RRX-001 is a novel systemically non-toxic small molecule with macrophage stimulating activity. An ongoing clinical trial in small cell non-small cell lung, ovarian, and neuroendocrine cancers has provided positive data for antitumor activity of RRX-001 in these difficult to treat cancers. In preclinical studies, RRX-001 induced immune-suppressive M2-like Tumor Associated Macrophages (TAMs) to express a series of cytokines and chemokines including transforming growth factor-β (TGF-β1), tumor necrosis factor-α (TNF-α), and interleukin-6 and -12, a profile that resembles the M1 activated macrophage state. Since tumor infiltrating myeloid cells in tumors produce large amounts of TGF-β1, which is poorly chemotactic for myeloid cells, the expression of TGF-β1 may serve as a marker for the presence of TAMs. Infiltration of tumor associated macrophages (TAMs) is hypothesized to be a sine qua non for the anticancer mechanism of RRX-001, which induces the polarization of TAMs from pro-tumor and anti-inflammatory to anti-tumor and pro-inflammatory. We therefore compared expression patterns of TGF-β1 and TGF-β1 receptor type 1 (TGF-βR1) in patient-derived biopsy samples obtained at screening and post RRX-001. We also investigated the presence of macrophages in the patient samples. Finally, using in vitro studies, we explored the effects of RRX-001 on cytokine and marker expression in cultured macrophages.

RRX-001 Tumor Specific Trojan Horse Mechanism of Delivery and Response

Minimal toxicity to normal tissue

BACKGROUND and METHODS

Background: RRX-001 is a novel systemically non-toxic small molecule with macrophage stimulating activity. An ongoing clinical trial in small cell non-small cell lung, ovarian, and neuroendocrine cancers has provided positive data for antitumor activity of RRX-001 in these difficult to treat cancers. In preclinical studies, RRX-001 induced immune-suppressive M2-like Tumor Associated Macrophages (TAMs) to express a series of pro-inflammatory cytokines and chemokines such as tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), interleukin-6 and -12, a profile that resembles the M1 activated macrophage state. Since tumor infiltrating myeloid cells including macrophages produce large amounts of TGF-β1, which is poorly chemotactic for myeloid cells, the expression of TGF-β1 is hypothesized to be a sine qua non for the anticancer mechanism of RRX-001, which induces the polarization of TAMs from pro-tumor and anti-inflammatory to anti-tumor and pro-inflammatory. We therefore compared expression patterns of TGF-β1 and TGF-β1 receptor type 1 (TGF-βR1) in patient-derived biopsy samples obtained at screening and post RRX-001. As RRX-001 is closely associated with the induction of fibrosis, we also examined key fibrosis markers.

Methods: Tumor biopsies were obtained before (screening) and after treatment with RRX-001 from consenting patients with non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), and neuroendocrine tumors in the QUADRUPLE THREAT Phase II clinical trial (NCT02489903). Post treatment biopsies were obtained following 6 weeks of once-weekly RRX-001 treatment co-incident with the final on-study CT scan. Tumor samples were evaluated immunohistochemically for TGF-β1 TGF-βR1 expression, as indications for the regulation of the TGF-β pathway. Tumors were also evaluated for the presence of CD68 positive macrophages, a pan-specific marker for macrophages, and for CD163 positive macrophages, a marker for M2 polarized macrophages. Finally, we performed in vitro assays using cultured macrophages to determine whether exposure to RRX-001 could affect macrophage polarization. J774.A1 murine macrophages were exposed to murine whole blood or 100 µM RRX-001 for 24 h at 37°C. After 16 h incubation, mRNA was collected, and qPCR was performed to determine cytokine and marker expression.

CONCLUSIONS

Our pre- and post-treatment patient data suggest that there is a correlation between TGF-β1 signaling activation and RRX-001 treatment response. Our data indicate that RRX-001 may correlate with increased numbers of macrophages, and their activation status, in the vicinity of the tumor which is validated in vitro Macrophage activation experiment. These data suggest that activation of the TGF-β1 pathway, as evident by expression of TGF-β1 and TGF-β1R1 by tumor cells, could be a predictive biomarker for RRX-001 treatment as a macrophage activating agent.