Phase 1 study of B440, an oral *Bifidobacterium*-engineered WT1 cancer vaccine, in patients with metastatic urothelial cancer.

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Background: B440 is an innovative oral cancer vaccine comprised of recombinant *Bifidobacterium* engineered to express WT1 tumor-associated antigen. By delivering the WT1 protein to dendritic cells in gut-associated lymphoid tissue, B440 is designed to induce a tumor-specific cellular immunity. Preclinical data demonstrated effective WT1-specific T-cell induction and anti-tumor activity in murine models of urothelial, prostate, and renal cancers. **Methods:** This open-label, single-arm, phase 1 study evaluated the safety and preliminary efficacy of B440 in patients with metastatic urothelial cancer who had progressed after all standard therapies, including cytotoxic chemotherapy, PD-1/PD-L1 inhibitors, and antibody-drug conjugates. Twelve patients were enrolled in two dose cohorts (800 mg or 1,600 mg, n = 6 each), administered once daily for five consecutive days per week over four weeks (20 total doses). The primary endpoint was dose-limiting toxicity (DLT), assessed during the treatment. Secondary endpoints included safety (adverse events [AEs] graded by CTCAE v5.0), best overall response (BOR), and progression-free survival (PFS) by RECIST v1.1. WT1-specific immune responses were measured via ELISPOT assays detecting interferon-γ-producing T cells. **Results:** All 12 patients completed the treatment: (median age: 74.5 years [range: 39–81]; primary tumors in bladder [n = 5], renal pelvis [n = 4], ureter [n = 3]). No DLTs were observed in either dose cohort. Treatment-related AEs were generally mild (Grade 1), with the most common events being transient IL-6 elevations and cold-like symptoms (n = 3 each). The disease control rate (DCR) was 50%, as six patients achieved stable disease (SD) as their BOR. Six patients also demonstrated WT1-specific T-cell induction confirmed by ELISPOT. ELISPOT-positive patients had a significantly longer PFS compared to ELISPOT-negative patients (median PFS: 113 days vs. 57 days; P = 0.0033). Although not included in the study protocol, six patients subsequently underwent pembrolizumab rechallenge at the discretion of their physicians. Of these, three achieved clinical responses (one complete response [CR] and two partial responses [PR]). Spider plot analyses indicated early tumor shrinkage among ELISPOT-positive patients, with maximum reductions of −100%, −49%, and −32.7% from baseline. Notably, three of the four ELISPOT-positive patients achieved objective responses upon rechallenge. **Conclusions:** B440 exhibited a favorable safety profile and no DLTs up to 1,600 mg. The induction of WT1-specific immunity correlated with improved PFS during B440 therapy and enhanced responses upon pembrolizumab rechallenge. These data support further investigation of B440 in larger, randomized trials and potential combination with other immunotherapies in WT1-expressing malignancies. Clinical trial information: jRCT2051220143. Research Sponsor: Japan Agency for Medical Research and Development (AMED); 23ym0126081h0002; Immunorock Co., Ltd.