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

Lynda Thyer[1], ***Jacopo J.V. Branca***[2]*, and Margit Taubmann[3]

[1]*Macro Innovations Ltd, CB4 0DS Cambridge, UK;*
[2]*Department of Experimental and Clinical Medicine, University of Firenze, 50134 Firenze, Italy;*
[3]*Naturheilzentrum, D-95444 Bayreuth, Germany.*

Corresponding Author: *Jacopo J. V. Branca (jacopo.branca@libero.it)*

INTERNATIONAL INSTITUTE OF ANTICANCER RESEARCH

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.

Here, we also describe for the first time the molecular structures that are responsible for the specific interactions between OA-GcMAF and oncogenes and tumour suppressor genes involved in human carcinogenesis.

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 (Fig. 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% (Fig. 1B).

The patient did not report any side effect: her body weight and lean mass increased during the two weeks of treatment.

Figure 1

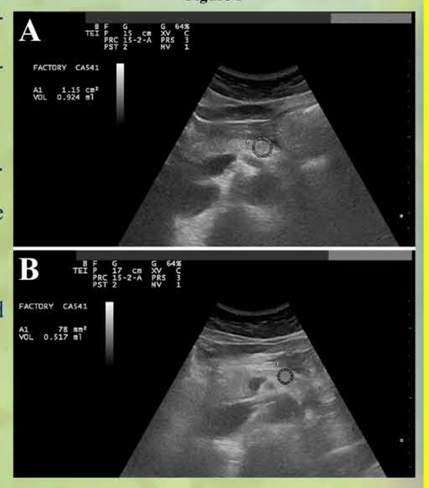
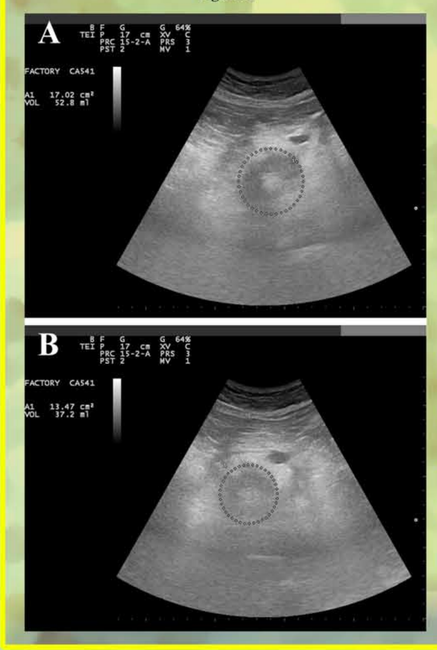


Figure 2



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, 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 (Fig. 2A). After two weeks of treatment, the calculated volume of this lesion was reduced by 30% (Fig. 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.

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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.

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].

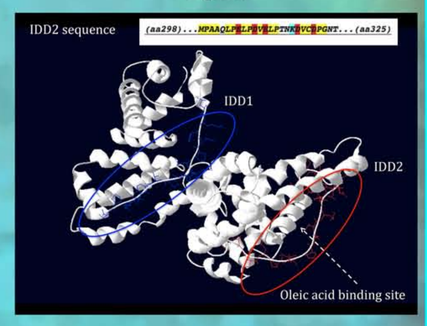
In this study we demonstrate that immunotherapy based on OA bound to GcMAF is effective in patients with localised or metastatic pancreatic adenocarcinomas. These results are consistent with clinical observation accumulated in the past six years demonstrating that immunotherapy based on GcMAF is highly 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.

The extreme therapeutic effectiveness of OA-GcMAF in a variety of human cancers has raised questions regarding its mechanism of action that apparently is far more more diversified and complex than simply stimulating macrophages as originally proposed.

Here we demonstrate for the first time that the plethora of biological activities observed when treating patients with Oa-GcMAF could be ascribed to the presence of intrinsically disordered domains (IDD) in the molecular structure of GcMAF.

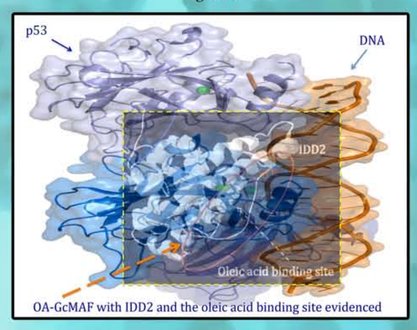
An IDD is a domain that lacks a fixed or ordered three-dimensional structure. IDDs cover a spectrum of states from fully unstructured to partially structured and include random coils, (pre-) molten globules, and large multi-domain connected by flexible linkers.

Figure 3



GcMAF shows two IDDs, one in the first domain (IDD1), and one in the second domain (IDD2), in the proximity of the oleic acid-binding domain (Fig. 3). As shown in Fig. 3, the sequence of the IDD2 shows a peculiar arrangement of hydrophobic aminoacids (in yellow) in the region that binds oleic acid as well as an IDD composed by negatively- (in red) and positively- (in blue) charged aminoacids.

Figure 4



OA-GcMAF with IDD2 and the oleic acid binding site evidenced

It is well assessed that IDDs have the capability to bind to IDDs of other proteins, thus influencing their activities. It is also well known that the major oncogenes and tumour suppressor genes responsible for human cancers, such as p53 and BRAC1, have IDDs that are responsible for mediating many of their interactions.

Therefore, the discovery that oleic acid binds exactly in the region of the IDD2 of GcMAF, explains the selective interaction between OA-GcMAF and the major oncogenes involved in human cancer. Quite obviously, such a specific interaction could not occur in the absence of oleic acid and this finding explains the reason why GcMAF not conjugated with oleic acid shows only a fraction of the biological activity of OA-GcMAF.

The molecular interaction between OA-GcMAF and p53, the most represented tumour suppressor gene in human cancer, is reported in Fig. 4 and elucidates the molecular mechanism underlying the astonishing anti-cancer properties of OA-GcMAF.