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CLINICAL EXPERIENCE OF IMMUNOTHERAPY BASED ON OLEIC ACID BOUND TO GLYCOSYLATED VITAMIN D-BINDING PROTEIN IN LOCALISED AND METASTATIC ADENOCARCINOMA OF THE PANCREAS

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Adenocarcinoma of the pancreas still carries a dramatically poor prognosis and the survival rate for this disease has not improved substantially in the past 40 years. Therefore, new treatment options are urgently needed and this need motivates oncologists to search for novel approaches such as immunotherapy. Here we report two clinical cases successfully treated with an integrative immunotherapeutic approach based on oleic acid bound to glycosylated vitamin D-binding protein (OA-GcMAF). Considering that immune suppression induced by pancreatic cancer is one of the main causes for resistance to chemotherapy and targeted therapy, this novel immunotherapeutic approach has the potential for revolutionising the field of pancreatic adenocarcinoma treatment. *Introduction:* Adenocarcinoma of the pancreas is, after colorectal cancer, the second most common digestive cancer in the USA where it represents the fourth leading cause of cancer-related death in both genders. In Europe, adenocarcinoma of the pancreas is the sixth most frequent cancer and is predicted to become the fourth cause of cancer death in both sexes in due course in the European Union (1, 2). Patients with pancreatic adenocarcinoma have an especially poor prognosis with a 5-year survival rate of <1% and a median survival of 4-6 months. Patients with a metastatic disease are usually treated with chemotherapy that is minimally effective (3). It has been demonstrated that pancreatic cancer-induced immune suppression is the main cause for this dramatically poor prognosis and, therefore, it has been proposed that immunotherapies may be particularly effective in this type of cancer (4). We recently demonstrated that immunotherapy based on oleic acid (OA) bound to glycosylated vitamin D-binding protein (OA-GcMAF) is effective in a variety of cancers (5); here we describe two clinical cases demonstrating that OA-GcMAF has therapeutic efficacy also in pancreatic adenocarcinomas. *Patients and Methods:* Patients with adenocarcinoma of the pancreas were treated with OA-GcMAF-based integrative immunotherapy according to the "Good practice in prescribing and managing medicines and devices" effective February 2013. The approaches described below, aimed at strengthening the immune system and reducing tumour growth, are considered complementary to other anti-neoplastic therapeutic procedures. OA-GcMAF complexes (GOleic) were prepared in-house at Immuno Biotech Ltd as previously described (5). The protocol for pancreatic adenocarcinoma was the following: OA-GcMAF (880 ng/day) was administered by subcutaneous injections in proximity of the inguinal lymphnodes (440 ng in each side) under ultrasound guidance. OA-GcMAF (880 ng) was also administered daily by nebulisation (880 ng dissolved in 5 ml saline). Suppositories containing 200 ng OA-GcMAF were administered daily. The total amount of daily OA-GcMAF was 1960 ng, an amount consistent with the procedure described by Nonaka *et al*. (6). Patients were provided with supplementation of vitamin D3, 20.000 IU per day, and they were taught to drink at least 2 litres of water per day. Patients followed a nutritional regime based on a diet very low in carbohydrates, and high in proteins (7). This regimen included supplementation with essential aminoacids (Master Aminoacid Pattern, dr. reinwald healthcare gmbh, Schwarzenbruck, Germany). Considering that probiotics are efficient immunopotentiators and have a role in cancer prevention (8), patients were provided with a probiotic fermented milk product containing colostrum and microorganisms known to produce natural OA-GcMAF during the fermentation process (Bravo Probiotic, Les Alpes, Wellington, NZ). Finally, considering the role of low-dose acetylsalicylic acid in cancer prevention (9), patients were provided with 100 mg of such a principle per day. *Results:* Clinical case #1. A 58-year-old lady was diagnosed with pancreatic adenocarcinoma localised in the body of the pancreas. The lesion could be detected by ultrasonography and it appeared as a roundish mass of 0.924 ml of calculated volume (Figure 1A). After two weeks of treatment as described above, the calculated volume of the mass was reduced to 0.517 ml that is about 39% (Figure 1B). The patient did not report any side effect: her body weight and lean mass increased during the two weeks of treatment. *Clinical case #2*. A 73-year-old man was diagnosed with metastatic pancreatic adenocarcinoma. Previous CT scans had evidenced peritoneal metastases. The patient was overweight and presented with an insulin-dependent diabetes. Due to the morphological constitution of the patient, the primary pancreatic lesion could not be evidenced by ultrasonography. However, a hypo-echoic roundish mass in the abdomen,

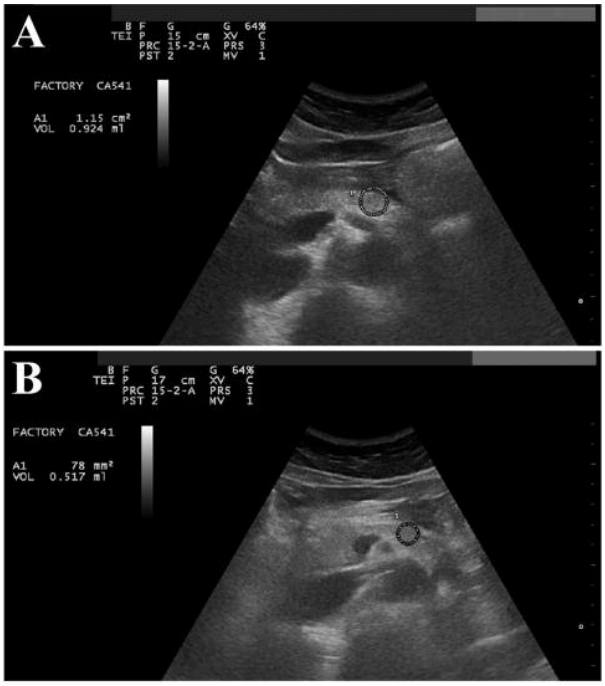

Figure 1. *Ultrasonography of adenocarcinoma of the body of the pancreas before and after OA-GcMAF-based immunotherapy. These images refer to clinical case #1 and were taken before treatment (panel A), and after two weeks (panel B).*

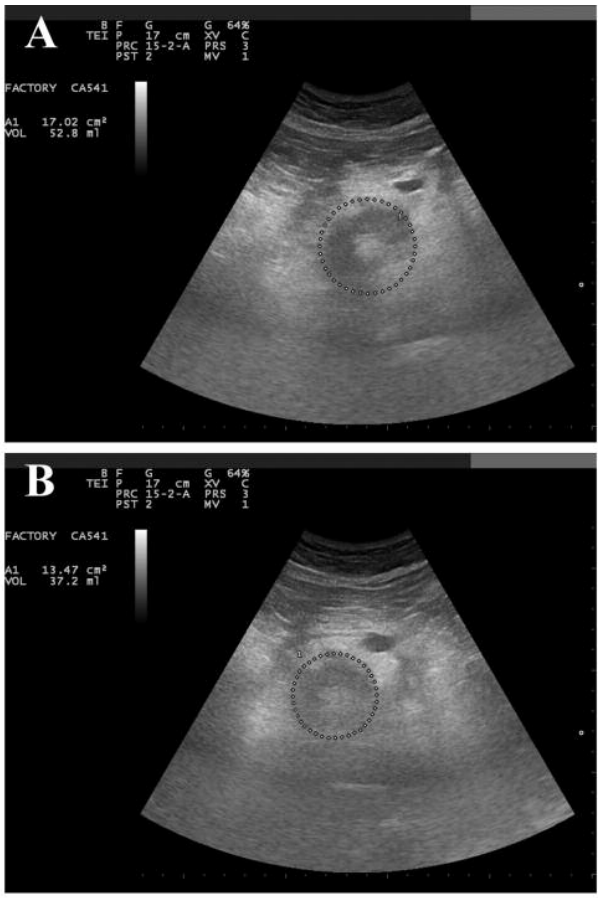

Figure 2. *Ultrasonography of a peritoneal lesion associated with metastatic adenocarcinoma of the pancreas before and after OA-GcMAF-based immunotherapy. These images refer to clinical case #2 and were taken before treatment (panel A), and after two weeks (panel B).*

interpreted as one of the metastases, was taken as reference. This mass was irregularly hypo-echoic with a relatively hyper-echoic central area and a calculated volume of 52.8 ml (Figure 2A). After two weeks of treatment, the calculated volume of this lesion was reduced by 30% (Figure 2B). In addition, thanks to the regimen described above, the patient was able to discontinue insulin administration and did not require oral anti-diabetic drugs. *Discussion:* Unlike other neoplasms, adenocarcinoma of pancreas is highly resistant to chemotherapy and targeted therapy (4). Therefore, new treatment options are urgently needed to improve the survival of patients with pancreas adenocarcinoma. Since the main reason for the resilience of pancreatic adenocarcinoma towards intensive treatment is the cancer-induced immune suppression, immunotherapy is probably the best candidate among new treatment strategies (10). In this study we demonstrate that immunotherapy based on OA bound to GcMAF is effective in patients with localised or metastatic pancreatic adenocarcinomas. These results are consistent with clinical observation accumulated in the past six years demonstrating that immunotherapy based on GcMAF is highly effective in a variety of cancers (5, 11-16). The approach described in this study targets immune suppression that is the main cause of pancreatic cancer poor prognosis; therefore, it has the potential for revolutionising the field of pancreatic adenocarcinoma treatment as it appears highly effective and devoid of harmful side effects.

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THREE DIMENSIONAL *IN VITRO* MODELS FOR STUDYING CANCER ANGIOGENESIS

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Introduction: Hydrogels prepared from star-shaped poly(ethylene glycol) (PEG) and maleimide-functionalized heparin provide a potential matrix for use in developing three dimensional (3D) models. We have previously demonstrated that these hydrogels support the cultivation of human umbilical vein endothelial cells (HUVECs). We extend this body of work to study the ability to create an extracellular matrix (ECM)-like model to study breast and prostate cancer cell growth in 3D. Also, we investigate the ability to produce a tri-culture mimicking tumour angiogenesis with cancer spheroids, HUVECs and mesenchymal stem cells (MSCs). *Materials and Methods:* The breast cancer cell lines MCF-7 and MDA-MB-231, and prostate cancer cell lines LNCaP and PC3, were seeded into starPEG-heparin hydrogels and grown for 14 days to analyze the effects of varying hydrogel stiffness on spheroid development. Resulting hydrogel constructs were analyzed *via* proliferation assays, light microscopy and immunostaining. Cancer cell lines were then seeded into starPEG-heparin hydrogels functionalized with growth factors as spheroids with HUVECs and MSCs and grown as a tri-culture. Cultures were analyzed *via* immunostaining and observed using confocal microscopy. *Results:* Cultures prepared in MMP-cleavable starPEG-heparin hydrogels display spheroid formation in contrast to adherent growth on tissue culture plastic. Small differences were visualized in cancer spheroid growth between different gel stiffness across the range of cell lines. Cancer cell lines were able to be co-cultivated with HUVECs and MSC. Interaction was visualized between tumours and HUVECs *via* confocal microscopy. Further studies intend to further optimize and mimic the ECM environment of *in situ* tumour angiogenesis. *Discussion:* Our results confirm the suitability of hydrogels constructed from starPEG-heparin for HUVEC and MSC co-cultivation with cancer cell lines to study cell-cell and cell-matrix interactions in a 3D environment. This represents a step forward in the development of 3D culture models to study the pathomechanisms of breast and prostate cancer.

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ANTIBODY-BASED TARGETING OF TNF-LIGANDS FOR CANCER THERAPY

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The tumor necrosis factor (TNF) ligand and cognate TNF receptor superfamily constitute an important immunoregulatory axis pivotal for the correct execution of immune responses. Members of these families among others are involved in induction of cell death in malignant cells as well as in providing co-stimulatory signals that help mount effective anti-cancer immune responses. This diverse and important regulatory role in immunity has sparked great