**THE IMMUNE STIMULANT PROPERTIES OF VITAMIN D BINDING PROTEIN - DERIVED MACROPHAGE ACTIVATING FACTOR CAN MINIMISE MORBIDITY IN GYNAECOLOGICAL CANCERS**

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**Introduction 1**

- Immunodeficiency and consequent increase in morbidity and common occurrence in advanced cancer patients (Comp Biochem Physiol A Mol Integr Physiol. 2002;132:1-8).
- One of the events responsible for immunodeficiency and increased morbidity in cancer patients is excess production of alpha-N-acetylglucosaminidase (nagalase) an enzyme that de-glycosylates (inactivates) vitamin D binding protein, which is the precursor of vitamin D binding protein-derived macrophage activating factor (GMAF).

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**Introduction 2**

- The increase in nagalase activity in cancer patients is due to the fact that cancer cells release nagalase (Cancer Lett. 2009; 283:222-9).
- Thus, nagalase activity reflects tumour burden, aggressiveness and progression of the disease (Cancer Lett. 2000;158:61-4) up to the point that determination of nagalase activity is a non-invasive way of evaluation of cancer severity (Cancer Lett. 2009;283:222-9).

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**Introduction 3**

- In these studies, the anti-cancer effects of GMAF were evaluated by measuring serum nagalase activity, a marker of tumour burden and progression as well as of immunodeficiency.

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**Introduction 4**

- Here we demonstrate that GMAF stimulates human monocytes/macrophages.
- Stimulation of human monocytes/macrophages restores a competent immune system.
- We elucidate the molecular mechanism of action responsible for GMAF signal transduction.
- We propose an integrated strategy to obtain the best effects from immuno-therapy with GMAF in order to minimize morbidity in cancer patients.

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**Materials and Methods**

- Purified, activity-assessed GMAF was from Immuno Biotech Ltd.
- Monocytes were collected from healthy volunteers. Separation procedure to obtain PMAEC was performed by Polymorphspop (Anti-Phel).  
- Assessment of cell viability was determined by Calceinomine Rapid Cell Proliferation Kit.
- cAMP levels were measured by a competitive EIA assay Cyclic AMP EIA kit, Cayman Chemical.
- Vitamin D receptor (VDR) restriction site polymorphism was evaluated on genomic DNA extracted from peripheral blood cells using a QIAamp DNA Blood Mini Kit.
- Absence or presence of the fixed restriction site was denominated "T" and "C", respectively.

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**Results 2**

- When we challenged monocytes from subjects harboring different VDR polymorphisms, we observed that the "A" allele of the VDR gene and the "A" genotype were associated with higher stimulation of proliferation by GMAF.
- Subjects with "A" genotype showed an intermediate response to GMAF.
- These data suggest that there is an association between the presence of "A" alleles and the degree of the response to GMAF.

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**Results 3**

- These results demonstrate that GMAF counteracts immunodeficiency by stimulating macrophage signaling and proliferation and these events are mediated through VDR.

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**Discussion 2**

- On the basis of the results presented here, it can be hypothesized that the patient’s genotype, as far as VDR gene polymorphisms are concerned, influences the individual response to GMAF.
- Therefore, the use of GMAF to restore a competent immune system and minimize morbidity in gynaecological cancers has to be calibrated on the individual patient’s genotype adopting an integrated approach.

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**Discussion 3**

- In fact, in order to minimise morbidity, it should be remembered that the prognosis for all types of cancers is dependent upon the nutritional and inflammatory status of the patient that can be monitored by the Prognostic Inflammatory and Nutritional Index (PINI) (Am J. Immunol. 2012: 8: 65-70).

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**Discussion 4**

- Proposed mode of interaction between GMAF and VDR in the plasma membrane. Vitamin D and oleic acid stabilize the complex by binding to the hydrophobic domains of the two vitamin D binding proteins.

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**Discussion 5**

- The observation that vitamin D and oleic acid participate in the GMAF/VDR interaction leads to propose an integrated individual approach, designed to exploit the characteristics of GMAF and reduce morbidity in cancer patients.

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**Discussion 6**

1. Determination of VDR genotype to assess the most appropriate individual dose for GMAF.
2. Determination of PINI score.
3. Design of individual nutritional plan to provide adequate amount of vitamin D and oleic acid.
4. Design of individual nutritional plan to lower PINI score.

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**Discussion 7**

- The observation reported here open the way to design GMAF/nutrition-based individual integrated approaches that will lead to:
  1. Minimise morbidity in cancer patients.
  2. Decrease the probability of cancer anorexia cachexia syndrome (CACS).
  3. Significantly increase survival.