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Multiple uses of tumor necrosis therapy (TNT) for the treatment and imaging of solid tumors: Preclinical considerations and progress

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ABSTRACT

In an attempt to identify reagents that can elicit an effective anti-tumor immune response, we produced a panel of fusion proteins consisting of the tumor necrosis therapy (TNT) antibody and potent cytokines/chemokines. At the center of this approach is the TNT antibody, which has several important characteristics that make it an ideal delivery agent for immune modulators. These include its applicability to a wide range of human and animal cancers, its inability to bind normal tissues, its long retention time in tumors, and its ability to target necrotic regions in primary and metastatic lesions. Because of these attributes, TNT has been used to deliver radionuclides, immunostimulatory molecules, and vasopermeability agents to treat tumors. Moreover, TNT can be used with imaging to provide critical information concerning the effects of cytoreductive therapy early in the course of treatment and to demonstrate the ability of vasoactive antibody reagents to improve the uptake of drugs used for the treatment of cancer. To date over 250 patients have been treated with ^{131}I-chTNT-3 demonstrating that all types of tumors can be targeted without significant uptake in normal tissues. TNT fusion proteins therefore have unique promise as second generation agents for the immunotherapy of solid tumors.

1. Overview of TNT approach to cancer targeting

A novel approach to cancer imaging and therapy utilizing necrotic cells as a target for the selective binding of monoclonal antibodies has been developed [1]. Designated tumor necrosis treatment (TNT), this approach represents a radical departure from current methods that employ monoclonal antibodies to bind to tumor-associated antigens. TNT is based on the hypothesis that monoclonal antibodies which target abundant and universal intracellular antigens retained by dying cells will preferentialy localize to necrotic regions of tumors. Since necrosis is a specific feature of malignancy and not found in normal tissues, TNT provides a targeting method to image and treat solid tumors in man.

It has long been recognized that rapidly growing tumors contain a proportion of degenerating or dead cells in addition to numerous proliferating cells but, with attention focused on attempts to kill the dividing cell population, the degenerating component has largely been ignored (Table 1). Calculations of tumor cell loss have revealed that, in contrast to normal tissues, 50% to 90% of the progeny of tumor cell divisions shortly undergo degeneration [2]. In tumors, the imperfect vasculature and impaired phagocytic response permit the accumulation of degenerating cells, often with the formation of large areas of necrosis. Thus, the accumulation within tumors of a high proportion of dying cells constitutes a major distinc-

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Table 1 – Major characteristics of tumor necrosis therapy

1. Recognizes abundant intranuclear antigens present in all cancers
2. Targets dead and dying cells (microregional targeting)
3. Long retention time in tumor
4. Capable of targeting solid tumors in experimental animals as well as man
5. Targets tumor at sites rich in tumor antigens and danger signals recognizable by an activated host immune system
6. Binds better after cytoreductive therapies which act to expand the necrotic fraction of the tumor

tion between malignant tumors and normal tissues where sporadic cell death occurs at a relatively low rate and is accompanied by a rapid and orderly removal of necrotic elements from the tissue [3].

Since degenerating cells have a permeable cell surface membrane not observed in viable cells, TNT monoclonal antibodies would enter and bind to their intracellular antigens in necrotic areas of the tumor. As shown in Figs. 1 and 2, 48 h after administration, TNT monoclonal antibodies accumulate in necrotic regions of tumors but are unable to bind to viable cells of the tumor and normal tissues. The first monoclonal antibody developed to test this approach, TNT-1, is directed against DNA/histone H1 complex and targets the cell nucleus of degenerating tumor cells [4]. Initial imaging and biodistribution experiments in tumor-bearing nude mice have shown that after radiolabeling, TNT-1 was able to target several different types of transplantable human tumors and did not accumulate in the normal tissues of the mouse [1]. Gross and microscopic autoradiographic studies demonstrated positive uptake over necrotic regions of the tumors in areas traditionally considered inaccessible to monoclonal antibodies [5]. Closer examination using microautoradiography revealed uptake of label over the nuclei of early degenerating cells adjacent to viable zones at the tumor periphery and over frankly necrotic areas more centrally located. After linkage to therapeutic radionuclides such as ^{131}I, radiolabeled TNT-1 was found to treat successfully 1 g size tumors produced by the ME-180 human cervical carcinoma after heterotransplantation in the nude mouse using doses non-ablative to the bone marrow (<1 mCi) [6].

1.1. *TNT antibodies*

The initial TNT monoclonal antibody, designated TNT-1, was directed against nucleosomal determinants consisting of histone H1 and DNA [1,5,6]. As shown in Fig. 2, this monoclonal antibody was shown by autoradiography to localize in the necrotic regions of solid tumors.[11] Since absolute tumor accretion of MAb is a critical indicator of antitumor efficacy, biodistribution studies were undertaken to measure the absolute count of radiolabeled antibody uptake in experimental tumors over time [1,6]. These studies showed high uptake in tumors compared to normal tissues and organs and a long retention time of radiolabeled TNT in tumors up to 10 days [6]. Because of these promising preclinical data, ^{131}I-labeled TNT-1 was used in an imaging study in which seven patients with different tumor types were treated with 2–5 mCi of radiolabeled antibody [7]. Five of seven patients demonstrated positive uptake in primary and metastatic sites and demonstrated no uptake in normal tissues of the patients. These preliminary clinical results suggested that TNT is a promising new approach for the radioimmunodetection and therapy of human cancers and that the data obtained in animal models appear valid in man.

Murine TNT-2 was also generated by immunizing mice with nuclear extracts from human lymphoma cells. Our findings from indirect immunofluorescence, immunoblot analysis, and competition radioimmunoassays demonstrated that TNT-2 binds heterochromatic DNA [4]. In addition, murine TNT-3 was developed and was shown to bind to a variety of human tumors in vivo. ELISA assays using numerous subcellular components have shown that TNT-3 binds single-stranded DNA and RNA preferentially [1]. Biodistribution studies showed that TNT-3 had a three-fold higher uptake in tumors than TNT-1 and TNT-2 [8].

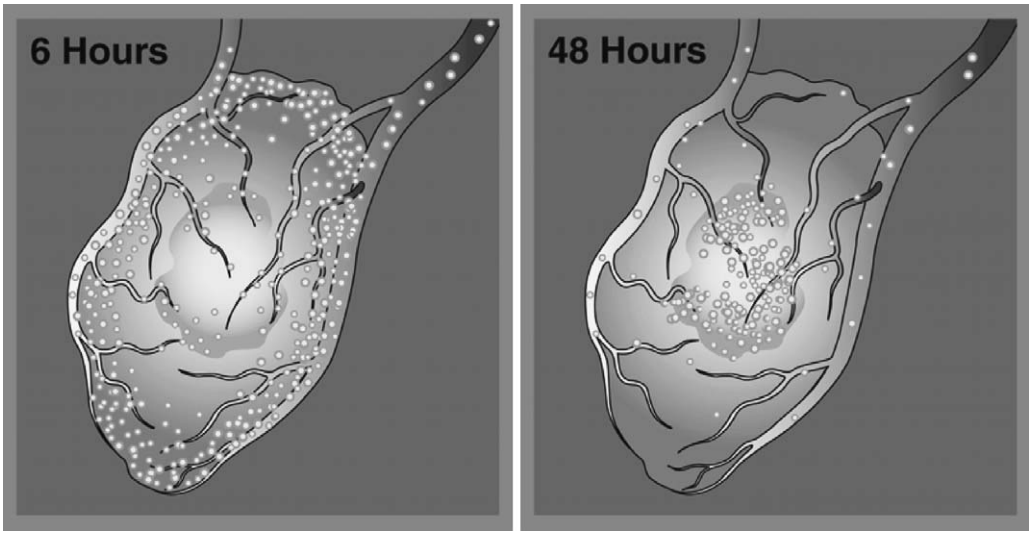

Fig. 1 – Schematic diagram of TNT monoclonal antibody (dots) uptake in tumor showing early entry of antibody at the tumor periphery and penetrating vessels at 6 h and localization of antibody in deep-seated necrosis at 48 h after migration through viable zones.