

Immunological monitoring of metastatic colorectal cancer patients undergoing active specific immunotherapy with a poly-epitope peptide vaccine (TSPP) to thymidylate synthase in combination with GOLFIG chemoimmunotherapy regimen

D. Mazza¹, C.E. Martino¹, L. Micheli¹, G. Giorgi¹, P. Correale²

¹Dept. of Medical Sciences, Surgery and Neuroscience, University of Siena

²U.O.C. of Radiotherapy, University Hospital AOUS of Siena

Background: TSPP is a 27 poly-epitope peptide anti-cancer vaccine which includes the amino acidic sequences of 3 known CTL-epitopes with HLA-A(*)02.01 binding motifs, contained in the thymidylate synthase (TS), a tumour-associated enzyme antigen critical for DNA synthesis and repair. The immunological and antitumor activity of TSPP has already been tested in preclinical studies, whose results offered the rationale to test the TSPP vaccine in cancer patients in a phase Ib trial. The study, designed on dose finding setting, was aimed to identify the maximal tolerated dose (MTD) and the most efficient biological dose (MEBD) of TSPP in different therapeutic conditions. **Material and method:** TSPP/VAC-1 (Eudract 2009-016897-33) is a mono-centric Phase Ib trial, approved by the *Istituto Superiore di Sanità* and Siena University ethical committee. The aim of the trial was to test the toxicity and immune-biological activity of TSPP alone (arm A) in combination with immune-adjuvant cytokines that are GM-CSF (Sargramostim) and IL-2 (Aldesleukine), according to the IG-1 regimen (arm B) or with a newest poly-chemo-immunotherapy regimen (GOLFIG) with gemcitabine, oxaliplatin, levofolinic acid, 5-FU, GM CSF and IL-2 (arm C). In the latter arm, TSPP was administered subcutaneously (diluted 1:2 with Montanide) on biweekly bases one week after the beginning of polychemotherapy. This report describes the results obtained of our immune-biological monitoring in twenty-seven patients enrolled in the arm C. The criteria for the enrollment were: metastatic colorectal carcinoma diagnosis, at least two previous chemotherapy lines, ECOG performance status ≤ 1 . The study was designed on dose-finding setting thus ten patients did not receive TSPP [Dose level (DL-0)]; three, TSPP at the dosage of 100 μ g (DL-1); three 200 μ g (DL-2) and eleven 300 μ g (DL-3). **Results:** TSPP vaccination resulted safe and did not enhance the frequency of adverse events associated with the GOLFIG regimen. Grade 1 and 2 (according with WHO criteria) chemotherapy-related haematological and gastrointestinal adverse events and 4 cases of oxaliplatin sensitizations were recorded. Ten cases of delayed hypersensitivity in the vaccine injection site and 12 cases of polyarthralgia were also recorded. We observed the ability of TSPP vaccination to induce systemic inflammation as shown by an increase in serum level of CRP (an average at the X cycle treatment of $3,10 \pm 0,09$ mm/h at DL-1,2 and 3 VS $0,70 \pm 0,59$ mm/h at DL-0; $P < 0.001$), ESR (an average at the X cycle treatment of $83,40 \pm 4,36$ mm/h at DL-1,2, and 3 VS $60,00 \pm 7,59$ mm/h at DL-0; $P < 0.001$) and myeloperoxidase and auto-antibodies like ENA (an average at the X cycle of treatment of $0,70 \pm 0,06$ ratio at DL-1,2 and 3 VS $0,30 \pm 0,035$ at DL-0; $P < 0.001$), p-ANCA (an average at the X cycle of treatment of $1,66 \pm 0,32$ U/ml at DL-1,2 and 3 VS $1,00 \pm 0,03$ at DL-0; $P < 0.01$) and c-ANCA (an average at the X cycle of treatment of $2,76 \pm 0,14$ U/ml at DL-1,2 and 3 VS $1,20 \pm 0,07$ at DL-0; $P < 0.001$). Our peripheral blood-cell monitoring showed a significant increase in lymphocyte counts (DL-1,2, and 3), which mainly concerned T cell subsets expressing a central memory ($CD3^+CD8^+CD45RA^-CCR7^+$) and effector memory ($CD3^+CD8^+CD45RA^-CCR7^-$) immunophenotype. Finally, a treatment related increase in the blood frequency of TSPP-specific cytotoxic T cells (as assessed by ELISPOT assay) and regulatory T cells (Treg, $CD3^+CD4^+CD25^+FoxP3^+$) confirmed the biological efficacy of the vaccine. **Conclusion:** TSPP vaccination resulted safe and its MTD could not be determined. TSPP MEBD was instead identified at 300 μ g, the dosage which was associated to the highest frequency of biological events. Our results also confirmed the hypothesis that the association of the vaccine with the GOLFIG has a significant immunomodulatory activity with potential anti-tumor activity.