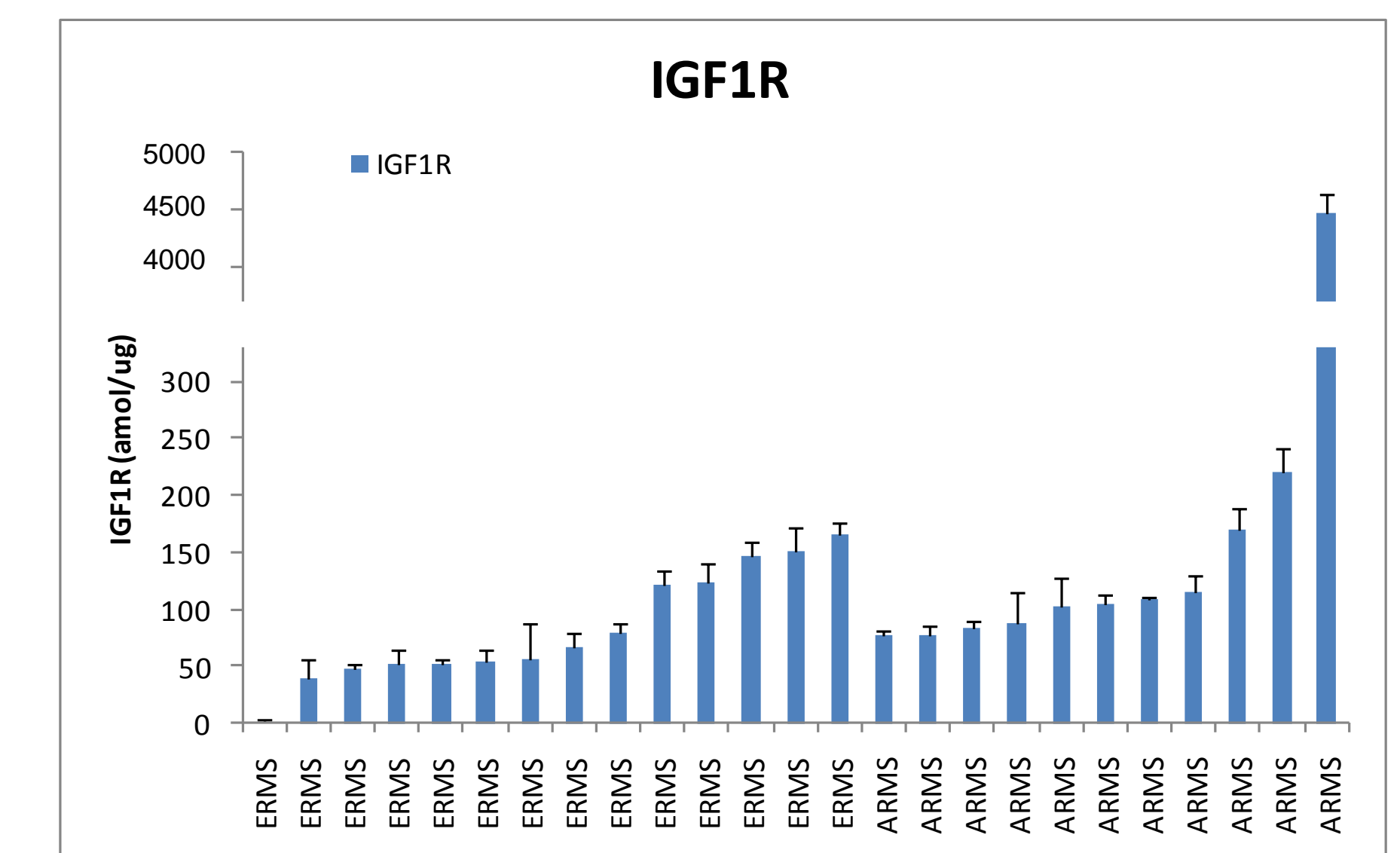


Figure 5. Absolute SRM quantitation of Liquid Tissue processed FFPE human RMS tumor samples. Quantitation of IGF-1R in embryonal or alveolar RMS tumor samples.

Conclusions and Future Directions

- As IGF-1R inhibitors are tested in clinical trials, understanding which patients will benefit most from this therapy is critical. Sensitivity of rhabdomyosarcomas to IGF-1R targeted therapies may be dependent on the level of IGF-1R expression. Here we demonstrated the ability to quantitate IGF-1R expression in FFPE tumor tissue using the Liquid Tissue-SRM assay.
- After correlation with IHC, one next step will be to evaluate IGF-1R expression in tumor tissue from patients enrolled in a clinical trial of an IGF-1R targeted therapy for RMS. The ability to correlate IGF-1R expression with response to IGF-1R targeted therapy may allow better selection of patients for future trials.
- In addition, assays for phospho-IGF-1R and Insulin Receptors A/B are under development. This should provide the ability to determine activation and attenuation of the IGF-1R target as well as an understanding of the contribution of the insulin receptor pathway in this disease.

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