nutraceuticals

MDPI

Article

Effects of Xylitol on Tumor Progression in Syngeneic Mice Cancer Models

Mark Cannon [1,2,*], Ashlee Cosantino [1], Lori Tran [3,†], Navdeep S. Chandel [4] and Nayereh Ghoreishi [3]

[1] Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL 60611, USA; acosantino@luriechildrens.org
[2] Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA
[3] Developmental Therapeutics Core, Northwestern University, Evanston, IL 60208, USA; nayereh.ghoreishi@northwestern.edu (N.G.)
[4] Department of Medicine, Biochemistry and Molecular Genetics, Northwestern University, Chicago, IL 60611, USA; nav@northwestern.edu
* Correspondence: drmarkcannon@outlook.com or markcannon@northwestern.edu
† Lori Tran is on leave from Developmental Therapeutics Core, Northwestern University.

Abstract: This study investigates the effects of xylitol, a natural sugar alcohol, on tumor progression in syngeneic mouse cancer models. Xylitol is known for its dental health benefits, but emerging evidence suggests broader biological roles, including potential anti-cancer properties. We explored xylitol's impact on two mouse cancer models: 4T1 mammary carcinoma and B16F10 melanoma. Xylitol's efficacy in inhibiting cancer cell lines and modulating tumor progression was assessed using immunocompetent female mice. The experiments involved intratumoral and peritumoral administration of a 20% xylitol solution in two mouse strains: BALB/c (4T1 mammary carcinoma) and C57BL/6 (B16F10 melanoma). Tumor volume, histopathology, and metabolomic analyses were conducted to gauge xylitol's influence. The study revealed that xylitol administration initially reduced tumor growth in the B16F10 melanoma model, accompanied by alterations in tumor metabolism. However, similar effects were not observed in the 4T1 mammary carcinoma model, and melanoma tumor growth re-commenced in the melanoma model after stroma deterioration caused xylitol solution leakage. These findings suggest that xylitol may have potential as an adjunct therapy in cancer treatment, specifically in melanoma. The differential response between the two cancer models underscores the complexity of cancer biology and the need for further investigation into xylitol's mechanisms of action and its role in cancer therapy.

Keywords: xylitol; tumor progression; syngeneic mice models; cancer treatment; melanoma; mammary carcinoma; mouse cancer models; sugar alcohol; oncology; metabolomics

Academic Editor: Masayoshi Yamaguchi

Received: 18 September 2024
Revised: 27 December 2024
Accepted: 3 January 2025
Published: 10 January 2025

Citation: Cannon, M.; Cosantino, A.; Tran, L.; Chandel, N.S.; Ghoreishi, N. Effects of Xylitol on Tumor Progression in Syngeneic Mice Cancer Models. *Nutraceuticals* **2025**, *5*, 4. https://doi.org/10.3390/nutraceuticals5010004

1. Introduction

Cancer continues to be the second most common cause of death in the USA [1]. Although progress has been made in cancer therapy, emerging therapeutics are considered economically unfeasible for many of the world's population [2]. Therefore, the potential use of an inexpensive and widely available prebiotic as an adjunct to cancer therapy would prove beneficial. Xylitol is a well-known preventative product used in dentistry for decades [3]. Recent research demonstrates the inhibitory properties of xylitol with many cancer cell lines when administered both dietary and systemically [4–6]. Because xylitol exhibits almost no side effects and is safely utilized by healthy human cells, it could be a beneficial natural supplement for potentially inhibiting cancer cell proliferation [7,8].

However, although the mechanisms of xylitol cancer cell inhibition were hypothesized in previous research, more information should be provided before human clinical trials.

The specific properties of xylitol and cancer inhibition may not have been entirely explained. Xylitol may inhibit cancer by reducing angiogenesis and decreasing the tumor's vascularization [9]. Increased vascularization would support tumor growth and possibly cancer metastasis. In addition, xylitol has reported anti-inflammatory properties that may reduce tumor progression [10,11]. The determination of the specific metabolic features of xylitol with tumor development needs further elucidation, requiring direct deposition of xylitol into tumors. Metabolomic analysis and histological evaluation of cancers with and without xylitol would provide the essential data. Metabolomics can be used to identify cancer biomarkers and the drivers of tumorigenesis [12]. Metabolism is dysregulated in cancer cells to support uncontrolled cell proliferation [13]. This dysregulation of cellular metabolism leads to specific metabolic phenotypes. These metabolic phenotypes can be used for earlier cancer diagnosis, clinical trials, patient selection, and as biomarkers of treatment response.

Previous research has shown that xylitol supplementation in animal models inhibits cancer cell lines and xenografts [6,14]. Combination treatments with xylitol were also reported as successful [15]. Understanding the mechanism of xylitol tumor inhibition should provide essential and usable information for adjunctive therapy that could prove to be cost-effective and readily available for millions of cancer patients. Xylitol is a prebiotic that shifts the host's microbiome [16]. This is important because the role of the human microbiome in cancer development and treatment is now well-recognized [17,18]. Indeed, the interactions between microbiome and cancer have generated research into the complex microbial communities and the possible mechanisms through which microbiota influence cancer prevention, carcinogenesis, and anti-cancer therapy. In addition, developing next-generation prebiotics and probiotics designed to target specific diseases is considered extremely urgent [19,20].

Animal models are considered the first phase of cancer research, looking for potential therapeutic agents [21]. This research study intends to use two mouse models to evaluate the efficacy of a higher concentration (20%) and the modality of direct delivery (intratumorally) of xylitol in cancer. The direct delivery of xylitol into tumors would rule out any systemic action of xylitol on the immune system via microbiome manipulation. The purpose was to evaluate the effects, including cell line inhibition of xylitol on tumor progression, in two syngeneic mouse cancer models. Specifically, this pilot study measured tumor growth inhibition from two cancer cell line implantations in two syngeneic mouse cancer models, 4T1 mammary carcinoma and B16F10 melanoma [22], with a 20% xylitol (prebiotic) solution intratumorally and peritumorally administered daily. The study of xylitol directly injected into tumors with metabolomic analysis and histological evaluation had yet to be investigated.

2. Materials and Methods

2.1. Experimental Mice and Study Design

This IACUC-approved study (IS000097440) included two strains of immunocompetent female mice: 20 C57BL/6 and 20 BALB/c mice for a total of 40 mice (see Table 1). The mice were acquired from Charles River Laboratories, Durham, NC, USA. The BALB/c group was injected with 4T1 mammary carcinoma cells (1×10^6 cells into the 4th mammary gland), and the C57BL/6 group was injected with B16F10 melanoma cells (1×10^6 cells into the flank). After the cancer cell injection, tumor growth became significant by the 14th day and treatment started by the 16th–19th day. When tumor sizes reached 50 to 100 mm^3, both treatment groups (10 each) were injected daily with 100 µL of a 20% xylitol solution

intratumorally (75%) and subcutaneously (25% peritumoral). The xylitol was acquired from Xlear, American Fork, Utah, USA. A 20% solution was the highest concentration of xylitol possible to formulate. Previously published research reported concentrations of 5 and 10%, but the principal investigator believed it was possible to use a higher percentage in this study. Control mice received sterile saline (10 each) with the same daily treatment frequency and amount (Monday–Sunday) as the xylitol group. All mice's body weight and tumor volume were measured every other day. All tumor volumes were measured by caliper and calculated by the following modified ellipsoidal formula [23]:

$$\text{Tumor volume} = 1/2\ (\text{length} \times \text{width}^2)$$

Table 1. Forty mice with two syngeneic models (twenty each) and ten control versus ten experimental mice in each group. The two syngeneic models were used to check the possible efficacy of xylitol therapy with both immune systems, adaptive and innate.

Mouse Strain	Cancer Cell Line	Immune System	Mice
BALB/C	4T1 mammary carcinoma	Adaptive immunity	20–10 controls and 10 experimental
C57BL/6	B16F10 melanoma	Innate immunity	20–10 controls and 10 experimental

Or $V = (L \times W^2)/2$; L is length, and W is width. L is the greatest longitudinal diameter (length), and W is the greatest transverse diameter (width). This formula is the standard used by the Developmental Therapeutics Core at Northwestern University, Evanston, IL, USA.

Euthanization Protocol

All mice were euthanized when the control tumor volumes were equal to or larger than 2000 mm^3 or if the mice lost more than 20% of their original body weight. In addition, euthanization was performed if there were other severe clinical health issues (e.g., paralysis) that would cause undue discomfort. Xylitol was injected daily until significant changes in the tumor sizes were observed (the study was to be terminated if no changes were seen per IACUC protocols). Euthanasia was also performed according to IACUC protocol. Animals were not combined from different cages, and when euthanizing some of the mice from a cage, the rest of the animals remained in their original cage. The maximum number of mice per cage was five, and the CO_2 flow rate per mouse cage was 3 L/min until 1 minute after breathing stopped. Euthanasia was confirmed by cervical dislocation.

2.2. Sample Collection

Immediately following euthanasia, tumors were harvested, sectioned, and one-half fixed in 10% neutral-buffered formalin for 48 h. The other half of the tumor was flash-frozen in liquid nitrogen for metabolomic analysis. Tumor tissues were embedded in paraffin, sectioned into 4–5 µm-thick sections, placed on microscopic slides, and stained with hematoxylin and eosin (H&E) by the Mouse Histology and Phenotyping Laboratory of Northwestern University. Microscopic slides were imaged using an Olympus BX45 microscope with an Olympus DP28 digital camera. Digital images were visualized using Olympus cellSens imaging software (version 4.2).

2.3. Metabolomic Analysis Procedure

Metabolomic samples were analyzed using high-performance liquid chromatography, high-resolution mass spectrometry, and Tandem mass spectrometry (HPLC-MS/MS). Specifically, the system consisted of a Thermo Q-Exactive in line with an electrospray source and an Ultimate3000 (Thermo, Waltham, MA, USA) series HPLC consisting of a binary pump, degasser, and auto-sampler outfitted with an Xbridge Amide column (Waters;

dimensions of 3.0 mm × 100 mm and a 3.5 µm particle size). The mobile phase A contained 95% (*v*/*v*) water, 5% (*v*/*v*) acetonitrile, 10 mM ammonium hydroxide, 10 mM ammonium acetate, pH = 9.0; B was 100% acetonitrile. The gradient was as follows: 0 min, 15% A; 2.5 min, 30% A; 7 min, 43% A; 16 min, 62% A; 16.1–18 min, 75% A; 18–25 min, 15% A with a flow rate of 150 µL/min. The capillary of the ESI source was set to 275 °C, with sheath gas at 35 arbitrary units, auxiliary gas at 5 arbitrary units, and the spray voltage at 4.0 kV. In positive/negative polarity switching mode, an m/z scan range from 60 to 900 was chosen, and MS1 data were collected at a resolution of 70,000. The automatic gain control (AGC) target was set at 1×10^6, and the maximum injection time was 200 ms. The top 5 precursor ions were subsequently fragmented in a data-dependent manner, using the higher energy collisional dissociation (HCD) cell set to 30% normalized collision energy in MS2 at a resolution power of 17,500. Besides matching m/z, metabolites are identified by matching either retention time with analytical standards and/or MS2 fragmentation pattern. Xcalibur 4.1 software and Tracefinder 4.1 software, respectively (from Thermo Fisher Scientific, Waltham, MA, USA), carried out data acquisition and analysis.

3. Results

3.1. Tumor Volume

After five days of 20% xylitol injections, tumor volumes were reduced by 40% in the C57BL/6 group with the B16F10 melanoma cells (see Figure 1), but with the BALB/c + 4T1 cancer line (adaptive immunity), the tumor growth was not significantly different between the control and the experimental (xylitol). With repeated intratumoral injections, the tumor stroma deteriorated in the B16F10 tumors, resulting in substantial xylitol leaking onto the skin surface. Afterward, experimental and control tumor volumes would become clinically comparable by study termination at day 14. Interstitial tumor pressure increased, preventing effective injection of the solution after five days. The 4T1 syngeneic model with the Balb/c mice demonstrated no statistically significant differences between the tumor volumes of the experimental (xylitol) group and the control (saline) (see Figure 2). Injection of the xylitol solution into the mammary tumors proved to be problematic.

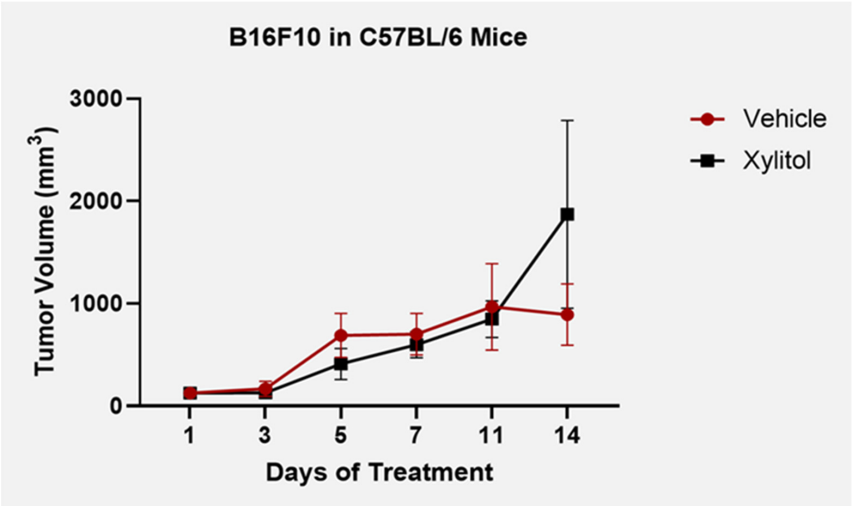


Figure 1. The tumor volumes of the B16F10 + C57BL6 melanoma syngeneic model until day 14 and study termination. The tumor volume increase was reduced by xylitol compared to the controls (saline) until the tumor stroma degraded, allowing the 20% xylitol solution to leak out. The black lines represent the xylitol-treated tumors, and the red lines are the vehicle-treated tumors. Standard deviation bars were calculated from tumor volumes. Statistical differences were seen on days 5 and 14 of the *t*-test analysis, with a p value < 0.05.

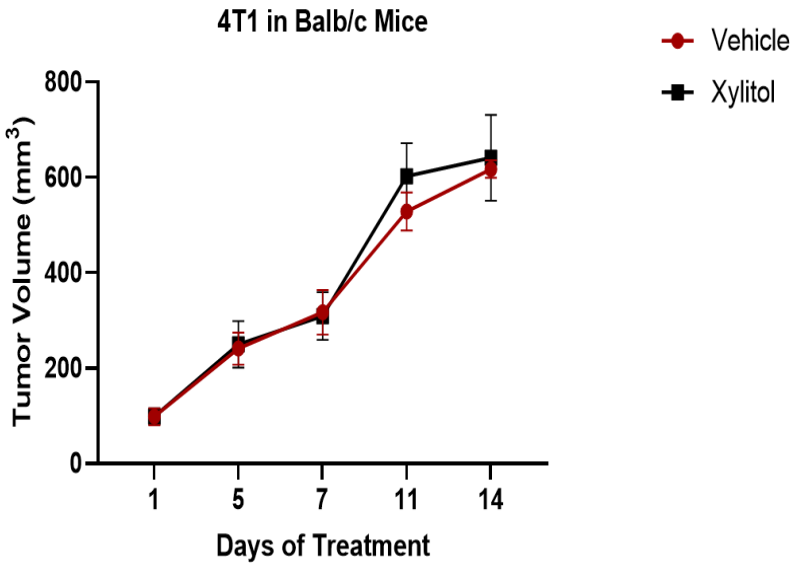


Figure 2. There were no statistically significant differences between the tumor volumes of the experimental (xylitol) group and the control (saline) in the 4T1 syngeneic model with the Balb/c mice (*t*-test analysis, p value > 0.05). The red lines represent the vehicle tumors, and the black lines represent the xylitol tumors.

3.2. Metabolomic Analysis

Metabolomic analysis revealed apparent differences between experimental and control tumor cellular metabolism (see Table 2). Lymph node histological analysis demonstrated metastasis in both groups by the time of euthanasia. The metabolomic analysis demonstrates that intratumoral xylitol reduces tumor cell production of histamine, NADP+, ATP, and glutathione thereby affecting the availability of reactive oxidative species and the host immune response (see Figure 3a,b). The xylitol group showed significantly decreased phosphocreatine, citrate, and pyruvic acid levels, signifying metabolic stress likely due to inhibiting mitochondrial metabolism within the tumor cells. Notably, xylitol levels were increased in tumors, demonstrating that xylitol accumulates in cancer cells and suggests that the effects of xylitol are likely due to cancer cell-intrinsic effects on metabolism (see Table 2). In addition, a decrease in tumor glutathione may affect the innate immune response, enhancing the impact of reactive oxidative species.

Table 2. Important features of the tumor cell metabolism listed by fold change values and *t*-test p values. Intertumoral xylitol presents changes to the metabolic products that may influence tumor development. These data were generated using MetaboAnalyst software version 6.0 NSERC Canada.

Metabolite	Fold Change	$\log^2$ (FC)	p Value
L-tyrosine methyl ester	0.13098	−2.9326	0.00055
tryptamine	0.43146	−1.2127	0.006415
2,3-bisphospho-D-glycerate	0.34972	−1.5157	0.017592
ATP/dGTP	0.29392	−1.7665	0.019468
cysteine	0.54949	−0.86384	0.02808
choline+	0.59869	−0.74012	0.032148
GDP	0.58635	−0.77016	0.03544
2-/3-phosphoglycerate	0.30686	−1.7044	0.037869
glutathione	0.42802	−1.2242	0.041884
GAP/DHAP	0.29889	−1.7423	0.041934
D-erythrose-4-phosphate	0.25721	−1.959	0.043739
CDP	0.34568	−1.5325	0.047149
FAD	0.3264	−1.6153	0.048366
putrescine	0.28675	−1.8022	0.048865

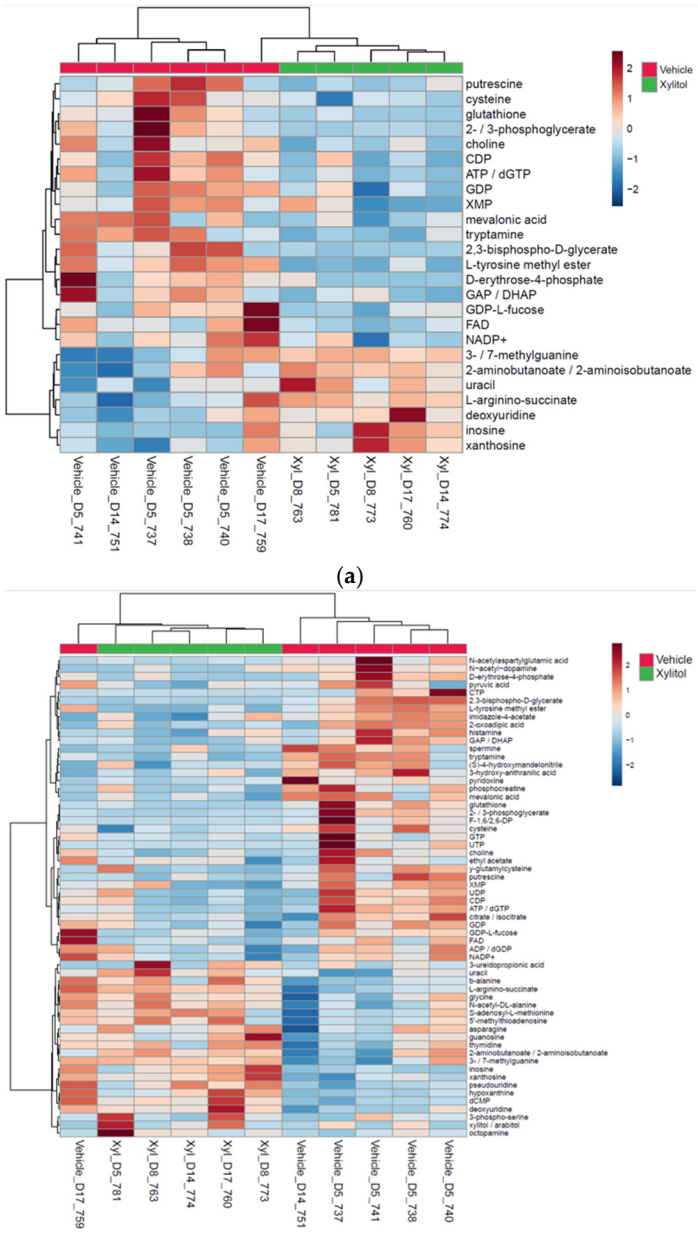
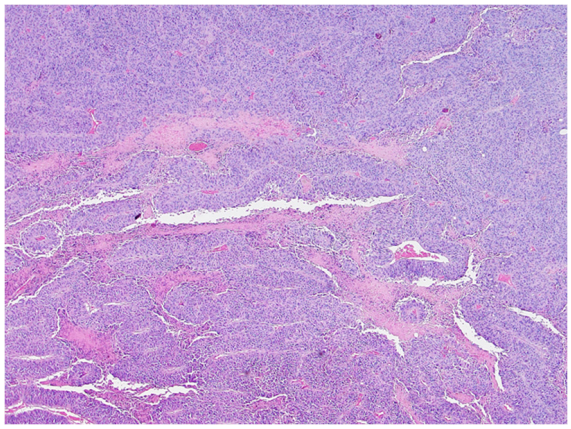


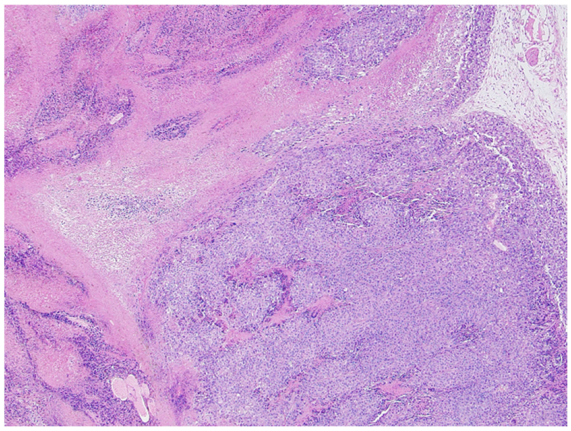
Figure 3. (**a**) Right legend: top 25 metabolic end products. Bottom legend: xylitol-injected or vehicle-injected tumor. Significant differences in tumor metabolism with xylitol are present compared to vehicle's. Xylitol group demonstrated significantly decreased phosphocreatine, citrate, and pyruvic acid levels, signifying metabolic stress within tumor cells. Putrescine, mevalonic acid, and tryptamine are elevated in tumor cells. (**b**) Heatmap displays top 50 metabolic end products in tumors treated with xylitol compared to vehicle control. Significant differences are noted between metabolites of xylitol-treated tumors and vehicle-treated tumors ($p = 0.05$).

3.3. Histological Analysis

Histological analysis did not reveal overall or consistent differences between the xylitol and the vehicle groups. Indeed, the vehicle group demonstrated significant necrosis, perhaps due to cancer growth. Tears or tracks were present in the xylitol samples, perhaps due to the xylitol injections. The vehicle injection group however did not demonstrate possible injection tracks (See Figure 4a,b).



(**a**)



(**b**)

Figure 4. (**a**) Xylitol-treated B16F10 melanoma tumor histology (H&E stain) demonstrates slight, patchy intratumoral necrosis (i.e., high viable fraction in tumor). Foci of degeneration/necrosis are evident. Several stromal blood vessels—overall vascularity in cancer is modest, with occasionally small hemorrhages. Section is from mouse 754 with 20% xylitol injections. Tears or tracks may represent areas where xylitol injections were placed and resulted in solution loss. (**b**) Vehicle-treated tumor representative image (H&E stain). Histology demonstrates extensive intratumoral necrosis, resulting in ~60% viable fraction in tumor based solely on image. Section from mouse 765 with B16F10 melanoma tumor. Scattered stromal blood vessels are present, and overall vascularity in tumor is low—with blood vessels in viable and necrotic areas.

4. Discussion

The previously published data suggests that xylitol inhibits cancer cell lines, although, in our study, tumor growth inhibition was only significant in the melanoma group (syngeneic model C57L/C, B16F10, innate immunity). Statistical differences were seen on days 5 and 14, with *t*-test analysis p value < 0.05. This result may be due to cancer cells not utilizing xylitol as normal human cells (and rodents such as rats and mice) in their mitochondria for energy [24]. Most cancer cells utilize glycolysis and mitochondrial metabolism to sustain growth in vivo [25]. Certain animal species cannot correctly utilize xylitol, especially carnivores that were not evolutionarily exposed to plants as foods [26]. One can theorize that homo sapiens cell mitochondria may have rapidly evolved to metabolize xylitol due to its presence in several survival foods, especially during periods of scarcity [27,28]. Cockroaches, rats, swine, and humans can metabolize xylitol [26–31]. The modern American diet utilizes sucrose and high fructose corn syrup as added sugars, which enhances cancer development [32,33]. Therefore, dietary considerations may become necessary in

preventing and treating cancer [33,34]. Xylitol and other natural sweeteners may be suitable substitutes for possibly oncogenic carbohydrates.

Xylitol reportedly enhances the innate immune system response to cancer cells. Cancer cell lines are effectively more sensitive to reactive oxidative species produced by killer T-cells [35]. The results of the metabolomics analysis support that theory. The metabolomic values were different between the vehicle and xylitol groups. Specifically, tumor cell production of glutathione and histamine was reduced by xylitol. Glutathione is an antioxidant that reduces the reactive oxygen species (ROS) effect on tumor cells [36,37]. Xylitol reduction in histamine may hypothetically reduce metastasis by reducing vascularization and proliferation [38,39]. In addition, mitochondrial metabolism is necessary to determine stem cell fate. Mitochondrial metabolism is responsible for the production of ATP and maintains the tri-carboxylic acid (TCA) cycle. The metabolites of the TCA cycle support stem cell survival and growth. Recent evidence shows that mitochondria control mammalian stem cells' fates and functions through reactive oxygen species (ROS) generation, TCA cycle metabolite production, NAD^+/NADH ratio regulation, pyruvate metabolism, and mitochondrial dynamics [40]. Xylitol affects the mitochondria and the TCA cycle, apparently with anti-oncogenic properties. The findings underscore the intricate relationship between metabolic interventions and tumor progression. Xylitol's impact on tumor metabolism, specifically its influence on reducing tumor cell production of crucial metabolites like histamine, $NADP^+$, ATP, and glutathione, paves the way for further exploration into its role as a metabolic modulator in cancer therapy. This is particularly relevant given the growing interest in metabolic pathways as targets for cancer treatment. Moreover, the differential responses observed between the 4T1 mammary carcinoma and B16F10 melanoma models illuminate the complex nature of cancer biology and the necessity for targeted therapeutic strategies. Our study also brings to light the challenges associated with intratumoral drug delivery, as evidenced by the complications in maintaining the structural integrity of the tumor stroma during xylitol administration.

This first protocol utilized intratumor injections, which proved less effective due to loss of stroma. Lack of structural integrity, interstitial fluid pressure, and possibly injection trauma resulted in xylitol solution leaking from the tumor, allowing for tumor progression [41]. This exact mechanism was reported with previous intratumoral injections using chemotherapeutics, such as 5-fluorouracil, until the development of smart hydrogels [42]. Studies in progress at the Developmental Therapeutics Core utilize Alzet mini osmotic pumps that deliver a consistent concentration of xylitol to the subject animals [43,44]. Our research team is now cautiously optimistic that this approach will advance techniques used in previously published studies with IV xylitol, which could be more challenging to implement in an animal group [5]. After submitting this manuscript, we learned that the consistency of the two tumors is quite different, which may have influenced the study results. Specifically, 4T1 tumors developed in the mammary fat pad are firm, a solid mass leaving no room for the xylitol solution. Meanwhile, the subcutaneous B16F10 melanoma tumor is a soft mass that allows space for the xylitol solution. This physical feature may explain the discrepancy in the effectiveness of xylitol in inhibiting the two cancer cell lines. As mentioned, ongoing studies use other delivery routes for the xylitol solution.

The role of the microbiome in cancer development/progression via several mechanisms, including immune response modulation and the promotion of a pro-inflammatory tumor environment, was very well studied and established [45]. It was also noted that xylitol significantly affects the composition of the gut microbiota, stimulates the propagation of beneficial bacteria, and produces short-chain fatty acids (SCFAs) [16,46]. This altered metabolism resulting from xenobiotic xylitol can potentially influence cancer growth and

progression [47–49]. However, this study relied upon the direct application of xylitol, negating any possible effect from the gut microbiome. Indeed, current research demonstrates the presence of specific tumor microbiomes with intracellular bacteria. Xylitol may influence those tumor-specific microbiomes.

Our study offers novel insights into the potential therapeutic role of xylitol in cancer treatment, specifically in melanoma. Therapy with a 20% xylitol solution demonstrated a significant reduction in the initial growth of B16F10 melanoma tumors in a syngeneic mouse model, highlighting xylitol's potential as an adjunctive treatment in oncology. Notably, this effect was only observed in the melanoma model, indicating a potential cancer-type specificity or differential mechanism of action. The efficacy of the xylitol injections decreased after five days in the BF16F10 model due to the degradation of melanoma tumor stroma, and the tumor's re-commenced growth. Previous research suggests that a lower concentration of xylitol is more effective than concentrations above 5% [5].

Future research should focus on optimizing the delivery method of xylitol to enhance its therapeutic efficacy. Extending these findings to other cancer models and eventually to clinical trials will be crucial to fully understanding xylitol's potential in cancer treatment. This research lays the groundwork for such future investigations, hoping to contribute to more effective and targeted cancer therapies. In addition, in vitro cytotoxicity studies of cancer and control cell line studies with xylitol and other carbohydrates in varying concentrations were accomplished and will be submitted for publication. Dietary considerations should be considered as many studies have documented carbohydrate consumption and microbiome shifts as possibly additional factors in cancer development and treatment.

5. Conclusions

In conclusion, cancer is a significant cause of death, requiring a readily available therapeutic treatment to inhibit tumor development and growth. Xylitol was previously studied and appears to be a safe adjunct in cancer inhibition, but the mechanism was not well described. This study reports on the metabolomics and histological analysis of xylitol directly injected into cancer cell growths in syngeneic mice models. Metabolomic changes were significant in the tumors injected with xylitol compared to the controls, providing insight into the specific metabolites associated with tumor inhibition. Importantly, the role of the innate immune system is crucial in tumor development, and understanding the tumor microenvironment will be essential for preventing and treating cancer.

Author Contributions: Conceptualization, M.C. and N.G.; methodology, N.G.; validation, A.C.; formal analysis, N.S.C.; investigation, M.C.; resources, L.T.; data curation, L.T.; writing—original draft preparation, M.C.; writing—review and editing; visualization, A.C.; supervision, M.C.; project administration, N.G.; funding acquisition, M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Developmental Therapeutics Core at Northwestern University and the Robert H. Lurie Comprehensive Cancer Center support grant (NCI CA060553). Funding was also provided by the Swanson Fund of Northwestern University, with special thanks to Michael Milligan, Lon Jones, and R. William Cornell for their contributions to the Swanson Fund.

Institutional Review Board Statement: This research was conducted in strict accordance with ethical standards and guidelines for animal experimentation. All procedures performed in animal studies complied with the ethical standards of the institution where the studies were conducted. Efforts were made to minimize animal suffering and reduce the number of animals used. The Institutional Animal Use and Control Committee approved the animal study protocol for Northwestern University, Center for Developmental Therapeutics. The IACUC protocol # is IS00009744.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are available at the Developmental Therapeutics Core and the Metabolomic Developing Core, Robert H. Lurie Comprehensive Cancer Center, Northwestern University: https://www.feinberg.northwestern.edu/research/cores/units/metabolomics.html (accessed on 27 December 2024).

Acknowledgments: Thank you to Mari Tesch, Fernando Valerio-Pascua, and Franck Rahaghi for contributing to the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Bray, F.; Laversanne, M.; Sung, H.; Ferlay, J.; Siegel, R.L.; Soerjomataram, I.; Jemal, A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2024**, *74*, 229–263. [CrossRef] [PubMed]
2. Vallano, A.; Pontes, C. Escalating costs of innovative medicines: Perspective and proposals. *Front. Public Health* **2024**, *12*, 1449707. [CrossRef] [PubMed]
3. Nayak, P.A.; Nayak, U.A.; Khandelwal, V. The effect of xylitol on dental caries and oral flora. *Clin. Cosmet. Investig. Dent.* **2014**, *6*, 89–94. [CrossRef] [PubMed]
4. Park, E.; Park, M.H.; Na, H.S.; Chung, J. Xylitol induces cell death in lung cancer A549 cells by autophagy. *Bio-Technol. Lett.* **2015**, *37*, 983–990. [CrossRef] [PubMed]
5. Tomonobu, N.; Komalasari, N.L.G.Y.; Sumardika, I.W.; Jiang, F.; Chen, Y.; Yamamoto, K.I.; Kinoshita, R.; Murata, H.; Inoue, Y.; Sakaguchi, M. Xylitol acts as an anticancer monosaccharide to induce selective cancer death via regulation of the glutathione level. *Chem. Interact.* **2020**, *324*, 109085. [CrossRef]
6. Sahasakul, Y.; Angkhasirisap, W.; Lam-Ubol, A.; Aursalung, A.; Sano, D.; Takada, K.; Trachootham, D. Partial Substitution of Glucose with Xylitol Prolongs Survival and Suppresses Cell Proliferation and Glycolysis of Mice Bearing Orthotopic Xenograft of Oral Cancer. *Nutrients* **2022**, *14*, 2023. [CrossRef]
7. Mehnert, H.; Förster, H.; Dehmel, K.H. The effect of intravenous administration of xylitol solutions in normal persons and in patients with liver diseases and diabetes mellitus. In Proceedings of the International Symposium on Metabolism, Physiology, and Clinical Use of Pentoses and Pentitols, Hakone, Japan, 27–29 August 1967; Springer: Berlin/Heidelberg, Germany, 1969; pp. 293–302.
8. Sato, J.; Wang, Y.M.; van Eys, J. Metabolism of xylitol and glucose in rats bearing hepatocellular carcinomas. *Cancer Res.* **1981**, *41*, 3192–3199. [PubMed]
9. Yi, E.Y.; Kim, Y.J. Xylitol inhibits in vitro and in vivo angiogenesis by suppressing the NF-κB and Akt signaling pathways. *Int. J. Oncol.* **2013**, *43*, 315–320. [CrossRef] [PubMed]
10. Kharaeva, Z.F.; Mustafaev, M.S.; Khazhmetov, A.V.; Gazaev, I.H.; Blieva, L.Z.; Steiner, L.; Mayer, W.; Luca, C.; Korkina, L.G. Anti-Bacterial and Anti-Inflammatory Effects of Toothpaste with Swiss Medicinal Herbs towards Patients Suffering from Gingivitis and Initial Stage of Periodontitis: From Clinical Efficacy to Mechanisms. *Dent. J.* **2020**, *8*, 10. [CrossRef]
11. Park, E.; Na, H.S.; Kim, S.M.; Wallet, S.; Cha, S.; Chung, J. Xylitol, an anticaries agent, exhibits potent inhibition of inflammatory responses in human THP-1-derived macrophages infected with *Porphyromonas gingivalis*. *J. Periodontol.* **2014**, *85*, e212–e223. [CrossRef]
12. Rinschen, M.M.; Ivanisevic, J.; Giera, M.; Siuzdak, G. Identification of bioactive metabolites using activity metabolomics. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 353–367. [CrossRef] [PubMed]
13. Vander Heiden, M.G.; DeBerardinis, R.J. Understanding the Intersections between Metabolism and Cancer Biology. *Cell* **2017**, *168*, 657–669. [CrossRef] [PubMed]
14. Trachootham, D.; Chingsuwanrote, P.; Yoosadiang, P.; Mekkriangkrai, D.; Ratchawong, T.; Buraphacheep, N.; Kijanukul, S.; Saekhow, S.; Pongpitchayadej, O.; Vongvachvasin, K.; et al. Partial substitution of glucose with xylitol suppressed the glycolysis and selectively inhibited the proliferation of oral cancer cells. *Nutr. Cancer* **2017**, *69*, 862–872. [CrossRef] [PubMed]
15. Qusa, M.H.; Siddique, A.B.; Nazzal, S.; El Sayed, K.A. Novel olive oil phenolic (−)-oleocanthal (+)-xylitol-based solid dispersion formulations with potent oral anti-breast cancer activities. *Int. J. Pharm.* **2019**, *569*, 118596. [CrossRef]
16. Xiang, S.; Ye, K.; Li, M.; Ying, J.; Wang, H.; Han, J.; Shi, L.; Xiao, J.; Shen, Y.; Feng, X.; et al. Xylitol enhances synthesis of propionate in the colon via cross-feeding of gut microbiota. *Microbiome* **2021**, *9*, 62. [CrossRef] [PubMed]
17. Meng, C.; Bai, C.; Brown, T.D.; Hood, L.E.; Tian, Q. Human Gut Microbiota and Gastrointestinal Cancer. *Genom. Proteom. Bioinform.* **2018**, *16*, 33–49. [CrossRef] [PubMed]
18. Zhou, C.B.; Zhou, Y.L.; Fang, J.Y. Gut Microbiota in Cancer Immune Response and Immunotherapy. *Trends Cancer* **2021**, *7*, 647–660. [CrossRef] [PubMed]

19. Tsai, Y.L.; Lin, T.L.; Chang, C.J.; Wu, T.R.; Lai, W.F.; Lu, C.C.; Lai, H.C. Probiotics, prebiotics and amelioration of diseases. *J. Biomed. Sci.* **2019**, *26*, 3. [CrossRef] [PubMed]
20. Yadav, M.K.; Kumari, I.; Singh, B.; Sharma, K.K.; Tiwari, S.K. Probiotics, prebiotics and synbiotics: Safe options for next-generation therapeutics. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 505–521. [CrossRef]
21. Ireson, C.R.; Alavijeh, M.S.; Palmer, A.M.; Fowler, E.R.; Jones, H.J. The role of mouse tumour models in the discovery and development of anticancer drugs. *Br. J. Cancer* **2019**, *121*, 101–108. [CrossRef] [PubMed]
22. Lofgren, J.; Miller, A.L.; Lee, C.C.S.; Bradshaw, C.; Flecknell, P.; Roughan, J. Analgesics promote welfare and sustain tumor growth in orthotopic 4T1 and B16 mouse cancer models. *Lab. Anim.* **2018**, *52*, 351–364. [CrossRef]
23. El-Ashmawy, N.E.; Khedr, E.G.; Ebeid, E.M.; Salem, M.L.; Zidan, A.A.; Mosalam, E.M. Enhanced anticancer effect and reduced toxicity of doxorubicin in combination with thymoquinone released from poly-N-acetyl glucosamine nanomatrix in mice bearing solid Ehrlish carcinoma. *Eur. J. Pharm. Sci. Off. J. Eur. Fed. Pharm. Sci.* **2017**, *109*, 525–532. [CrossRef] [PubMed]
24. Ylikahri, R. Metabolic and nutritional aspects of xylitol. *Adv. Food Res.* **1979**, *25*, 159–180. [CrossRef] [PubMed]
25. DeBerardinis, R.J.; Chandel, N.S. We need to talk about the Warburg effect. *Nat Metab.* **2020**, *2*, 127–129. [CrossRef] [PubMed]
26. Cortinovis, C.; Caloni, F. Household Food Items Toxic to Dogs and Cats. *Front. Vet. Sci.* **2016**, *3*, 26. [CrossRef]
27. Marlowe, F.W.; Berbesque, J.C. Tubers as fallback foods and their impact on Hadza hunter-gatherers. *Am. J. Phys. Anthropol.* **2009**, *140*, 751–758. [CrossRef]
28. Marean, C.W. When the sea saved humanity. *Sci. Am.* **2010**, *303*, 54–61. [CrossRef]
29. Iannotti, L.L.; Gyimah, E.A.; Reid, M.; Chapnick, M.; Cartmill, M.K.; Lutter, C.K.; Hilton, C.; Gildner, T.E.; Quinn, E.A. Child dietary patterns in Homo sapiens evolution: A systematic review. *Evol. Med. Public Health* **2022**, *10*, 371–390. [CrossRef] [PubMed]
30. Olm, M.R.; Dahan, D.; Carter, M.M.; Merrill, B.D.; Yu, F.B.; Jain, S.; Meng, X.; Tripathi, S.; Wastyk, H.; Neff, N.; et al. Robust variation in infant gut microbiome assembly across a spectrum of lifestyles. *Science* **2022**, *376*, 1220–1223. [CrossRef] [PubMed]
31. De Filippo, C.; Cavalieri, D.; Di Paola, M.; Ramazzotti, M.; Poullet, J.B.; Massart, S.; Collini, S.; Pieraccini, G.; Lionetti, P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 14691–14696. [CrossRef]
32. Fan, X.; Liu, H.; Liu, M.; Wang, Y.; Qiu, L.; Cui, Y. Increased utilization of fructose has a positive effect on the development of breast cancer. *PeerJ* **2017**, *5*, e3804. [CrossRef] [PubMed]
33. Ting, K.K.Y. Fructose-induced metabolic reprogramming of cancer cells. *Front. Immunol.* **2024**, *15*, 1375461. [CrossRef] [PubMed]
34. Nakagawa, T.; Lanaspa, M.A.; Millan, I.S.; Fini, M.; Rivard, C.J.; Sanchez-Lozada, L.G.; Andres-Hernando, A.; Tolan, D.R.; Johnson, R.J. Fructose contributes to the Warburg effect for cancer growth. *Cancer Metab.* **2020**, *8*, 16. [CrossRef]
35. Trachootham, D.; Alexandre, J.; Huang, P. Targeting cancer cells by ROS-mediated mechanisms: A radical therapeutic approach? Nature reviews. *Drug Discov.* **2009**, *8*, 579–591. [CrossRef] [PubMed]
36. Niu, B.; Liao, K.; Zhou, Y.; Wen, T.; Quan, G.; Pan, X.; Wu, C. Application of glutathione depletion in cancer therapy: Enhanced ROS-based therapy, ferroptosis, and chemotherapy. *Biomaterials* **2021**, *277*, 121110. [CrossRef] [PubMed]
37. Wen, Y.; Chen, H.; Zhang, L.; Wu, M.; Zhang, F.; Yang, D.; Shen, J.; Chen, J. Glycyrrhetinic acid induces oxidative/nitrative stress and drives ferroptosis through activating NADPH oxidases and iNOS, and depriving glutathione in tri-ple-negative breast cancer cells. *Free Radic. Biol. Med.* **2021**, *173*, 41–51. [CrossRef]
38. Hellstrand, K.; Brune, M.; Naredi, P.; Mellqvist, U.H.; Hansson, M.; Gehlsen, K.R.; Hermodsson, S. Histamine: A novel approach to cancer immunotherapy. *Cancer Investig.* **2000**, *18*, 347–355. [CrossRef] [PubMed]
39. Stoyanov, E.; Uddin, M.; Mankuta, D.; Dubinett, S.M.; Levi-Schaffer, F. Mast cells and histamine enhance the proliferation of non-small cell lung cancer cells. *Lung Cancer* **2012**, *75*, 38–44. [CrossRef]
40. Chakrabarty, R.P.; Chandel, N.S. Mitochondria as Signaling Organelles Control Mammalian Stem Cell Fate. *Cell Stem Cell* **2021**, *28*, 394–408. [CrossRef] [PubMed]
41. Simonsen, T.G.; Gaustad, J.V.; Leinaas, M.N.; Rofstad, E.K. High interstitial fluid pressure is associated with tumor-line specific vascular abnormalities in human melanoma xenografts. *PLoS ONE* **2012**, *7*, e40006. [CrossRef] [PubMed]
42. Shin, G.R.; Kim, H.E.; Kim, J.H.; Choi, S.; Kim, M.S. Advances in Injectable In Situ-Forming Hydrogels for Intra-tumoral Treatment. *Pharmaceutics* **2021**, *13*, 1953. [CrossRef] [PubMed]
43. Gould, H.J., 3rd; Paul, D. Targeted Osmotic Lysis: A Novel Approach to Targeted Cancer Therapies. *Biomedicines* **2022**, *10*, 838. [CrossRef] [PubMed]
44. Liu, Y.X.; Liu, W.J.; Zhang, H.R.; Zhang, Z.W. Delivery of bevacizumab by intracranial injection: Assessment in glioma model. *OncoTargets Ther.* **2018**, *11*, 2673–2683. [CrossRef]
45. Algrafi, A.S.; Jamal, A.A.; Ismaeel, D.M. Microbiota as a New Target in Cancer Pathogenesis and Treatment. *Cureus* **2023**, *15*, e47072. [CrossRef]
46. Wu, H.J.; Wu, E. The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes* **2012**, *3*, 4–14. [CrossRef]

47. Halley, A.; Leonetti, A.; Gregori, A.; Tiseo, M.; Deng, D.M.; Giovannetti, E.; Peters, G.J. The Role of the Microbiome in Cancer and Therapy Efficacy: Focus on Lung Cancer. *Anticancer Res.* **2020**, *40*, 4807–4818. [CrossRef] [PubMed]
48. Zuo, Q.L.; Cai, X.; Zheng, X.Y.; Chen, D.S.; Li, M.; Liu, Z.Q.; Chen, K.Q.; Han, F.F.; Zhu, X. Influences of Xylitol Consumption at Different Dosages on Intestinal Tissues and Gut Microbiota in Rats. *J. Agric. Food Chem.* **2021**, *69*, 12002–12011. [CrossRef]
49. Koppel, N.; Maini Rekdal, V.; Balskus, E.P. Chemical transformation of xenobiotics by the human gut microbiota. *Science* **2017**, *356*, eaag2770. [CrossRef] [PubMed]