Tumor suppressor p53 is a pro-apoptotic molecule frequently inactivated in cancer by gene mutations or defective signaling. Mutated p53 is uncommon in many childhood cancers and may have therapeutic benefit in the pediatric setting. RG7112 is a selective inhibitor of p53-MDM2 binding that frees p53 from negative control, activating the p53 pathway in cancer cells and leading to cell cycle arrest and apoptosis. RG7112 is a member of the Nutlin family of MDM2 antagonists that has improved potency and pharmacological properties. It is currently in clinical evaluation in adults with leukemias and selected solid tumors.

Methods
In vitro: in vitro testing was performed using DMSO (2.5%) and MTS Assay (Promega) in 96-well flat bottom, 384-well format and in a black plate format. For MTS assay, IC50 values were calculated from 1 nM to 10 µM. Relative IC50 (rIC50) values were used to measure the potency of the inhibitor to each xenograft. RG7112 and its inactive enantiomer RG7112i were evaluated against the PPTP in vivo panel using the 2x25 µM dose level and in concentrations from 1 nM to 10 µM. Relative IC50 (rIC50) values were used to measure the potency of the inhibitor to each xenograft.

In vivo: standard PPTP methods for in vivo testing were employed [10]. Briefly, 8-12 week-old female BALB/c nu/nu mice, were inoculated with 3-5 x 10^6 mononuclear cells expressing MDM2 and p53 expression from each PPTP xenograft line, 8 mice were inoculated with 3-5 x 10^6 mononuclear cells expressing MDM2 and p53 expression from each PPTP xenograft line. In vivo testing was supported by NCI NO1CM42216. Testing was supported by Drs. Lyubomir Vassilev and Steven Middleton regarding experimental design and interpretation of results. RG7112 was provided for testing by Hoffmann-La Roche. The PPTP acknowledges helpful discussions with Drs. Lyubomir Vassilev and Steven Middleton regarding experimental design and interpretation of results. Testing was supported by NCI NO1CM42216.

RG7112 induced significant differences in EFS (event-free survival) distribution compared to the control in 10 of 13 evaluable p53 WT solid tumor xenografts (p<0.001) in 10 of 12 evaluable p53 WT solid tumor xenografts (p<0.001). RG7112 induced a two-fold or greater delay in time to event (EFS T/C > 2) in 10 of 25 (40%) p53 WT solid tumor xenografts, including: 2/2 rhabdoid tumor, 2/2 Wilms tumor, 2/2 Ewing, and 3/8 (38%) alveolar rhabdomyosarcoma models.

Neuroblastoma (n=5) or osteosarcoma (n=6) models showed EFS T/C > 2, and one solid tumor xenografts with mutant p53 (Rh30 and EWS) showed no response to RG7112 as expected. Objective responses were observed in 5 solid tumor xenografts: maintained complete response (MCR) or complete response (CR) for a medulloblastoma and an alveolar rhabdomyosarcoma, respectively, and partial responses (PR) for a Wilms tumor, rhabdoid tumor, and Ewing tumor xenograft.

For the ALL panel, among 13 xenografts there were 11 CR, 1 MCR and 1 PR. Each of the 7 ALL xenografts with MLL-rearrangement was highly responsive to RG7112 with 6 CR and 1 MCR.

Two additional MLL-rearranged xenografts (MV4:11 and RS4:11) grown subcutaneously were also tested, with the former showing MCR and the latter showing tumor growth delay (PD2).

The consistent high level activity of RG7112 against ALL models, particularly those with MLL-rearrangement, supports prioritization of RG7112 for in vivo testing in the acute leukemia setting. Preclinical evaluations of RG7112 with standard agents are planned for both solid tumor and ALL models.

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