Bryostatin 1, A New Anti-tumor Agent: From Bench to Bedside

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Abstract

Bryostatin 1 represents a novel natural agent that appears to exert pleiotropic biological effects via protein kinase C (PKC). Bryostatin 1 has been found to have anti-neoplastic and immunomodulatory activity in a variety of *in vitro* and *in vivo* systems. Although Bryostatin 1 exhibits its dose dependent inhibitory response on solid tumor lines as well, it has more potential against human leukemias and lymphomas. Extensive studies over the past several years have recently led Bryostatin 1 to enter phase 1 clinical trails for patients with lymphoma and leukemia tumors in our institute.

Keywords: **Bryostatin 1, B-cell tumors, differentiation, apoptosis, bcl2, c-myc, p53.**

Among the new anti-tumor agents of the modern era, Bryostatins represent the most promising therapeutic compounds isolated from marine animals.[1] The most extensively studied of these compounds is Bryostatin 1, a macrocyclic lactone derived from an Eastern Pacific colonial marine filter feeder, *Bugula neritina* of phylum Bryozoa, often mistaken for coral, seaweed and hydroids (Figure 1).

The difficulties in isolation are indicated by the extremely low natural abundances of these compounds, ranging from less than one parts/billion to 600 parts/billion of the wet weight of the animals, and by the fact the tedious purification procedures had to be monitored by lengthy bioassay of the anti-leukemic activity in animals. Figure 2 shows the chemical structure of Bryostatin 1.

In 1982, G. R. Pettit et al. isolated and characterized Bryostatin 1[2] based on its *in vivo* anti-neoplastic activity demonstrated in the National Cancer Institute P388 lymphocytic leukemia screening system. Bryostatin 1 has been shown to have pleiotropic cellular effects associated with activation of PKC. It also acts as a biologic response modifier by stimulating the production of cytokine, by stimulating bone marrow progenitor cells and by activating the neutrophils. Bryostatin 1 displays anti-tumor activity in solid tumors as well as hematopoietic malignancies.

Our focus, in this article, will be on human B-cell tumors. B-cell tumors in man, represent a spectrum of heterogenous diseases, extending from the tumors of immature "stem cell" to the most mature "plasma cell" of B-cell lineage. These disorders vary in natural histories, presentations and responsiveness to therapy. It has long been hypothesized that disturbance in the differentiation pathway plays a vital role

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in pathophysiology of malignancies.[3] Each tumor therefore may represent a monoclonal population of cells arrested at a certain state of maturation. Unlike granulocystic series, morphology is not a reliable measure of differentiation state in lymphoid lineage. Various lineage-specific and stage-restricted antibodies developed over the last three decades are used to detect the surface markers and the state of differentiation as shown in Figure 3. Classic examples of B-cell tumors included chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma (NHL). CLL is the most common adult type of leukemia in the western world and remains incurable [4] while the incidence of NHL is increasing by 3% - 4% annually.[5] More than 40,000 new cases of NHL are diagnosed every year in the United States[6] and despite great advances in the treatment, more than half of the patients still die of their disease.[7,8] New and novel approaches are therefore being tried to improve the outcome. Bryostatin 1 is one of the those unique and promising agents.

Anti-tumor activity

Bryostatin 1 inhibits significant anti-tumor activity against leukemias and lymphomas. It inhibits clonogenic growth of K562 cells (a myeloid leukemia cell line), Reh cells (a pre-B-lymphoblastic cell line) and fresh ANLL cells (acute nonlymphocytic leukemia cell line).[9] Bryostatin 1 also demonstrates growth inhibition of hematopoietic progenitor cells from patients with myelodysplastic syndrome (MDS).[9] In 1993, our group studied the effects of Bryostatin 1 on human non-Hodgkin's B-lymphoma tumor lines *in vitro* and demonstrated that it had differentiation effects on low-; intermediate- and some high-grade lymphomas.[10]

The *in vitro* experiments were translated into *in vivo* animal model studies. Prolonged survival has been demonstrated in animals bearing the M5076 reticulum sarcoma, B16 lung metastases and L10A B-cell lymphoma tumors after treatment with Bryostatin 1. In one of our studies, severe combined immunodeficient (SCID) mice with human Waldenstrom's macroglobulinemia xenograft were successfully treated with a combination of Bryostatin 1 given 24 hours prior to vincristine (VCR) or melphalan (Melph).[11] Bryostatin 1 given before VCR or Melph resulted in the highest tumor growth inhibition, tumor growth delay and tumor cell kill. Forty percent receiving Bryostatin 1/VCR combination were free of tumors > 200 days after treatment and were considered cured. In the light of our findings, we recommend that Bryostatin 1 be considered for clinical investigation in human B-cell tumors and might best given combined with other chemotherapy agents used in the treatment of that disease

Differentiation

In addition to its anti-proliferative effects, Bryostatin 1 induced differentiation on various stages of B-cell lineage. It brings up macrophage-like differentiation of human peripheral chronic myelogenous leukemia (CML) cells[12] and

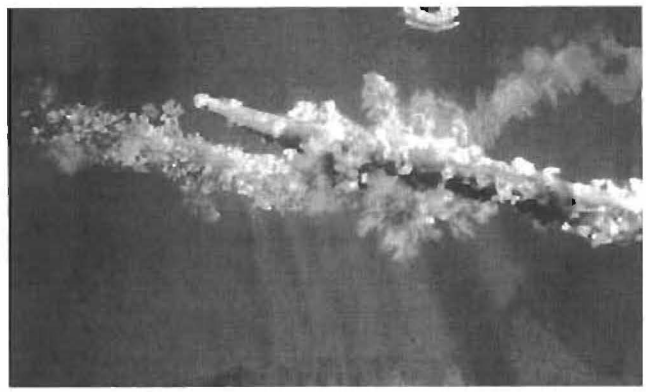

Figure 1. Bugula neritina growing on a seaweed plant.

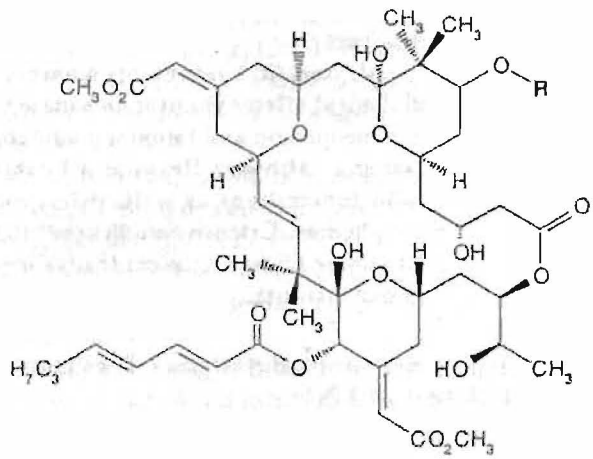

Figure 2. Chemical structure of Bryostatin 1.

triggers activation and terminal differentiation of B-chronic lymphocytic leukemia (CLL) cells as assessed by morphological changes, increased RNA synthesis and immunoglobin production.[13] Differentiation and growth modulation are also observed with HL-60 human myeloid clones.[14] Varying responses are seen with Bryostatin 1 on different human B-cell tumor lines as shown in Figure 4. Most responsive to Bryostatin 1 are those that are IgM^+/IgG^- and least responsive re IgG^+/IgM^- or weakly IgM^+, suggesting that Bryostatin 1 is more effective in earlier stages of differentiation.[15]

Earlier, a high grade lymphoma tumor (MANCA) line was shown to convert to intermediate grade after treatment with Bryostatin 1, using polypeptide analysis on 2D gel electrophoresis.[16] In later experiments flow cytometry and cell markers and other enzymatic studies like acid phophatase (AP, Tartrate Resistant Acid Phophatase (TRAP, were used to demonstrate the differential effects of Bryostatin 1 on human non-Hodgkins's lymphoma cells and lines.[10] In a separate study Bryostatin 1 was shown to induce Hairy cell

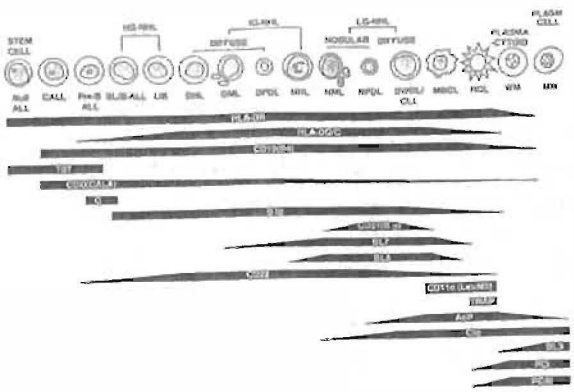

Figure 3. A hypothetical schema of B-cell differentiation.

features on chromic lymphocytic leukemia cells *in vitro*.[17] Another report demonstrated significant dose dependent growth inhibition, and further differentiation of acute lymphoblastic leukemia cell line (Reh), by phenotypic studies, flow cytometry and surface markers.[18]

Mechanism

Protein Kinase C (PKC)

Although the exact mechanism responsible for anti-tumor activity of Bryostatin 1 is presently unclear, the wide variety of its biological effects appear to be mediated through activation of Protein Kinase C (PKC).[19] Bryostatin 1 constitutes perhaps the most unusual and least understood class of PKC modulators. Because of its striking properties, such as extremely high potency and its unique effects of PKC physiology. The PKC pathway is of great interest in cancer research because PKC is the likely receptor for phorbol esters, which are tumor promoters.[20] Bryostatin 1, like phorbol esters, induces translocation of PKC from cytosol to the cell membrane, but has no tumor-promoting activity and when present with phorbol esters it blocks their tumor-promoting capabilities as well.[21] At lower concentrations, Bryostatin 1 induced phophorylation and down regulation of transferrin receptors,[22] down regulation of c-myc expression and induction of c-fos, c-fms and TNF transcripts in HL-60 cells,[13] activation of neutrophil oxidative bursts and degranulation[23] platelet aggregation and dense granular release.[24,25] At higher concentrations, it inhibits epidermal growth factor (EGF) binding and arachidonic acid release from murine fibroblastic cell line C-3H 10T 1/2[26] and strongly antagonizes other action of phorbol esters.[27]

Immunomodulation

At lower concentrations, Bryostatin 1 appears to have immuno-enhancing properties towards T-lymphocytes. It activates their IL-2R expression and exhibits IL-2-induced development of cytoxotic T-cells when combined with calcium ionophores,[28,29] as well as increasing their cytoxicity against target cells lacking antigenic determinants. At higher concentration, Bryostatin 1 partially inhibits both antigen-specific cytoxicity[19] and antibody-dependent cell-mediated cytotoxicity (ADCC).[30]

Despite these inhibitory effects, Bryostatin 1 can promote immuno-rejection of tumors *in vivo*. Tuttle et al.[31] showed that T lymphocytes from draining lymph nodes of MCA-105 tumor-bearing mice can be activated and expanded 130-fold *in vitro* with Bryostatin 1, a calcium ionophore and low dose IL-2. When these cells are adoptively transferred to mice bearing-MCA-105 lung metastases, they induced complete regression of lung nodules. *In vitro* and *in vivo* depletion studies and phenotype analysis suggest that CD-8+ T-lymphocysts are responsible for tumor regression, but the exact mechanism is unclear.

Gene expression and apoptosis

Expression of the multi-drug resistance (mdr-1) gene is a common mechanism by which cancer cells evade the cytotoxicity of chemotherapeutic agents. Early data supports that Bryostatin 1 down regulates the expression of mdr-1.[32] A diffuse large cell lymphoma xenograft in SCID mice was analyzed for quantitation of mdr RNA by competitive PCR before and after treatment with Bryostatin 1, and a decline was noted.[33] Those findings may lead to the fact that Bryostatin 1 can make tumor cells more vulnerable to the standard chemotherapeutic agents and inhibit the resistance against them.

Apoptosis has been recognized as a fundamental tissue homeostatic mechanism for a wide range of physiological and pathological conditions including cancer. In one of our recent studies, results show that both Bryostatin 1 and Vincristine induced apoptosis in diffuse large cell lymphoma. Immunocytochemistry revealed that relative bcl-2 oncoprotein expression was decreased in cells treated with Bryostatin 1, or Vincristine separately and was abolished by combining both drugs. However, upon treatment with the above drugs, the expression of p53 was moderate on Bryostatin 1- or Vincristine-treated cells and strong on cells treated with the Bryostatin 1/Vincristine combination. Various other changes in genetic expression like down-regulation of c-myc are also noted.

Clinical application

The initial Phase 1 trial of 60% ethanol: 40% saline (0.9%) formulation of Bryostatin 1 has been performed in the United Kingdom.[34] Bryostatin was given as a one-hour intravenous infusion at the beginning of each two-week cycle. A maximum of three treatment cycles were given. Doses were escalated from 5 to 65 $\mu g/m^2$ in successive patient groups. The maximum tolerated dose was 50 $\mu g/m^2$. WHO grade 3 Myalgia was the dose limiting toxicity in all three patients treated at 65 $\mu g/m^2$. Headaches, WHO grade 3 anemia, thrombocytopenia, and leukopenia were seen at the highest dose levels. Other side effects are tenderness/cellulitis/phlebitis at infusion site, flu-like symptoms, rhinitis, fever, nausea, lethargy and dysphagia. Cellulitis

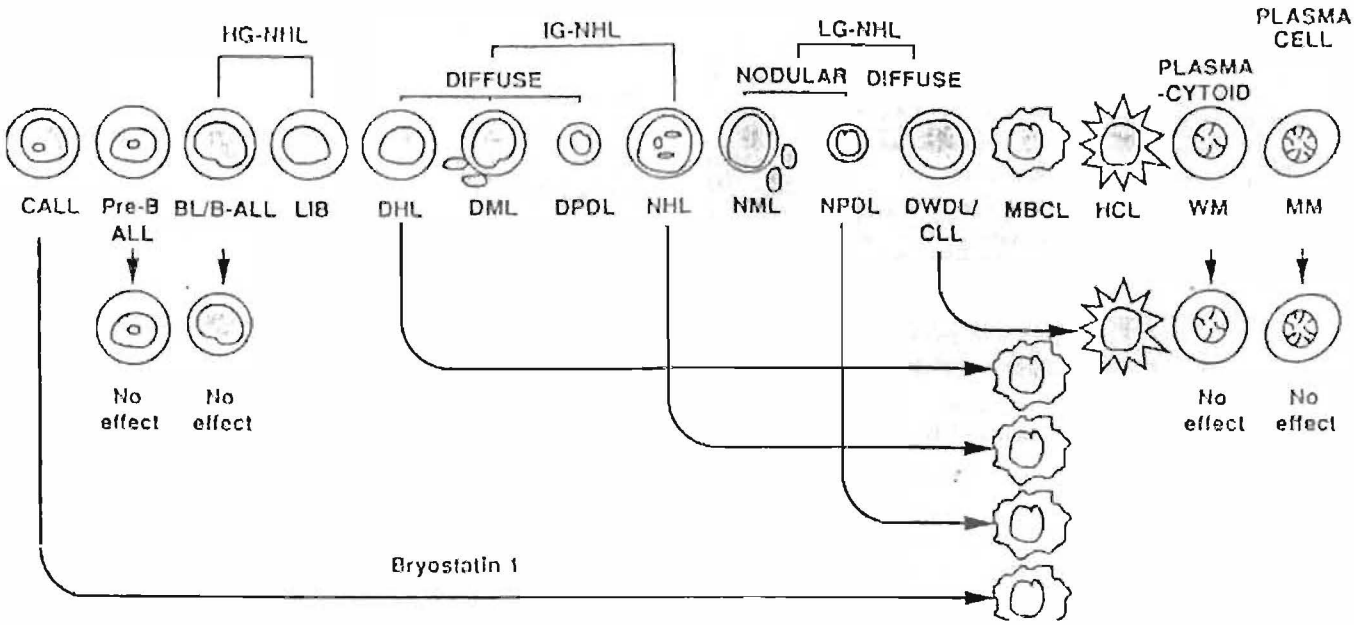

Figure 4. A schematic overview of the differentiating activity of Bryostatin 1 on human B-cell tumors in vitro. Monitoring of differentiation was determined according to the marker's expression.

and Phlebitis at the site of injection occurred as a result of the 60% ethanol diluent.

Ample preclinical data from extensive bench research provided us the privilege to start Phase 1 clinical trials at our institute for patients in relapsed lymphoma and chronic lymphocytic leukemia (CLL). A significant number of patients are currently receiving Bryostatin 1 in serially increased doses with close surveillance of their disease.

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