Dependence of Wilms tumor cells on signaling through insulin-like growth factor 1 in an orthotopic xenograft model targetable by specific receptor inhibition

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AUTHOR SUMMARY

Wilms tumor is an embryonal renal tumor that accounts for nearly 6% of all pediatric cancers and more than 90% of kidney tumors in children. Although survival is good for the majority of patients, for children with anaplastic or relapsed disease, the clinical outcome remains poor. The insulin-like growth factor 2 (IGF2) may be produced in large amounts in these tumors as a result of loss of heterozygosity or loss of imprinting (1, 2), genetic and epigenetic mechanisms that result in increased expression of the ligand as a result of preferential targeting of the active allele at this locus. Previous work in our laboratory identified that an increased copy number of the gene encoding the receptor for IGF2, IGFR1, is associated with a poor clinical outcome (3). Upon IGF2 binding IGFR1, downstream intra-cellular signaling cascades are initiated that drive tumor cell proliferation and survival. Novel therapeutic strategies targeting the IGF1 receptor, alone or in concert with existing chemotherapies, may therefore be beneficial for patients with anaplastic and relapsed Wilms tumor. We identified single-agent activity in orthotopic, but not subcutaneous (s.c.), xenografts in vivo, thus demonstrating the importance of using models that accurately recapitulate the disease phenotype.

To evaluate the utility of targeting the IGF1R in Wilms tumor, we first screened five cell lines to test the efficacy of the small-molecule IGF1R inhibitor NVP-AEW541 in vitro, and measured the levels of activated IGFR1, i.e., phosphorylated IGFR1. Protein analysis and gene expression profiling of downstream targets of IGFR1 signaling pathways revealed coinhibition of mitogenic/antiapoptotic PI3-kinase and MAP kinase pathways upon pharmacological and genetic inhibition of IGFR1. Importantly, IGFR1 inhibition affected cell cycle arrest in the G1 phase and apoptosis (as measured by flow cytometry). Given the known chemotherapy-sensitizing effects of IGF1R inhibition in other cell systems, with similar effects on cell signaling and gene expression, we investigated the effects of the IGF1R inhibitor NVP-AEW541 in combination with clinically relevant chemotherapeutic strategies in Wilms tumors. Median effects analysis revealed that combined treatment with NVP-AEW541 and doxorubicin or topotecan results in highly synergistic interactions in vitro. To test the efficacy of the drugs in vivo, we developed both heterotopic (injection of cells into the flank, i.e., s.c.) and orthotopic (at the site of origin of the tumor cells, i.e., the kidney) xenograft models. s.c. tumors were poorly reflective of Wilms tumor histology, and did not respond to NVP-AEW541. In contrast, cells implanted within the kidney formed tumors with morphological features consistent with human Wilms tumors, and showed significant reduction in tumor weight and volume with the single-agent NVP-AEW541 treatment (Fig. P1). While investigating the mechanisms behind these observations, we noted a significantly enhanced inhibition of activated IGFR1 and the downstream signaling pathways.


Conflict of interest statement: V.R., S.J., and F.H. are employees of Novartis Pharma.

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downstream PI3-kinase and MAP kinase pathways in orthotopic compared with s.c. tumors at the equivalent drug dose. However, as these data are reliant on xenograft experiments performed in a single cell line, they require validation. The microenvironment in which the cells are grown clearly exerts a significant effect on the phenotype of the tumor growth as well as the response to the targeted therapeutic inhibition.

Ectopic models are widely used because they provide a simple, rapid, and reproducible means to evaluate emerging therapies. However, they do not imitate the natural tumor environment, as they omit organ-specific host–tumor interactions that may influence responses to therapy. In the context of Wilms tumor, the predictive values of the IGF2-stimulated in vitro models were critically dependent on IGFI-R-mediated signaling, and were recapitulated only in the orthotopic setting. A recently developed genetically engineered mouse model combined loss of Wt1 function and Igf2 up-regulation to drive tumor initiation and proliferation through IGFI-R signaling may be a biologically relevant model for testing the efficacy of agents that target the receptor (4).