Effective Treatment of Preexisting Melanoma with Whole Cell Vaccines Expressing α(1,3)-Galactosyl Epitopes

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Abstract
The hyperacute immune response in humans is a potent mechanism of xenograft rejection mediated by complement-fixing natural antibodies recognizing α(1,3)-galactosyl epitopes (αGal) not present on human cells. We exploited this immune mechanism to create a whole cell cancer vaccine to treat melanoma tumors. B16 melanoma vaccines genetically engineered to express αGal epitopes (B16αGal) effectively treated preexisting s.c. and pulmonary αGal-negative melanoma (B16Null) tumors in the α(1,3)-galactosyltransferase knockout mouse model. T cells from mice vaccinated with B16αGal recognized B16Null melanoma cells measured by detection of intracellular tumor necrosis factor-α. We showed successful adoptive transfer of immunity to recipient mice bearing lung melanoma metastases. Mice receiving lymphocytes from donors previously immunized with B16αGal had reduced pulmonary metastases. The transfer of lymphocytes from mice vaccinated with control vaccine had no effect in the pulmonary metastasis burden. This study unequivocally establishes for the first time efficacy in the treatment of preexisting melanoma tumors using whole cell vaccines expressing αGal epitopes. Vaccination with B16αGal induced strong long-lasting cell-mediated antitumor immunity extended to B16Null. These data formed the basis for the testing of this therapeutic strategy in human clinical trials currently under way. (Cancer Res 2005; 65(22): 10555-61)

Introduction
Despite multiple preventive and therapeutic approaches, cancer is one of the major causes of death worldwide. The American Cancer Society estimated >1.3 million new cases of cancer for 2005. Among other therapies, manipulation of the immune system in different types of immunotherapies has shown encouraging results in human clinical trials (1, 2). However, new immunotherapies are greatly needed because currently available treatments are still partially effective in cancer eradication (3). Different modalities of cancer vaccines have shown some degree of clinical efficacy. Whole tumor cell vaccines, administered in presence of adjuvant and genetically engineered tumor cells that express cytokines (i.e., granulocyte macrophage colony-stimulating factor and interleukin 2), are being studied extensively (1, 3). These vaccines have the advantage of expressing relevant tumor-associated antigens shared by the patient’s cancer cells. However, one of the disadvantages of these vaccines is the weak antigen presentation and poor ability to stimulate a potent immune response (3). In the present study, we have used a different approach to treat preestablished tumors based on the mechanisms responsible for hyperacute rejection of xenotransplants.

The major barrier for xenotransplantation to humans is the α(1,3)-galactosyl epitope (αGal). The gene encoding α(1,3)-galactosyltransferase (αGT), which catalyzes the synthesis of αGal epitopes on glycoproteins and glycolipids, is inactive in humans and Old World monkeys but is functional in other mammalian cells (4, 5). The human immune system is continuously stimulated by intestinal and pulmonary bacterial flora to produce natural antibodies that recognize αGal epitopes. These anti-αGal antibodies initiate a "hyperacute rejection" on tissues and cells expressing αGal. The hyperacute rejection of a xenotransplant is characterized by a rapid and acute organ destruction occurring within minutes to hours after transplantation. This reaction is mediated by the binding of anti-αGal antibodies from the recipient to αGal epitopes expressed on the xenograft and complement activation through the classic pathway (5). In addition, noncomplement fixing natural anti-αGal antibody induces antibody-dependent cell-mediated cytotoxicity that initiates tissue damage in xenotransplants mediated by natural killer cells (6–9).

We and others have suggested that the hyperacute rejection of whole cell cancer vaccines expressing αGal epitopes could be exploited as a new therapeutic approach to treat human malignancies (10–14).

The hypothesis that humoral immunity to αGal epitopes may induce anticancer immunity was tested using the α(1,3)-galactosyltransferase knockout (αGT KO) mouse model (15). These mice do not express αGal epitopes in their cell surfaces and can be induced to develop high titers of anti-αGal antibodies (16, 17). We and others have shown that mice with anti-αGal antibodies are protected when challenged with αGal-expressing cancer cells, whereas no protection was observed in mice without anti-αGal antibodies (13, 18). Moreover, the rejection of melanoma cells expressing αGal epitopes conferred protection against melanoma cells lacking the expression of αGal epitopes. Mice that rejected the first challenge with live B16αGal cells were protected from a second rechallenge with B16Null. Moreover, strong CTLs were induced in melanoma protected mice recognizing B16Null (14). In addition, vaccination with B16 melanoma cells expressing αGal epitopes prevented tumor development (19).

These data support the hypothesis that cancer vaccines designed to express αGal epitopes could induce strong antitumor immunity. Undoubtedly, the key challenge for the future clinical use of this anticancer vaccine is effective treatment of preestablished tumors. In this study, we show for the first time that mice with preexisting s.c. melanoma tumors or pulmonary melanoma metastases treated...
with melanoma vaccine cells expressing αGal epitopes exhibited greater survival and fewer pulmonary metastases. Evidence presented here shows that the rejection of αGal expressing melanoma vaccine cells in mice with preexisting humoral immunity against this epitope, induces strong, T cell–mediated antitumor immunity that recognizes native αGal-negative melanoma cells.

Materials and Methods

Cell lines. The B16.BL6 melanoma cell line (referred here as B16Null, H-2 b/b) does not express αGal epitopes (20). The αGal-negative CA320M (H-2 b/b) is a colon carcinoma chemically induced in αGT KO mouse generated and generously provided by Daniel Hellrung from the Iowa Cancer Research Foundation. B16.BL6 cells were transduced with pLNCKG, encoding the αGT gene under control of the cytomegalovirus (CMV) promoter (LTR-NeoR-CMV-αGT-LTR). The expression of αGal epitopes was detected in transduced cells by staining with the FITC-labeled Griffonia simplicifolia isoelectin B4 (IB4) as previously described (14, 18). B16.BL6 cells transduced to express αGal epitopes were referred here as B16αGal cells. B16.BL6 cells transduced with pLN (LTR-NeoR-LTR) yielded αGal-negative control cells referred as B16Neo0 cells. The pLNCKG vector was provided by Dr. Won-Bin Young (NewLink Genetics, Ames, IA). The activity of αGT was determined by fluorescence-activated cell sorting (FACS). No αGal epitopes were detected in B16Neo0 cells and B16Null cells. About 60% to 80% of B16αGal cells express αGal epitopes (data not shown). All cells were maintained at 37°C in a 5% CO2 incubator in complete medium consisting of DMEM supplemented with 10% fetal bovine serum (FBS), L-glutamine, sodium pyruvate, penicillin, streptomycin, HEPES, 2-ME, and nonessential amino acids. Cell vaccines were prepared by γ-irradiation (200 Gy) of transduced or nontransduced cells.

Mouse and animal treatments. Female and male αGT KO mice of H-2 b/b haplotype, 8 to 14 weeks old, were used in this study (15). The original αGT KO mouse expressed both alleles H-2 b and d haplotypes. These animals were generated by crossing C57BL/6 × DAB/J × 129sv mice (H-2 b × H-2d). In an effort to obtain homozygous colonies for the H-2 loci, by breeding and selection, we generated two αGT KO mouse colonies homozygous for both H-2 b/b and H-2 d/d haplotypes. Animals used in this study express H-2 Kb and H-2Dd haplotypes and do not express H-2Kd or H-2Db (14).

All mice were immunized i.p. with 1 × 106 rabbit RBC (RRBC) 2 or 3 weeks apart to generate anti-αGal antibodies as previously described (18). All mice developed high titers (>1:200) of anti-αGal antibodies (IgG and IgM) measured by ELISA as previously shown (18). One week following the last RRBC immunization, mice were injected s.c. with 1 × 105 B16Null and randomized. Starting on days 3 to 5 after tumor injection, mice received two or three doses of 5 × 105 irradiated whole cell vaccines 1 week apart from each other (treatment of preestablished s.c. tumors). Developing tumors were measured twice weekly in a blinded manner.

For the treatment of preestablished pulmonary melanoma metastases, mice were injected i.v. with 1 to 5 × 106 B16Null cells. After 4, 11, or 21 days of tumor establishment, mice received either B16αGal or B16Neo0 vaccines. Each vaccine consisted of 1 to 5 × 106 irradiated cells, administered s.c. Mice were euthanized after 30 days, and pulmonary tumors were enumerated in a blinded manner. All animal experiments were conducted according to the Institutional Animal Care and Use Committee–approved protocols. Histopathology sections of s.c. tumors and lung tissue were stained with H&E.

In vitro detection of T-cell reactivity and adoptive cell transfer experiments. Mice were preimmunized by i.p. injections of RRBC and subsequently vaccinated with three s.c. injections of 2 × 106 irradiated B16Neo0 or B16αGal cells 1 week apart. Spleen mononuclear cells (Spm) were harvested as previously described (14) and stored in freezing medium (90% FBS and 10% DMSO) in liquid nitrogen until used. Spm cells were rapidly thawed, washed with complete media, and either used for in vitro recognition studies or as donor lymphocytes during adoptive cell transfer experiments. CD8+ T cells were isolated by magnetic purification from Spm using Dynal mouse CD8-negative isolation kit (Oslo, Norway) following manufacturer’s instructions. Efficacy of purification was determined by FACS staining with an anti-CD8 monoclonal antibodies (BD PharMingen, San Diego, CA). After purification, cell preparation consisted of >90% CD8+ T cells and >95% CD3+ T cells (data not shown).

Intracranial tumor necrosis factor-α (TNF-α) was detected using a mouse detection kit (BD PharMingen) following manufacturer’s instructions. Briefly, 1 × 105 target cells/mL (CA320M or B16Null) were cultured overnight with 100 ng/mL of IFN-γ to up-regulate MHC class I (data not shown). After washing target cells to remove nonadherent cells, effector cells (Spm or purified CD8+ T cells) were added to the culture for activation and cytokine production. After 6 hours of incubation in presence of Golgi Plug (BD PharMingen), effector cells were harvested and stained for 

Figure 1. Treatment of preestablished s.c. melanoma by vaccination with B16αGal. A, mice were challenged s.c with B16.BL6 (B16Null) and randomized. Three days after the challenge, they received no treatment (n = 11, ●) or were vaccinated twice 7 days apart, with B16Neo0 (n = 24, ▲) or with B16αGal (n = 24, ●). Tumor sizes were determined by measuring three perpendicular diameters. Tumor growth over time is plotted for each group. Points, mean; bars, ± SE. Slope comparison using straight line model B16Neo0 versus B16αGal groups (P = 0.0054) and no treatment versus B16Neo0 (P = 0.84). B, tumors from control-vaccinated mice had minimal infiltrates of lymphocytes at the periphery of the tumor masses and few lymphocytes around blood vessels that permeated throughout the mass. Some areas showed active proliferation of melanoma cells (arrowhead). C, tumors from mice receiving B16αGal vaccines had increased infiltrates around blood vessels that permeated the mass. These infiltrates were present in the vascular adventitia and extended a short distance between tumor cells. In some areas, tumors from B16αGal–vaccinated mice had intense infiltrates of lymphocytes mixed with degenerate and/or necrotic neoplastic cells (C and D, arrows). Abbreviations: MEL, melanoma cells; BV, blood vessels; TIL, tumor-infiltrating lymphocytes.
intracellular TNF-α and CD3 expression. Up-regulation of cellular activation markers was measured after 24 hours of culture in the absence of brefedlin A. Cells were harvested and stained with PE-labeled anti-CD25 or anti-CD69 monoclonal antibody (BD PharMingen). Acquisition was done using Coulter flow cytometer Epics Ultra (Miami, FL) using Expo32 Software.

For cell transfer experiments, recipients oGT KO mice were iv injected with 1 x 10⁵ B16Null cell and randomized 4 to 5 days before cell transfer. They received 1 x 10⁵ irradiated purified CD8+ T cells administered iv. Pulmonary melanoma burden was determined 25 days after tumor inoculation.

Statistical analysis. GraphPad Prism software was used for statistical analysis, Kaplan-Meier survival analysis and log-rank tests were used for curve comparisons. One-way ANOVA test was used when appropriate.

Results

Effective treatment of established s.c. melanoma tumors by vaccination with B16αGal. We first determined whether B16αGal vaccination could retard the growth of preexisting s.c. melanoma tumors. Tumors from mice vaccinated with B16αGal had significantly reduced growth rate compared with the growth rate of tumors from control mice (Fig. 1A). More than 2-fold reduction in the average of the tumor size was observed at 30 days after challenge following B16αGal treatment. Vaccination with B16Neo3 had no effect on tumor growth rate. Histopathologic examination of s.c. tumors showed noticeable differences in the quantities and quality of inflammatory cellular infiltrates. Melanoma tumors from nonvaccinated and mice vaccinated with control vaccines (B16Null and B16Neo3) showed minor cellular infiltrates at the edges of the tumor, with only scant lymphocytes around i.t. vessels (Fig. 1B) and no visible granulocytic cells. Tumors from B16αGal-vaccinated mice showed mononuclear cell infiltrates at the edges of the tumor and infiltrates within the tumor, consisting of granulocytes, mast cells, and eosinophils. Numerous perivascular lymphocytes were found within the αGal-vaccinated tumor mass (Fig. 1C and D).

Mice vaccinated with B16αGal cells had significantly prolonged survival when compared with mice receiving no vaccination of B16NeoR control vaccine (Fig. 2A). Five of 19 mice treated with B16αGal vaccine remained tumor free for 70 days. In contrast, only 1 of 20 control mice survived the s.c. challenge with melanoma. The median survival time of the B16αGal vaccine group was increased to 39 days compared with only 27 and 29 days for respective controls.

The efficacy of B16αGal vaccines was improved by increasing the number of vaccine doses administered in a period of 3 weeks after tumor establishment. As before, mice receiving B16αGal vaccines developed smaller tumors that grew significantly slower, whereas

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**Figure 2.** Survival analysis of mice bearing s.c. melanoma treated with B16αGal vaccine. A, mice were challenged s.c with B16Null. On days 3 and 10 after challenge, they received either no treatment (n = 9, ■) or were vaccinated with B16Neo3 (n = 20, ●) or with B16αGal (n = 19, ●). Log-rank test for mice vaccinated with B16Neo3 compared with no treatment mice (P = 0.84). Log-rank test for B16Neo3 mice compared with B16αGal-treated mice (P = 0.02). B, mice were challenged s.c with B16Null. On days 3, 10, and 17 after challenge, they received either no treatment (n = 12, ▲) or were vaccinated with B16Neo3 (n = 23, ●) or B16αGal (n = 26, ●). Log-rank test for no treatment compared with B16Neo3 vaccine (P = 0.43). Log-rank test for all group comparison (P = 0.0045).

**Figure 3.** Reduction of lung melanoma metastases by treatment with B16αGal vaccine. Pulmonary metastases were established by i.v. injection of B6Null cells. Mice received either 10⁵ (A and B) or 5 x 10⁵ (C) B16Null cells. Mice were randomized and vaccinated with 5 x 10⁵ irradiated B16Neo3 or B16αGal vaccines on days 4, 11, and 21 after challenge. Thirty days following B16Null challenge, pulmonary melanoma metastases burden was determined. A, mice receiving the B16Neo3 vaccine. B, mice treated with B16αGal vaccine. C, lungs weighed in block. No vaccine (n = 10), B16Neo3 (n = 11), and B16αGal (n = 11) vaccines. Bars, SE. *, P < 0.02 (one-way ANOVA).
tumor growth rates were very similar in control mice (data not shown).

Treatment with three doses of the B16oGal vaccine significantly increased the survival of treated mice, whereas vaccination with B16NeoR had no effect on the survival of mice compared with non-vaccinated mice (Fig. 2B). Importantly, surviving mice that received the B16oGal vaccine were tumor free at the time the experiment was terminated. These data showed that oGal-expressing vaccines induce efficient regression of preexisting s.c. melanoma tumors.

**Effective treatment of pulmonary melanoma metastases by vaccination with B16oGal.** We next evaluated the efficacy of B16oGal vaccine for the treatment of pulmonary metastases as a model of disseminated disease. This experiment is very relevant from the clinical point of view, because cancer patients usually die from disseminated, non-resectable disease. Three mice treated with B16NeoR vaccine had lungs with “too-numerous-to-count” (arbitrary value, >200) tumors, one mouse had 30 pulmonary metastases, and two mice were tumor free (Fig. 3A). Moreover, one mouse in this control group had three additional metastatic tumors in the peritoneal cavity showing further dissemination of melanoma cells outside the lung tissue. In striking contrast, all mice treated with B16oGal vaccine were tumor free by macroscopic observation (Fig. 3B). Histopathologic examination of lungs from B16oGal-vaccinated mice revealed no evidence of micrometastases and absence of residual abnormal inflammatory lesions. This suggests the clearance of lung metastasis early after establishment of the pulmonary tumors.

To increase the stringency of these experiments, the i.v. dose of B16Null challenge was increased 5-fold. Numerous pulmonary melanoma metastases developed in the lungs of control groups receiving no treatment or B16NeoR control vaccine. On the contrary, a significant reduction of pulmonary metastasis was shown in mice treated with B16oGal vaccine (Fig. 3C).

These data provide strong evidence that the B16oGal vaccine can effectively treat preexisting disseminated metastatic melanoma. Furthermore, the vaccination with B16oGal prevented spreading of melanoma metastasis outside of the lung cavity.

**Induction of T-cell precursors specifically recognizing B16Null after vaccination with B16oGal.** A fundamental question concerning this novel anticancer vaccine is whether or not T cell–mediated antitumor immunity to the oGal-negative melanoma tumor is generated. We addressed this question by harvesting Spm cells from mice vaccinated with B16oGal or vaccinated with B16Null and testing their reactivity against B16Null. Purified CD8+ T cells were used as effector cells and cultured with CA320M or B16Null as target cells. We detected significant amount of double-positive cells (TNF-α+CD3+ cells) in cultures using purified CD8+ T cells from mice vaccinated with B16oGal stimulated with B16Null (Fig. 4A, top right; 5.3% TNF-α+CD3+ cells). Intracellular TNF-α was not detected when purified CD8+ T cells from B16oGal-vaccinated mice were stimulated with a nonmelanoma oGal-negative cancer cell line (CA320M, H-2 b/b; Fig. 4A, top left; 0.3% TNF-α+CD3+ cells), indicating specific recognition of melanoma targets. Only background intracellular TNF-α staining was observed when purified CD8+ T cells from control vaccinated mice were cultured with either B16Null (0.3% TNF-α+CD3+ cells) or CA320M (0.7% TNF-α+CD3+ cells) as targets (Fig. 4A, bottom right and left, respectively). These results indicate that the observed T-cell reactivity is melanoma specific and only induced when mice are vaccinated with oGal-expressing melanoma vaccines. Similarly, Spm cells from mice vaccinated with B16NeoR produced very low intracellular TNF-α when stimulated with B16Null cells (Fig. 4B; mean fluorescence intensity, MFI = 17). In contrast, Spm from B16oGal-vaccinated mice exhibited a 4-fold increase in TNF-α after stimulation with B16Null cells (Fig. 4B; MFI = 69). Spm from mice vaccinated with B16oGal and cultured with B16Null cells had
significantly increased up-regulation of cell surface activation markers CD25 and CD69 (Fig. 4C and D). This activation was not observed when these Spm were cultured with control CA320M cells. These results show that the up-regulation of T-cell activation molecules was due to specific recognition and activation of cultured lymphocytes by B16Null cells. Control mice vaccinated with B16Neo\(^{\alpha}\) and stimulated with B16Null cells had fewer activated splenocytes (Fig. 4C and D). These results show for the first time the induction of melanoma-specific T cells that can recognize αGal-negative tumor cells after vaccination with B16αGal.

**Antitumor immunity adoptively transferred to syngeneic recipients bearing melanoma metastasis.** To confirm that the presentation of αGal epitopes on whole cell cancer vaccines induces T cells that recognize melanoma cells lacking the expression of αGal epitopes, we did adoptive T-cell transfer experiments. A significant reduction of pulmonary melanoma metastases was observed in mice receiving total spleen mononuclear (Spm) cells harvested from B16αGal-vaccinated mice (Fig. 5A). The transfer of Spm cells from B16Neo\(^{\alpha}\)-vaccinated mice had no effect on the pulmonary tumor burden (Fig. 5A). To confirm that CD8\(^{\text{T}}\) T cells with antitumor activity are induced by B16αGal vaccines, CD8\(^{\text{T}}\) cells were purified and transferred into mice bearing 5-day pulmonary metastases (Fig. 5A). Mice receiving no CD8\(^{\text{T}}\) cells or receiving purified CD8\(^{\text{T}}\) T cells from mice vaccinated with B16Null control vaccine had significant numbers of pulmonary metastases. In striking contrast, 11 of 12 mice receiving CD8\(^{\text{T}}\) T cells from mice vaccinated with B16αGal were free of pulmonary tumors, and 1 of 12 had only a small number of small tumors (Fig. 5B).

The significant reduction of established pulmonary melanoma metastasis by adoptive transfer of CD8\(^{\text{T}}\) T cells from B16αGal-vaccinated mice provides further evidence for the induction of a strong, specific CD8\(^{\text{T}}\) T cell–mediated antitumor immunity by αGal\(^{\text{Gal}}\) melanoma vaccines. The results suggest that the presence of αGal epitopes on cell surface proteins and lipids of melanoma cells function as a strong immune adjuvant able to induce cell-mediated antitumor immunity against melanoma cells lacking the expression of αGal epitopes.

**Discussion**

In the present report, we clearly show for the first time efficacy in the treatment of established melanoma tumors using a novel concept of immunotherapy based on hyperacute rejection of whole cancer vaccine cells expressing αGal xenoepitopes.

Mice with either preexisting s.c. tumors or pulmonary melanoma metastases were successfully treated with αGal-expressing melanoma vaccines. This vaccine preparation differed from control vaccines only by its glycosylation pattern. Without other external cytokines and/or previously described adjuvants, the combination of αGal epitopes on vaccine cells and preexisting anti-αGal antibodies triggered a cascade of events that ultimately concludes in a potent cell-mediated antitumor immune response. T cells generated by this mean recognized native melanoma cells lacking the expression of αGal epitopes, indicating that the specificity of the immune reaction was extended to αGal-negative target antigens. This concept of whole cell vaccination differs from other similar studies in several mechanistic steps. First, it takes advantage of preexisting antibodies to the whole cell vaccine, favoring uptake and presentation of tumor-associated antigens. Second, complement activation results in the recruitment and maturation of antigen-presenting cells. Third, antigen uptake relies on Fc receptor–mediated phagocytosis. These elements combined together are unique compared with other whole cell vaccines previously described.

With results presented here and other published information, we propose a model that describes some elements important for this vaccine to generate such strong antitumor immunity (Fig. 6). Allogeneic or autologous tumor cells (from human or αGT KO mice origin) genetically engineered to express the αGT enzyme express αGal epitopes in multiple proteins and lipids that traffic through the Golgi apparatus. These αGal-coated cells constitute a multivalent vaccine containing several αGal molecules and multiple tumor-associated antigens, which can be α-galactosylated or not. Glycosphingolipids and glycoproteins are potential tumor antigens (e.g., melanoma-specific tyrosinase and gp100; ref. 21).
Immediately after the injection of αGal-positive whole cell vaccines in αGal-negative subjects with high titers of anti-αGal antibodies, such as humans or αGT KO mouse, vaccine cells are opsonized by these anti-αGal antibodies. Antibody opsonization of αGal epitopes induces complement activation through the classic pathway and cell lysis (22). Also opsonization promotes Fc receptor–mediated phagocytosis (23), which is a very efficient mechanism of antigen uptake and processing important for the cross-presentation of antigens to CD8+ T cells (24, 25). Complement-mediated cell lysis generates a “danger signal,” which has numerous implications for the kind of immune response that will be generated at the injection site (26, 27). Danger signals (necrosis, tissue injury, and release of heat shock proteins) are recognized as crucial components for antigen-presenting cell activation and differentiation to mature dendritic cells. Cell debris for example, acts as immune adjuvant by releasing among others, heat shock proteins. These molecules activate antigen-presenting cells through TOLL-like receptors. Antigen-presenting cells activated by these receptors are empowered for efficient T-cell activation (28, 29). In addition, complement degradation products have chemotactant properties that result in recruitment of macrophages, granulocytes, and eosinophils (data not shown). Moreover, natural antibodies and complement are endogenous adjuvants for vaccine-induced CD8+ T-cell responses (30). Two main mechanisms of antigen uptake take place during this phase of hyperacute rejection. The exogenous pathway, which results in presentation of the processed antigen on MHC class II surface molecules and activation of CD4+ T helper cells (31), and the uptake of immunocomplexes involving Fc receptors and anti-αGal antibodies. This will favor the cross-presentation pathway resulting in antigen presentation in the context of MHC class I molecules that will preferentially activate CD8+ cytotoxic T cells (24, 25, 32, 33). These cascade of events will ultimate induce activation, proliferation, and expansion of T cells recognizing tumor antigens expressed in tumor cells lacking the αGT enzyme proliferate. 7, these CD8+ T cells migrate within the αGal-negative tumor mass and eradicate preestablished tumors. Abbreviations: Gp, glycoproteins; Gr, glycoproteins; GL, glycolipids; ER, endoplasmic reticulum; DC, dendritic cells; TAA, tumor-associated antigens.

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