ABSTRACT

Background: RX-5902 (necitumumab) is a novel anti-cancer compound that targets phosphatidylinositol-3 kinase (PI3K). The ability to inhibit PI3K in the Westerman model has been shown previously to result in tumor growth inhibition in vivo. We have previously shown RX-5902 inhibits cell growth in a dose-dependent fashion in the triple-negative breast cancer (TNBC) model MDA-MB-231. In the current study, we have expanded on investigations of the therapeutic potential of RX-5902 against TNBC using both in vitro and in vivo preclinical models.

Methods: RX-5902 was provided by Rexahn Inc. (Rockville, MD). Cell proliferation was measured using the Cell-Titer-Glo luminescent cell viability assay (Promega). Apoptosis was assessed using fluorescent Caspase 3/7 flow-assay (Roche). Immunoblot analysis of the MDA-MB-231 cell line were probed by 4-catechol (Cell Signaling). SYTox Green (Molecular Probes) and DNase I were used as positive controls. Fluorescence images of nuclei were taken in multiple fields. The MTT assay was used to measure the viability of cells in the presence or absence of RX-5902. Cells were either grown as a single cell or in combination with anti CTLA-4 or anti PD-1 are commonly observed in this tumor model over a period of 20 days. Both treatments were significantly superior to either treatment alone.

Conclusions: RX-5902 showed efficacy against several in vitro and in vitro preclinical models of TNBC. RX-5902 resulted in G2/M arrest and induced apoptosis in sensitive TNBC cell lines and decreases in nuclear beta-catenin. In vivo, RX-5902 demonstrated anti-tumor effects when combined with either anti-CTLA-4 or anti-PD-1 immunotherapies. In a preclinical mouse model, RX-5902 resulted in G2/M arrest and induced apoptosis in sensitive TNBC cell lines and decreases in nuclear beta-catenin. In vivo, RX-5902 demonstrated anti-tumor effects when combined with either anti-CTLA-4 or anti-PD-1 immunotherapies. In a preclinical mouse model, RX-5902 is combined with the anti-PD-1 checkpoint inhibitor nivolumab. Together, these findings indicate that RX-5902 may have important clinical implications for the treatment of TNBC.

MATERIALS AND METHODS

Design: RX-5902 was provided by Rexahn Inc. (Rockville, MD). Anti-PD-1 and anti-PS-3/1 checkpoint inhibitors were obtained from Selleck and the University of Colorado Hospital Pharmacy, respectively.

Cell proliferation was measured using the Cell-Titer-Glo luminescent cell viability assay (Promega). Apoptosis was assessed using fluorescent Caspase 3/7 flow-assay (Roche). Immunoblot analysis of the MDA-MB-231 cell line were probed by 4-catechol (Cell Signaling). SYTox Green (Molecular Probes) and DNase I were used as positive controls. Fluorescence images of nuclei were taken in multiple fields. The MTT assay was used to measure the viability of cells in the presence or absence of RX-5902. Cells were either grown as a single cell or in combination with anti CTLA-4 or anti PD-1 are commonly observed in this tumor model over a period of 20 days. Both treatments were significantly superior to either treatment alone.

Conclusions: RX-5902 showed efficacy against several in vitro and in vitro preclinical models of TNBC. RX-5902 resulted in G2/M arrest and induced apoptosis in sensitive TNBC cell lines and decreases in nuclear beta-catenin. In vivo, RX-5902 demonstrated anti-tumor effects when combined with either anti-CTLA-4 or anti-PD-1 immunotherapies in 4T1 syngeneic mouse models. In a humanized immune-cell model bearing MDA-MB-231 cell line xenografts, we observed a tumor growth inhibition in RX-5902 is combined with the anti-PD-1 checkpoint inhibitor nivolumab. Together, these findings indicate that RX-5902 may have important clinical implications for the treatment of TNBC.

CONCLUSIONS

• RX-5902 demonstrated efficacy against several in vitro and in vivo preclinical models of TNBC
• RX-5902 resulted in G2/M arrest and induced apoptosis in sensitive TNBC cell lines and decreases in nuclear beta-catenin.
• In vivo, RX-5902 demonstrated additive anti-tumor effects when combined with either anti-CTLA-4 or anti-PD-1 immunotherapies in 4T1 syngeneic mouse models.
• In a humanized immune-cell model bearing MDA-MB-231 cell line xenografts, we observed a tumor growth inhibition in RX-5902 is combined with the anti-PD-1 checkpoint inhibitor nivolumab.
• Together, these findings indicate that RX-5902 may have important clinical implications for the treatment of TNBC.

Cellular Ploidy (24h)

Figure 1: Mechanism of RX-5902 action

Figure 2: Immunofluorescence of MDA-MB-231 cell line extracts from cell exposed to increasing doses of RX-5902 (1uM)

Figure 3: ICCS x3 plates for a panel of TNBC cell lines exposed to RX-5902 for 72 hs. as measured by Cali Titre Glo assay

Figure 4: Caspase 3/7 activity over time as measured by Incucyte for the indicated doses of RX-5902

Figure 5: Measurement of ploidy by Incucyte at the indicated doses of RX-5902 after 24 hrs treatment

Figure 6: Schema for generation of humanized mice for testing combinations with immunotherapies with cell lines or PDX samples

Figure 7: Dose dependent inhibition of tumor growth was observed with RX-5902 and additive effect in combination with anti-PD-1 immunotherapy

Figure 8: Tumor growth curves of humanized mice bearing MDA-MB-231 TNBC cell line xenograft tumors at the indicated doses