INTRATUMORAL (IT) INJECTION OF THE TOLL-LIKE RECEPTOR 4 (TLR4) AGONIST G100 INDUCES A CLINICAL RESPONSE AND A T CELL RESPONSE LOCALY AND SYSTEMICALLY

Y. David Seo, Jing Zhou, Kevin Morse, Jeremy Patino, Sean Mackay, Edward Y. Kim, Ernest U. Conrad III, Ryan B. O’Malley, Lee Cranmer, Halling Lu, Frank J. Hsu, Yuexin Xu, Elizabeth Loggers, Taylor Hain, Venu G. Pillarisetty, Gabrielle Kane, Stanley R. Riddell, Jan ter Meulen, Robin L. Jones and Seth M. Pollack

Introduction

Solid tumors (STS) are heterogeneous microenvironmental space which are both morphologically and biochemically distinct. G100 is a stable sIgM in water-emulsion of glucosylceramide (41, A highly potent TLR4 agonist, which has been utilized for intratumoral (IT) injections within a variety of solid tumor xenograft models. The mAb-induced microenvironment reorganization of STS generally leads to the rapid infiltration and activation of immune cells seen in other solid tumors. In the setting of a “cold” tumor microenvironment, we hypothesized that G100 would induce local tumor infiltration and subsequently stimulate immune responses. By using the STS immunologic microenvironment test (sIT), we postulated that G100 would allow for the identification of optimal chemotherapy regimens as well as provide a novel vaccination agent for future clinical trials using other immunotherapeutics (such as immune checkpoint, signaling) agents.

Methods

Patients and methods: STS patients who had a superficially inoperable lesion were treated with weekly T100 for 8-12 weeks; 12 patients received concurrent radiation for 2 weeks at the start, while 8 got IT G100 alone for 5 weeks prior to radiation (See Figure S for scheme). This cohort also received 20µg of IT G100, which is thought to be the dose that has been previously studied as a vaccine adjuvant. Fresh tumor biopsies and PBMC were collected pre- and post-treatment, and flow cytometry was performed on biopsies. Tumor infiltrating lymphocytes (TIL) expanded in vitro, and PBMC were analyzed with TCR deep sequencing. Cytokine producing T cells in PBMC were selected using immunization with CD3 COOH bead cocktail (76). PBMC were also analyzed by single cell multispectral cytokine profiling. Clonality was calculated in 2- Patient’s vaccine using single clonal fragments from each sample. RECEPTOR v1.1 and the Common Terminology Criteria for Adverse Events (CTCAE) were used to monitor clinical outcomes.

Results

Patients had a median of 10-30 days prior ease of therapy and mean tumor size of 5cm (1-24cm) (Table 1). No grade 3 or higher treatment-related adverse events (AEs) was observed; and local tumor control was achieved in all evaluable patients, as shown in Table 2. One patient (Patient #10-1) withdrew from trial because of treatment-related symptoms. One patient (Patient #10-1) developed fever, chills, and hypotension, which was not considered to be an adverse event (AE). AE was noted as minor and was not considered to be related to the local control data. If the treated tumors had been treated independently of the post-treatment for 10-30 days prior ease of therapy and mean tumor size of 5cm (1-24cm) (Table 1). No grade 3 or higher treatment-related adverse events (AEs) was observed; and local tumor control was achieved in all evaluable patients, as shown in Table 2. One patient (Patient #10-1) withdrew from trial because of treatment-related symptoms. One patient (Patient #10-1) developed fever, chills, and hypotension, which was not considered to be an adverse event (AE). AE was noted as minor and was not considered to be related to the local control data.

The treated tumors were tracked beyond the post-treatment period for 30 days following the first injection for the 14 evaluable patients with adequate imaging; 35 (86%) had minimal or no residual tumor present. A post-treatment evaluation revealed significant differences in local control based on cohort. In all patients with long-term follow-up post 1-4 months, treated patients had maintained or improved control versus other index lesions which did not receive G100 (19% in vs. 42% at mean 265 days, p=0.02). Patient #4 had complete regression of the irradiated tumor, as seen in the CT scan (Figure 3). In the trial period, another irradiated lesion which did not receive G100 (10-30 days) showed only stable disease with the untreated index lesion (in green) showed growth. When looking at other patients, irradiated lesions and following their response, again local maintained a partial in complete response (Figure 3). This suggests that G100 could have been used as a radiation in this patient. Other patients with long-term follow up also maintained a persistent local control at the irradiated tumor (shown in red), better than at other irradiated (blue) or untreated (green) tumors (Figure 3).

Conclusions

Our pilot study of IT G100 demonstrates that it is well-tolerated (with no CTCAE grade 3-4 AEs) and effective, with impressive local control of metastatic STS (100% control of disease at 12 months). Only 1 patient with complete tumor necrosis occurred. G100 may also serve as a radiosensitizer, as evidenced by patient #5-6 with long term CR. With or without radiation, G100 pushes the tumor microenvironment into a more inflammatory state, driven largely by an increase in T cell infiltration as well as an increase in the functional CD4 T cells in the peripheral blood. The T cell response may be specific to tumor antigens, as evidenced by the high degree of clone overlap between polyclonal CD4 and CD8 T cells and the pre-treatment memory T cells in patients with good local response; conversely, the patients had low local responses and had no detectable overlap, suggesting a potential for a clinical predictor of response to G100. The robust rate of local control, tolerability, and ability to turn the STS microenvironment into a robust immune response is a novel immune-oncology agent that warrants further mechanistic and clinical evaluation; future trials combining IT G100 with systemic immune checkpoint therapies are currently under review.