The references and development of cellular and humoral response against WT1 protein vaccination in mice

To the Editor: Many anti-cancer vaccination strategies have been tested in mice and humans in the attempt to eradicate leukemia cells [1]. The vast majority of clinical trials are based on peptide vaccination which allows the induction of cellular response to specific tumor associated antigens [2]. WT1(Wilms tumor-1) gene is located on chromosome 11p13 and encodes a zinc finger transcription factor that plays an important role in cell growth and differentiation. WT1 was originally described as a tumor suppressor gene although many evidences demonstrated that it plays an oncogenic function in the setting of leukemia. WT1 protein represents an optimal tumor antigen since it is highly expressed in acute leukemias, myelodysplastic syndromes (MDS) and myeloproliferative neoplasms [3]. By contrast, it is expressed in acute leukemias, myelodysplastic syndromes (MDS) and myeloproliferative neoplasms [3]. By contrast, it is expressed at very low levels in normal hematopoietic progenitors. Expression of the WT1 protein is restricted to a limited set of tissues, including the gonads, uterus, kidney, and spleen.

The success of a particular peptide vaccine to elicit an immune response is influenced by many parameters, including the presence of helper T cell epitopes, processing and presentation by professional antigen presenting cells (APCs), biodistribution, peptide length, peptide affinity, and route of administration. Recently Brayer [4] and colleagues published in this journal the results of WT1 peptide vaccination in AML and MDS. The conclusion from this study and many others based on WT1 peptide vaccination is that this strategy is safe, feasible but, at least in this study, it is not able to induce a consistent and measurable WT1 specific T cell response. In the majority of the clinical trials WT1 peptide elicited CD3\(^+\) CD8\(^+\) T cells. Additional trials showed that the combination of short and long peptides induced also CD3\(^+\) CD4\(^+\) T cells. Interestingly, it was shown that long peptide elicited the strongest immunological response against WT1. The clinical results are overall encouraging, describing several patients obtaining molecular remission, partial responses or stable disease. The main limits are the immune tolerance and immun evasion. Two main strategies have been tested to overcome these limits, the use of long sequence peptides preferentially processed by APCs in the lymph node, circumventing some of the tolerance mechanisms, and the addition of adjuvant to stimulate APC. Here, we report the results of WT1 protein vaccination in mice.

The complete WT1 murine coding sequence cloned in an expression vector (pGEX-4T-1) together with GST protein has been amplified. The fusion protein GST-WT1 has been transfected in E.Coli and purified. Thirty C57Bl/6J mice have been utilized according to the scheme represented in Fig. 1 panel A. The first group (10 mice) was vaccinated performing a first injection with 30\(\mu\)g of GST-WT1 protein + 50 \(\mu\)g of complete Freund adjuvant (AD) at Week 0. After 2 and 4 weeks, a second and third dose 30 \(\mu\)g of GST-WT1 protein + 50 \(\mu\)g of AD were injected. The second group (5 mice) was vaccinated with 30 \(\mu\)g of GST-WT1 protein only at week 0, 2 and 4. The third group (5 mice) was vaccinated with 30 \(\mu\)g of GST-WT1 protein only at week 0, 2 and 4. The fourth group (10 mice) was treated with PBS only and used as control. After 2 additional weeks (weeks 6) 200 000 TRAMP-C cells, a senescent prostatic cancer cell line overexpressing WT1, were injected subcutaneously in all animals. After 8 weeks from the first injection half the mice were sacrificed to evaluate the immune response, both cytotoxic and humoral.

Figure 1: Panel A: Scheme of vaccination. Panel B: Response in terms of tumor bourden in vaccinated mice (ii) in which the tumor is undetectable compared to control mice (i) which developed a measurable tumor mass of 1.5 cm after 8 weeks from the first vaccination. Panel C: Dot blot analysis for the detection of specific antibodies against WT1. The analysis has been performed after 8 weeks from the first vaccination. Panel D: Quantification of the dot blot results. Panel E: \(^{51}Cr\) release test for the evaluation of cytotoxicity. Panel F: Evaluation of organ toxicity before and after vaccination, respectively, in lymphnode (a,b), spleen (c,d), kidney (e, f), and ovary (g,h).

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4. Musallam KM, Cappellini MD, Daar S, et al. Serum ferritin level and morbidity risk in humans in the attempt to eradicate leukemia cells [1]. The vast majority of clinical trials were based on peptide vaccination which allows the induction of cellular response to specific tumor associated antigens [2]. WT1(Wilms tumor-1) gene is located on chromosome 11p13 and encodes a zinc finger transcription factor that plays an important role in cell growth and differentiation. WT1 was originally described as a tumor suppressor gene although many evidences demonstrated that it plays an oncogenic function in the setting of leukemia. WT1 protein represents an optimal tumor antigen since it is highly expressed in acute leukemias, myelodysplastic syndromes (MDS) and myeloproliferative neoplasms [3]. By contrast, it is expressed at very low levels in normal hematopoietic progenitors. Expression of the WT1 protein is restricted to a limited set of tissues, including the gonads, uterus, kidney, and spleen.

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and the tumor burden, while half of them were sacrificed after 15 weeks to evaluate immune response, tumor burden, and organ toxicity.

Dot blot analysis on mouse serum showed the presence of IgG antibodies against WT1 after vaccination with GST-WT1 protein + AD and GST-WT1 protein alone. By contrast, the antibodies were not present after injection of GST + AD and PBS. (Fig. 1 panel C and D). Furthermore, cytotoxicity of T cells was evaluated by 51Cr release test. In mice injected with GST-WT1 + AD the level of cytotoxicity was 30% ± 2 compared to 2% ± 0.5 (background level) in control mice. Finally, we examined the toxicity in organs which physiologically express WT1 at low levels: lymphnode, spleen, ovary, and kidney in vaccinated mice and controls. No toxicity was observed (Fig. 1 panel F). Hemocromatometric analysis as well as BM smears (data not shown) excluded any kind of hematological toxicity. The mean Hb level was 13.9 g/dl in vaccinated mice and 14.2 g/dl in controls (P > 0.05), the median WBC count was 5135/µl in vaccinated mice and 6357/µl in controls (P > 0.05), the median platelet count was 1182000/µl in vaccinated mice and 1020000/µl in controls (P > 0.05). In conclusion, vaccination with WT1 protein induces a significant cytotoxic response and a potent antibody response. This results, at least in mice, in a significant reduction of the tumor burden. The median reduction of the volume of the tumor after 8 weeks of vaccination is 62%. (Fig. 1 panel B). This strategy may allow to overcome some of the limits associated with peptide vaccination including the restriction of the HLA typing of the patient and the prevalent T CD8+ response. This strategy allows to exploit the whole reactive potential of the immune system, both cytotoxic and humoral.

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Author Contribution
S.S.C. conceptualized the assay design and revised the manuscript. M.C. performed the assay optimization and validation and wrote the manuscript. S.L.Y., Sa.N., S.C.N., H.J.H.T., Su.N., P.C.W., and S.F.L. co-ordinated patient care, and E.B.P. the IVF/ICSI and embryo transfer procedures, as well as reviewed and approved the manuscript. A.S.C., F.S.H.C., and E.E.L.S. performed the clinical PGD testing and revised the manuscript.

CORRESPONDENCE

MIP CHEN1,2, SONG FEI LIMO1, SHI LING YU1, SURESH NAIR1, HENG HAO TAN1,2, SADHANA NANDARASAM1, PENG-CHIANG WONG1,2, SOON CHYE NEO1, ETHABE B. PRAASATH1,2, ARNOLD S.C. TAN1,2, EVELIA S.H. CHIN1,2, EUGENE E.L. SAW1,2, SAMUEL S. CHONG1,2,4,6

1Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 2Kho Teck Puat-National University Children's Medical Institute, National University Health System, Singapore; 3Thomson Fertility Centre, Thomson Medical Centre, Singapore; 4Centre for Assisted Reproduction, Department of Obstetrics & Gynaecology, Singapore General Hospital, Singapore; 5Gynecology Consultants Clinic and Surgery, Singapore; 6KKIV, Department of Reproductive Medicine, KK Women’s and Children’s Hospital, Singapore; 7Clinic for Human Reproduction, Department of Obstetrics and Gynecology, National University Hospital, Singapore; 8Sincere IVF Center, Sincere Healthcare Group, Singapore; 9Preimplantation Genetic Diagnosis Centre, Kho Teck Puat–National University Children’s Medical Institute, National University Health System, Singapore; 10Department of Laboratory Medicine, National University Hospital, Singapore

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