119

ONYX-015: AN ADENOVIRUS ATTENUATED IN THE E1B-55KD GENE SELECTIVELY REPLICATES IN, AND CAUSES LYSIS OF, P53- TUMOR CELLS.

D. Kirn, C. Heise, A. Sampson-Johannes, A. Williams, M. Muna, P.W. Trown and F. McCormick. ONYX Pharmaceuticals Inc., Richmond, CA 94806.

ONYX-015 is a human adenovirus construct (Ad2/Ad5) with a deletion in the E1B-55kD region that results in selective replication in, and killing of, p53-deficient tumor cells (Science, 274:373-376,1996). We have further defined the selectivity and efficacy of this agent both in vitro and in vivo. Cytopathic effect (CPE) and viral burst studies have shown that ONYX-015 replication was significantly attenuated in normal endothelial or epithelial cells (p53+). In contrast, ONYX-015 induced complete cytolysis in p53-deficient tumor cells from the following tissues: brain, breast, colon, cervix, larynx, liver, lung, ovary, pancreas and prostate. The therapeutic index between tumor cells and normal cells was 100-1000:1. In contrast, wild-type adenovirus replicated in and lysed both normal and tumor cells regardless of the p53 status. Direct intratumoral injection of ONYX-015 increased survival and resulted in tumor regression in several types of p53- tumor xenografts in nude mice. Complete, durable (>6 months) regression was observed in 10 of 18 solid tumors derived from C33A cells (p53- cervical carcinoma). Tumor growth inhibition was observed in HLaC (p53+ sequence but p53- function) tumors after treatment with ONYX-015. Viral replication and distribution studies after systemic delivery to cotton rats, which are permissive hosts for human adenoviruses, demonstrated that ONYX-015 replicated to significantly lower titers than a wild-type virus. These results demonstrate that injections of ONYX-015 directly into solid tumors resulted in anti-tumor efficacy in nude mice. Phase I clinical trials are underway.

120

SUICIDE GENE THERAPY IN CANCER

Craig A. Mullen, M.D., Ph.D.
Dept.'s of Experimental Pediatrics & Immunology, Univ. of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Box 88, Houston, TX 77030, TEL 713-792-3314, FAX 713-794-4373, mullen@utmdacc.mda.uth.tmc.edu

A suicide gene encodes an enzyme that can metabolically activate a relatively nontoxic prodrug to a highly toxic form that can kill the cell. Examples include the herpes simplex virus type 1 thymidine kinase that can activate ganciclovir (GCV) to the toxic GCV-monophosphate, and the E. coli cytosine deaminase that can deaminate 5-fluorocytosine to the potent cytotoxic 5-fluorouracil. Unmodified mammalian cells do not express these enzymes and thus are not killed by the prodrugs. However, cells modified by gene transfer vectors containing such genes are killed both in vitro and in vivo by the respective prodrugs. Curiously, prodrug treatment both in vitro and in vivo of mixed populations consisting of suicide gene expressing and unmodified cells often results in killing of both populations. The killing of the unmodified cells has been termed the "bystander effect". In vitro the process has been extensively studied and it appears that prodrugs activated in suicide gene-expressing cells can leave these cells and be taken up by unmodified cells in the microenvironment. In vivo an additional component of bystander effect killing may be contributed by the immune system. Evidence for this has come from studies in immunodeficient mice in which bystander effect killing of unmodified tumor cells is less effective, and in normal mice which may develop systemic immunity to rechallenge with wild type tumor after prior exposure to live suicide gene-expressing tumor cells and prodrug. Suicide gene transfer may have several possible applications in cancer therapy. The first is direct transfer of suicide genes to tumors using gene transfer vectors, followed by systemic treatment with prodrug. This approach may be of value in locally invasive tumors that are not surgically resectable or in which radiotherapy may be too toxic. A second approach is the use of suicide gene-containing vectors to purge residual tumor cells contaminating autologous bone marrow. A third involves control of graft versus host disease by transferring suicide genes to allogeneic T lymphocytes used in allogeneic bone marrow transplantation.

121

RETROVIRUS VECTORS

Wolfram Ostertag, Christopher Baum, Carol Stocking and Meike-Dorothee von Laer
Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, Martinistraße 52, 20251 Hamburg, Germany

Retrovirus derivatives are the most commonly used vectors for somatic gene therapy in malignancies. They are also suitable for correction of genetic diseases. We have developed retrovirus vectors that can be utilized within the haematopoietic system, both for stem cell protection in case of malignant diseases, and for genetic diseases. For both applications it is required that the vector can infect stem cells, and, if possible,that these cells can be used to expand haematopoietic cells. We have noticed three major deficiencies in haematopoietic stem cells that makes the use of retrovirus infection of haematopoietic stem cells for gene therapy difficult: 1) We have noticed, that common retrovirus vectors do not have the appropriate number of receptors on the target cells. 2) Stem cells usually lose the property for self-renewal if placed under culture conditions. 3) However, excellent expression can be obtained by use of vectors that contain control regions of the Friend F-MCF and the primer site of the MESV which we both developed in the last ten years.
We can overcome problems with infectivity by use of viral pseudotypes and are working on obtaining expansion of stem cells by employing genes that guarantee survival in culture. Selective expansion of such stem cells can be obtained by inserting selectable marker genes in the vector designed.

122

Pilot Trial of MDX-H210 and GM-CSF for Patients with Advanced erbB-2 Positive Malignancies

Posey J., Verma U., [1]DeoY., Marshall JL., Raspet R., Mazumder A., Hawkins MJ.
Georgetown University Medical Center, Washington, D.C. 20007 and [1]Medarex, Inc., Annandale, N.J. 08801.

MDX-H210 is a chemically, cross-linked bispecific half humanized antibody composed of MoAb H 22 that binds to the high affinity receptor Fc RI and MoAb 520C9 that recognizes the erbB-2 oncoprotein.In a previous trial, the murine bispecific MoAb MDX-210 at a dose of 7mg/m2 was well tolerated and activated monocytes and macrophages in vivo at doses as low as 0.35mg/m2. In our trial, GM-CSF 250mcg/m2 s.c. was given on days 1-4 and MDX-H210 was given on day 4 to thirteen patients at dose levels of 1,3.5,7.10,15,20 mg/m2 without dose limiting toxicity. Fever, chills, and rigors occurred during and up to 2hrs. post-infusion and correlated with the time to peak levels of TNFà (median 88.2 pg/ml; range 15.6 to 887 pg/ml) and IL-6 (median 371 pg/ml; range175 to 2149 pg/ml). By the fourth consecutive week of treatment the side effects and cytokine levels decreased significantly. HABA levels increased 512-fold in 3 patients and were associated with decreased MHX-H210 levels in two of these patients. The monocyte and granulocyte population increased on days 4 and 11 (median 44%; range 18%to 68% and 42%; 19%to 71%) respectively for monocytes and (60%; 43%to 75% and 74%; 54%to 82%) on days 4 and 11 for granulocytes. There was a significant decrease in the monocyte populations immediately after MDX-H210 administration (median decrease 73%; range 42%to94%) and (52%; 12%to 72%) on days 4 and 11 respectively. All patients have been reevaluated after four weeks. One patient had a 38% reduction in an index lesions, 8 patients had stable disease and 4 patients progressed before the fourth week. In an effort to increase effector cell cytotoxicity, we are currently evaluating 11 days of GM-CSF pretreatment prior to MDX-H210 administration.