

## **Regulatory T Cells in Cancer Treatment – The Role of Low Dose Cyclophosphamide in Sonodynamic Photodynamic Therapy and Immunotherapy**

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In good health, our immune system is in a constant state of surveillance, balanced between clearance of damaged cells and pathogenic organisms, and tolerance to our own healthy tissue, dietary intake and environment.

It has long been recognised that in certain cancer cases the body spontaneously generates an immune response capable of clearing even advanced and widespread cancers. At present, some of the most exciting approaches to cancer treatment such as immunotherapy with dendritic cell vaccination aim to harness this powerful immune potential by increasing recognition of tumour cells by our immune system.

Likewise, treatment such as Sonodynamic Photodynamic Therapy (SPDT) has been shown to generate a beneficial “vaccine” response due to cell breakdown (necrosis) in the tumour site. This releases tumour-associated antigens with an inflammatory ‘danger’ signal to the immune system that attracts immune cells. This is capable of producing body-wide cancer-specific immunity (1, 2). So, how can this immune response be optimised?

Firstly, effective cell-mediated immune function appears of prime importance in cancer prevention and management. Alongside the right diet, this is best achieved with certain 1-3 1-6 Beta Glucans refined from the cell wall of *saccharomyces cerevisiae* (Baker’s yeast). These prime CR3 receptors on immune cells making them respond faster and more effectively to immune challenges (increases chemotaxis and phagocytosis). Many studies have shown significant benefits in combining this well-tolerated food-based supplement with immune approaches to cancer treatment (3, 4). However, the quality varies greatly amongst the category of immune-priming 1-3 1-6 Beta Glucans. **Immiflex** contains by far the most researched and refined extract, with published safety studies and proven effectiveness (6, 7, 8, 9, 10, 11)

One question that must now be considered during cancer treatment asks, “what prevents the immune system recognizing the cancer in the first place?” Several factors have been studied. One very important factor appears to be **Regulatory T cells** (sometimes known as TReg’s or suppressor T cells) which are a specialized subpopulation of T cells that suppress activation of the immune system. These cells normally act to maintain immune system homeostasis and tolerance to self-antigens. (5)

In other words, it is essential that our immune system knows when to “switch off” after an immune challenge such as infection has been effectively cleared, and this enables

us to tolerate the tissues of our own body. The immune regulatory function of these cells has been related to their ability to secrete immune-suppressive messenger proteins (cytokines) such as IL10 and TGFβ (12)

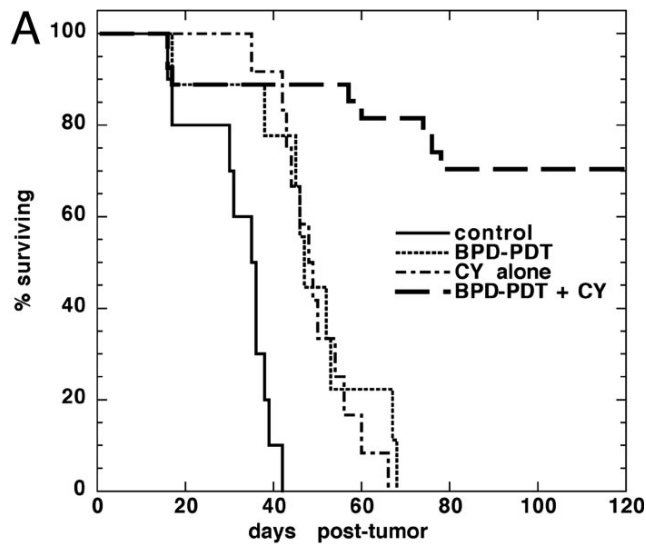
However in people with cancer, it has been observed that numbers of TReg cells are significantly increased in blood, bone marrow and at tumour sites (13). It is becoming clear that cancer growth, development and resistance to treatment may be aided by this element of our own immune systems (14,15). In this situation the TReg's act to prevent our killer immune cells recognising the cancer as abnormal and potentially dangerous. Thus, if regulatory T cells become dominant around the tumour they form an immune barrier or shield that can reduce the effectiveness of our body's ability to mount an effective anti-tumour response.

Research indicates however that there are well-tolerated approaches that can reduce the dominant effect of TReg's and bring their population numbers back to the normal reference range, increasing immune recognition of the tumour and making immunotherapy treatments more effective. (16.Ghiringhelli F, *et al.* (2004) CD4, CD25 regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *EurJ Immunol* 34:336–344.)

One such approach uses a very low dose of a chemotherapy medication called cyclophosphamide. At low doses of cyclophosphamide a specific effect of lowering the number of TRegs is noted without the general immunosuppression caused by higher doses. (17)

This treatment is very well tolerated, and at the extremely low dose prescribed is not associated with the side-effects usually seen with chemotherapy. The reduction in TReg cell numbers is measured using an approach called flow cytometry. This measures the amount of cells in the blood with specific surface markers that are found on TReg cell populations. These include CD4, CD25, CD39 and CD127.

TReg's and the environment around the tumour appear to be highly relevant in cancer management, including sonodynamic photodynamic therapy (18). Previous studies, as shown below, have demonstrated the potential benefit of low dose cyclophosphamide with photodynamic therapy and immunotherapy.



Reference:(19) Photodynamic therapy plus low-dose cyclophosphamide generates antitumor immunity in a mouse model. Ana P. Castano et al. Proceedings of the National Academy of Science.2008.105(14). p5495–5500.

Diagram A. This graph highlights the effect of combining low dose cyclophosphamide (CY) with photodynamic therapy (BPD-PDT). Either treatment alone generated a benefit in survival from the baseline-control, but when applied together the survival of animals injected with malignant cells significantly improved. Interestingly this effect was not seen with high doses of cyclophosphamide such as the doses used conventionally, which may be a result of immune suppression of functional cytotoxic T cells required for the effective immune response. This highlights the importance of the low dose regimen.

Blood tests looking at TReg populations are taken prior to commencing treatment and the effect of low dose cyclophosphamide is usually re-tested after approximately four weeks.

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