# TREATMENT STRATEGIES
# ONCOLOGY

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Poster Synopsis

# Effects of Vitamin D Binding Protein-derived Macrophage Activating Factor (GcMAF) on Human Neuroblastoma Cells and Predicted Molecular Interaction with the Vitamin D Receptor

M. Ruggiero,[1] M.G. Fiore,[1] S. Magherini,[2] G. Morucci,[2] J.J.V. Branca,[2] M. Gulisano,[2] L. Thyer,[3] R. Smith,[3] E. Ward[3] and S. Pacini[2]

1. Department of Experimental and Clinical Biomedical Sciences, University of Firenze, Italy; 2. Department of Experimental and Clinical Medicine, University of Firenze, Italy; 3. Macro Innovations Ltd, Cambridge, U.K.

## Introduction

As of today, there are 57,821 published papers on the immunotherapy of cancer, with an exponential growth in the number of publications. According to a recent study, "immunotherapies require activation of macrophages to be effective"[1]. Since 1994, it has been demonstrated that macrophage activation requires vitamin D binding protein-derived Macrophage Activating Factor (GcMAF)[2]. Therefore, GcMAF has become a stronghold in the immunotherapy of cancer, and there are scores of studies on this subject including a very recent peer-reviewed report describing successful immunotherapy of patients with advanced cancer treated with GcMAF[3]. In this study we present data concerning the effect of GcMAF on human neuroblastoma cells.

## Materials and Methods

Highly active purified GcMAF was obtained from Immuno Biotech Ltd, Guernsey, Channel Isles. GcMAF was purified according to the procedure described in Autism Insights[4]. Biological analysis demonstrated that this GcMAF had the highest activity in comparison with other preparations obtained from major researchers [5]. Human neuroblastoma cells SH-SY5Y were obtained from the Istituto Zooprofilattico Sperimentale, Brescia, Italy. SH-SY5Y cells originally derived from a metastatic bone tumour biopsy[6], and represent a model system to study the effects of anti-cancer therapies aimed at neuroblastoma[7]. However, since they are able to differentiate, they also represent a model system to study the neurobiology of neurodegenerative diseases[8]. Experiments, designed to study inhibition of cell proliferation, were conducted in the presence of 1% serum, whereas experiments designed to study differentiation were conducted in serum-free medium.

## Results

GcMAF treatment of SH-SY5Y cells resulted in different effects depending on the proliferative activity of the cells. In actively proliferating cells, GcMAF inhibited cell proliferation in a dose-dependent manner and induced their apoptosis. In serum-starved, quiescent cells, GcMAF induced morphological changes indicating differentiation (Figure 1). The effects of GcMAF were mediated by cAMP production, possibly through cross-talk with the vitamin D receptor (VDR).

## Discussion

Our results demonstrate that GcMAF inhibits actively proliferating human neuroblastoma cells, whereas it induces the differentiation of serum-starved (quiescent) human neuroblastoma cells. The concentration of GcMAF necessary to inhibit proliferation of actively proliferating cells was 10 fold higher than that required to induce differentiation of quiescent cells. The effect of GcMAF on actively proliferating human neuroblastoma cells was qualitatively superimposable to that observed when treating the same cell type with vitamin D3. In fact, GcMAF is a member of the so-called vitamin D axis since it derives from de-glycosylation of Vitamin D-Binding Protein or Gc protein. Consistent with this notion, we had previously demonstrated that polymorphisms of the VDR gene, known to be associated with the highest responses to VDR agonists, were associated also with the highest responses to GcMAF[9]. The interaction between GcMAF and VDR helps explaining the multiplicity of biological effects observed with GcMAF as well as the variety of clinical applications ranging from cancer to autism. Thus, VDR is expressed in a great number of cell types (including SH-SY5Ycells), and regulates a wide array of genes involved in the control of the major cell functions.

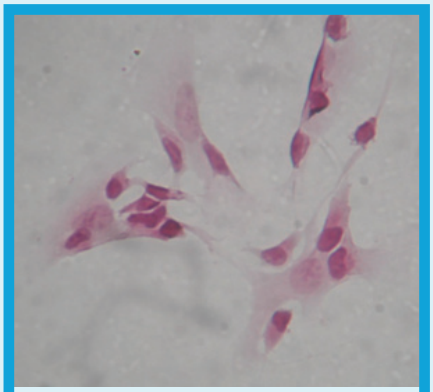

Figure 1. After 24 h stimulation with 8 pM GcMAF, serum-starved, quiescent cells showed a significant change in morphology that was consistent with the induction of differentiation. The cytoplasm was enlarged and several cytoplasmic elongations could be observed. The effect was dose- and time-dependent.

Marco Ruggiero holds a PhD in molecular biology, is a certified medical doctor specialised in clinical radiology, and is full professor of molecular biology at the Department of Experimental and Clinical Biomedical Sciences of the University of Firenze, Italy. He worked at Burroughs Wellcome Co. North Carolina, USA, where he published a seminal paper with Nobel Laureate Sir John Vane and, subsequently, at the National Cancer Institute of the NIH in Bethesda, working with Dr. Stuart A. Aaronson and Dr. Peter Duesberg. Since 1992 he has been Chair of Molecular Biology at the University of Firenze. He has published more than 150 peer-reviewed scientific papers on signal transduction in a variety of experimental and spontaneous pathologic system related with cancer, chronic kidney disease, chronic fatigue syndrome and neurological conditions. In the past 20 years he has worked on the vitamin D axis, a metabolic pathway that includes GcMAF. In the past 3 years he has published studies on the immunotherapeutic effects of GcMAF in cancer, chronic fatigue syndrome and neurological conditions.

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