# FOCUSSED TRANSCRANIAL ULTRASOUNDS: APPLICATION TO THE DELIVERY OF GLYCOSYLATED OLEIC ACID/VITAMIN D-BINDING PROTEIN TO BRAIN TUMOURS AND METASTASES


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## INTRODUCTION

✓ Glycosylated oleic acid/vitamin D-binding protein, also known as oleic acid/Gc protein-derived Macrophage Activating Factor (OA-GcMAF), has proven effective in the immunotherapy of solid neoplasms and haematological malignancies [1].

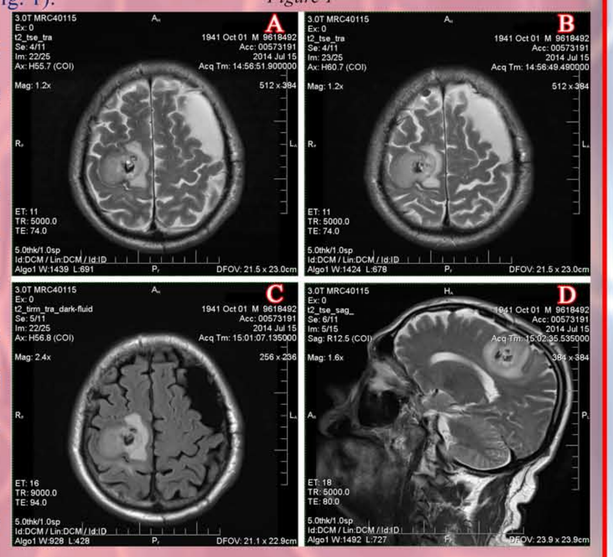
✓ The limited permeability of the blood brain barrier (BBB), however, is an obstacle to its widespread use in primary brain tumours as well as in brain metastases. In order to overcome this limitation, we developed a procedure involving the use of focussed ultrasounds that allow selective permeabilisation of the BBB and targeted delivery of OA-GcMAF.

✓ Here we describe the clinical case of a patient with malignant glioblastoma treated with OA-GcMAF-centred integrative immunotherapy. We also describe for the first time the molecular mechanism of action underlying the powerful biological effects of OA-GcMAF in human cancer.

## PATIENT and METHODS

➲ A 73-year old man was admitted at a major specialised hospital of London, UK, with the diagnosis of malignant (stage 4) glioblastoma in the right emisphere, spread in the temporo-parietal region (Fig. 1).

*Figure 1*




➲ In the weeks preceding admission to this hospital, the patient suffered of progressive paralysis of his left side beginning with the upper limb and rapidly progressing to the entire right emisoma. Before being admitted, the patient had been previously admitted to another hospital in London where he had been labelled as "incurable/inoperable". During his stay at the Oncology Ward of the second hospital, the patient was treated with conventional therapies (radiotherapy with "gamma knife") as well as with the so-called Swiss Protocol® for brain cancer immunotherapy that was performed in the hospital by his private consultant.

➲ Essentially, the Swiss Protocol® for brain cancer is based on the the procedure described in [1] with the addition of ultrasound-guided permeabilisation of the BBB in order to specifically deliver OA-GcMAF to the affected areas. The Swiss Protocol® for brain cancer is designed so that one day before the procedure, OA-GcMAF (Goleic, Immuno Biotech Ltd, Guernsey, Channel Islands) is injected in the proximity of inguinal nodes (440 ng in each side) under ultrasound guidance (Fig. 2).

*Figure 2*

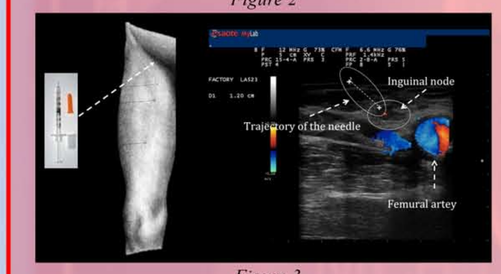
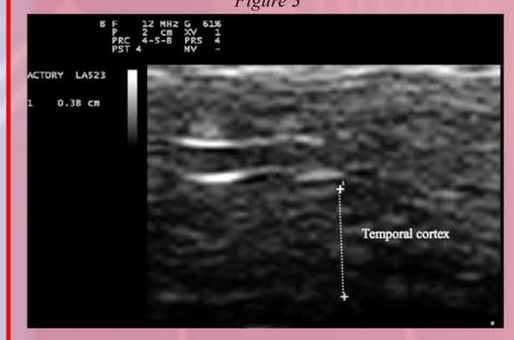



*Figure 3*



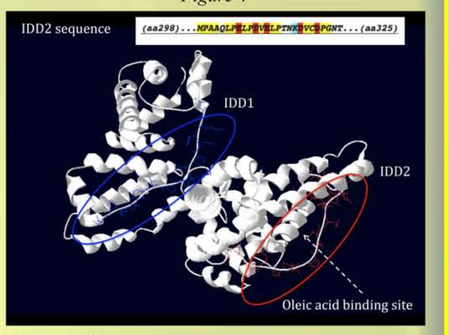

➲ The same day, OA-GcMAF, naturally produced during the fermentation process of a proprietary food product (Les Alpes Ltd, Wellington, NZ) is administered orally as a drink (120 ml). The same functional food, with 5 g of essential amino acids (MAP, Master Aminoacid Pattern, dr. reinwald healthcare gmbh, Schwarzenbruck, Germany) dissolved into it, can be administered as enema (25-50 ml) and/or as suppositories. As part of the Swiss Protocol® for brain cancer, the patient was instructed to follow a strict nutritional regime based on a very low carbohydrate/very high protein content aimed at preventing the onset of the cancer cachexia anorexia syndrome and a lowering the prognostic inflammatory nutritional index (PINI) [2]. On the following day, OA-GcMAF (880 ng dissolved in 5 ml saline) is administered by nebulisation. Measurement of blood pressure variations, and assessment of splenic blood flow after nebulisation are used to determine the individual response [1]. 5 min after nebulisation, transcranial ultrasounds are applied 5 min after nebulisation, transcranial ultrasounds are applied through the temporal acoustic window on the same side where the lesion is located. With proper setting, the cerebral cortex can be visualised (Fig. 3), thus indicating that the ultrasound waves reach the gray matter [3].

➲ Ultrasound could then be directed toward the anatomical regions where the lesion is located. Most regions of the brain can be accessed though the temporal acoustic window, with the exception of distal areas of the frontal and occipital lobes.

## RESULTS

► Focussed ultrasounds are known to transiently increase the permeability of the BBB [4] and, therefore, to allow passage of OA-GcMAF that is a lipophilic molecule thanks to the presence of oleic acid bound to a specific region of the molecule which is characterised by the presence of an Intrinsically Disordered Domain (IDD2) interspersed in an hydrophobic region (Fig. 4).

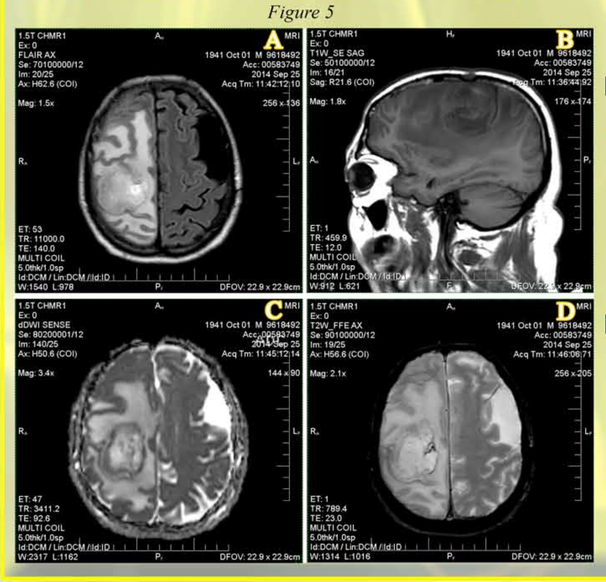
*Figure 4*




► The procedure lasts 5-15 min, causes no discomfort or side effect, and can be repeated continuously or in cycles. The procedure can be safely performed before, during or after other anti-neoplastic treatments as in this clinical case.

► After two weeks of treatment, the patient showed significant signs of clinical improvement with partial resolution of the paralysis in the right emisoma and, with the help of conventional FKT, he was able to regain his mobility.

*Figure 5*




► The MRI scan, performed 8 weeks after implementing the Swiss Protocol® for brain cancer showed a significant change in the structure of the lesion that was interpreted as a blockade of the rapid and aggressive growth as well as the appearance of a capsule around the lesion that was interpreted as fibrotic reaction (Fig. 5).

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

## DISCUSSION

✸ 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.

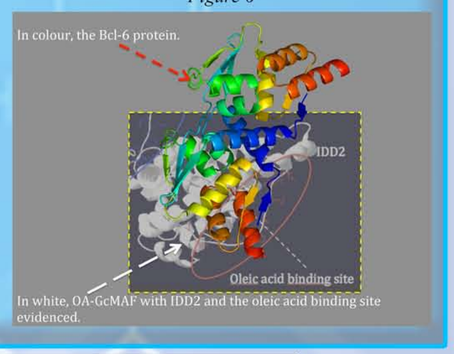
✸ GcMAF shows two IDDs, one in the first domain (IDD1), and one in the second domain (IDD2), in the proximity of the oleic acid-binding domain (Fig. 4). As shown in Fig. 4, the sequence of the IDD2 shows a peculiar arrangement of hydrophobic aminoacids (in yellow) in the region that binds oleic acid as well as an IDD composed by negatively- (in red) and positively- (in blue) charged aminoacids.

✸ 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.

✸ As far as this particular clinical case is concerned, it has been recently demonstrated that the oncogene Bcl-6 is involved in the pathogenesis of glioblastoma [5]. Translocation of the proto-oncogene Bcl-6 in human glioblastoma multiforme). Fig. 6, shows the molecular interaction occurring between the IDD2 of OA-GcMAF and the recognition helices of the Bcl-6 protein. This interaction could be responsible for the observed clinical effects.

*Figure 6*





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