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Original Research

Nitrosylcobalamin Selectively Targets Tumors via Cobalamin Uptake and Lysosomal Processing

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Abstract

Background: Nitrosylcobalamin (NO-Cbl) is a vitamin B_{12} analog designed to exploit the "Trojan horse" vulnerability created by the heightened need of cancer cells for cobalamin and one-carbon metabolism. Building on our recent biophysical studies confirming the affinity of NO-Cbl for intrinsic factor, this work aimed to investigate the mechanistic basis for the selective anticancer activity of NO-Cbl through the cobalamin transport axis and lysosomal processing. **Materials and Methods**: Human cancer cell lines (NIH-OVCAR-3, MCF-7, WM9, and DU145) were cultured and transfected to overexpress transcobalamin II (TCII). Cell proliferation and cytotoxicity were measured using the sulforhodamine B (SRB) assay. TCII-R (CD320) expression was quantified by flow cytometry. The impact of anti-CD320 antiserum and lysosomal alkalization (chloroquine) on NO-Cbl activity was assessed. **Results**: Antiserum inhibition of the TCII receptor resulted in dose-dependent inhibition of NIH-OVCAR-3 and MCF-7 cell proliferation. Lysosomal alkalinization by chloroquine pretreatment abrogated NO-Cbl-induced cytotoxicity in OVCAR-3 cells. Flow cytometric analysis demonstrated an inverse correlation between TCII-R (CD320) expression (MFI ratio) and NO-Cbl ID_{50}. TCII overexpression significantly reduced NO-Cbl ID_{50} in NIH-OVCAR-3 cells. **Conclusion**: NO-Cbl utilizes tumor cell cobalamin transport and processing pathways to deliver nitric oxide selectively to cancer cells. These results, integrated with recent binding studies, validate NO-Cbl as a cobalamin-based targeted anticancer agent with efficacy in tumors expressing high levels of TCII and CD320.

Keywords: nitrosylcobalamin; cobalamin; transcobalamin; TCII; CD320; lysosome; cancer

1. Introduction

The pivotal role of vitamin B_{12} (cobalamin, Cbl) in human health was first highlighted in 1926 when Minot and Murphy demonstrated that a liver-rich diet could effectively treat pernicious anemia, suggesting the presence of a specific therapeutic agent in liver tissue [1]. Subsequent research led to the isolation and characterization of this "anti-pernicious anemia" factor. By 1948, Karl Folkers and colleagues achieved the further purification of vitamin B_{12}, identifying it as a cyano-cobalt coordination complex, subsequently named cyanocobalamin [2].

Early modifications of vitamin B_{12} involved substituting the cyano group with various ligands, resulting in derivatives such as hydroxo-, chloro-, bromo-, sulfato-, nitro-, and cyano-cobalamin [3]. These studies laid the groundwork for the concept of modifying vitamin B_{12} to create biologically active analogs. The rationale behind this approach is supported by numerous studies demonstrating that cobalamin is essential for maintaining cellular differentiation, proliferation, and metabolic functions [4]. Therefore, derivatization of cobalamin could yield pharmacologically effective analogs [5].

Cancer cells, by virtue of their relentless proliferation and metabolic rewiring, exhibit a dramatically increased demand for cobalamin and one-carbon metabolism [6–8]. Uptake and intracellular processing of cobalamin are orchestrated by a highly specific molecular relay: absorption with intrinsic factor, plasma transport via transcobalamin II (TCII), cellular internalization through the TCII receptor (CD320), and trafficking into lysosomal compartments for cobalamin release and utilization [9–13].

This cobalamin-centric metabolic axis presents a unique vulnerability, a "gateway", that can be exploited for therapeutic purposes. Tumors upregulate TCII, CD320, and downstream one-carbon metabolic pathways, increasing their reliance on cobalamin for DNA synthesis, methylation, and redox homeostasis [14,15]. Thus, cobalamin-dependent malignancies represent ideal targets for "Trojan horse" strategies that masquerade as cobalamin, infiltrating cancer cells through their own transport machinery.

Nitrosylcobalamin (NO-Cbl), a nitric oxide–modified cobalamin analog, is designed to capitalize on one carbon metabolism in cancer cells [16]. Our recent biophysical studies established that NO-Cbl maintains high-affinity binding to intrinsic factor, indicating preservation of key recognition sites for physiological cobalamin transport proteins [17]. This enabled NO-Cbl to mimic natural B12 culminating in the selective delivery and release of cytotoxic nitric oxide within tumor cells [18].

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The specific aims of this study were to assess possible mechanisms associated with cobalamin uptake, specifically NO-Cbl, in human cancer cell lines. Our goal was to assess the functional blockade of TCII-mediated uptake along with the overexpression of TCII and the effects of lysosomal processing to encourage continued research into one-carbon metabolism as potential exploits for future anticancer treatments.

2. Materials and Methods

2.1 Synthesis of Nitrosylcobalamin

Nitrosylcobalamin was synthesized as previously described [16]. Hydroxocobalamin (vitamin B12a) acetate (Hebei Huarong Pharmaceutical Co. Ltd., Shijiazhuang, Hebei, China) was dissolved in dichloromethane (Omni-Solv, EMD Chemicals, Gibbstown, NJ, USA) and exposed to CP grade NO gas (Praxair, Bethlehem, PA, USA) at 150 PSIG (1034 kPa). The reaction proceeded in a closed system within a high-pressure stainless-steel reactor (Parr Instrument Co., Moline, IL, USA). The system was purged daily and evacuated prior to NO exposure. The NO gas was scrubbed prior to entering the system using a stainless-steel cylinder (Midwest Process Controls, Bay Village, OH, USA) containing NaOH pellets. The solid NO-Cbl product was collected following rotary evaporation of the solvent and stored under argon at –80 °C prior to use.

2.2 Cell Culture and Reagents

Human tumor cell lines: HTB-161, HTB-22, TIB-152 (ATCC, Manassas, VA, USA) and WM9-01-0001 (Rockland Immunochemicals, Inc. Limerick, PA, USA) were grown in RPMI 1640 and Dulbecco's modified Eagle medium (DMEM, Cellgro, Mediatech, Herndon, VA, USA) as appropriate, both supplemented with 10% fetal bovine serum (FBS, Mediatech) and 1% Antibiotic-Antimycotic (GIBCO BRL, Invitrogen, Carlsbad, CA, USA). Cells were maintained in 5% CO_2 at 37 °C in a humidified incubator. Cells were confirmed to be mycoplasma free using a MycoAlert® Mycoplasma Detection Kit (LT07-118; MycoAlert, Lonza, East Rutherford, NJ, USA). The cell lines were purchased from American Type Culture Collection (ATCC) and validated by STR profiling.

TCII cDNA (a gift from Dr. Rothenberg, SUNY Brooklyn, NY, USA) [19,20] was sub-cloned into the pCXN2myc mammalian expression vector using XhoI and BstXI restriction sites. Cells were transfected using Lipofectamine Plus Reagent (Invitrogen); GFP was used as a positive control. Chloroquine diphosphate salt was purchased from MP Biomedicals, Inc. (Solon, OH, USA).

2.3 Sulforhodamine B Cell Growth Assay

Cells were harvested with 0.5% trypsin/0.53 mM EDTA, washed with PBS and resuspended in media containing 10% FBS. Cells were plated in 96-well plates in 0.2 mL aliquots containing 2000 cells. Cells were allowed to adhere to the plate for 4 h and then NO-Cbl was added in different dilutions to the assay plate. Replicates of eight were performed for each treatment. Growth was monitored using a modified sulforhodamine B (SRB, Sigma Chemical, St. Louis, MO, USA) colorimetric assay [21,22].

After 96 h growth, the medium was removed, and cells were fixed with 10% trichloroacetic acid and stained with SRB. Bound dye was eluted from cells with 10 mM TRIS (pH 10.5) and absorbance was measured at 570 nm using a Lab Systems Multiskan RC 96-well plate reader (Lab Systems Multiscan RC, Thermo Lab Systems, Franklin, MA, USA).

To quantify growth of the cells, experimental absorbance values (A_{exp}) were compared with initial absorbance readings representing the starting cell numbers (A_{ini}). To determine the starting cell number, an additional 96-well plate was seeded with cells and fixed at the beginning of the experiment. The absorbances derived from the initial plate and from the untreated cells at the end of the growth period (A_{fin}) were defined as 0% and 100% growth, respectively. The percentage control growth ($100\% \times [A_{exp}–A_{ini}]/[A_{fin}–A_{ini}]$) was expressed as a percentage of untreated controls.

2.4 Antibody Blockade and Lysosomal Alkalization Experiments

Cells were treated with increasing dilutions of anti-TCIIR (CD320) antiserum [23] (1:40 to 1:2000; a gift from Dr. Bellur Seetharam) for 72 hours to assess effects on proliferation. For lysosomal alkalization, cells were pretreated with chloroquine (20 µM) for 30 min prior to NO-Cbl exposure and quantified by SRB.

2.5 Flow Cytometric Analysis of TCII-R

Trypsinized cells (250,000) were resuspended in PBS pH 7.4 and incubated with rabbit anti-human TCII-R polyclonal antibody 1:20 (a gift from Dr. Bellur Seetharam) [23] on ice for 30 min. Cells were washed three times and incubated with Goat Anti-Rabbit IgG (H+L) Antibody (Alexa Fluor® 568 Conjugate, 1:20; #39572; Cell Signaling Technology, Danvers, MA, USA) for 30 min on ice. Normal rabbit serum was used as a negative control. Cells were washed and fixed with 1% paraformaldehyde in PBS pH 7.4. The total cell population was examined for TCII-R positivity analyzed as mean fluorescent intensity (MFI) compared to controls and analyzed by flow cytometry (EPICS XL-MCL using EXPO32 ADC software, Beckman Coulter, Miami, FL, USA).

2.6 Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM) unless otherwise specified. To evaluate the effect of TCII-R (CD320) blockade on cell proliferation, Pearson correlation coefficients were calculated to quantify the relationship between antibody dilution and per-

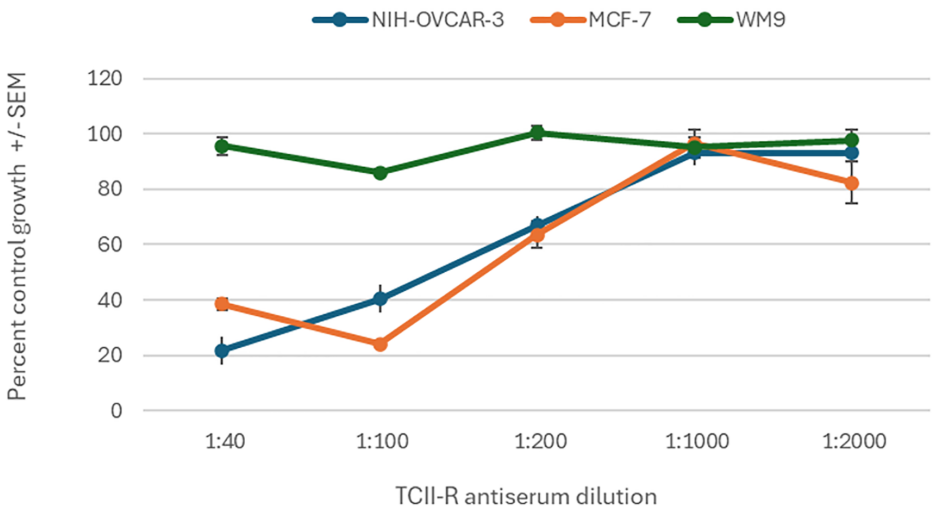

Fig. 1. Effect of TCII-R antiserum on percent control growth in NIH-OVCAR-3, MCF-7, and WM9 cell lines. Cells were treated for 72 hours with serial dilutions of anti-TCII-R (CD320) antiserum (1:40 to 1:2000), and cell proliferation was measured by the SRB assay. Percent control growth (mean ± SEM) is shown for each cell line at each dilution. Increasing concentrations of TCII-R antiserum (lower dilution numbers) resulted in a concentration-dependent inhibition of cell growth, most notably in NIH-OVCAR-3 and MCF-7 cells. SEM, standard error of the mean; SRB, sulforhodamine B.

cent control growth for each cell line in the SRB assay. For lysosomal alkalization studies, comparisons between NO-Cbl alone and chloroquine plus NO-Cbl groups at each time point were analyzed using unpaired *t*-tests. Flow cytometric data were analyzed by calculating the mean fluorescence intensity (MFI) ratio (primary + secondary compared to secondary only) for each cell line, and the correlation between TCII-R expression and NO-Cbl ID_{50} was assessed using Pearson correlation with two-tailed significance testing. Differences between wild-type and TCII-overexpressing groups were determined using Student's *t* test. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using Microsoft Excel.

3. Results

3.1 Blockade of TCII-R (CD320) Inhibits Cancer Cell Growth in a Dose-Dependent Manner

Treatment of NIH-OVCAR-3, MCF-7, and WM9 cell lines with serial dilutions of anti-TCII-R (CD320) antiserum (1:40 to 1:2000) for 72 hours resulted in a marked, concentration-dependent inhibition of cell proliferation as measured by the SRB assay (Fig. 1). The greatest inhibition was observed at the highest antibody concentrations (lowest dilution), with OVCAR-3 and MCF-7 exhibiting the most pronounced response.

3.2 Lysosomal Alkalization Protects Against NO-Cbl–Induced Cytotoxicity

To investigate the role of lysosomal processing in NO-Cbl cytotoxicity, OVCAR-3 cells were pretreated with chloroquine (CQ, 20 μM) for 30 minutes, followed by pulsed NO-Cbl exposure (3, 4, or 5 hours). After 96 hours, cell growth was assessed by the SRB assay (Fig. 2). Chloroquine pretreatment significantly protected against NO-Cbl-induced cell death at all time points, with percent control growth restored to baseline levels in CQ+NO-Cbl groups compared to robust cytotoxicity from NO-Cbl alone ($p < 0.001$, unpaired *t*-test at each time point).

3.3 TCII-R (CD320) Expression Predicts NO-Cbl Sensitivity Across Cancer Cell Lines

Flow cytometric analysis of Jurkat, NIH-OVCAR-3, MCF-7, and WM9 cells revealed variable expression of TCII-R (CD320), measured as the ratio of mean fluorescence intensity (MFI) for primary + secondary antibody versus secondary antibody alone. Representative histograms are shown for each cell line (Fig. 3A–D). Quantitative analysis (Fig. 3E) demonstrated a negative correlation between TCII-R relative fluorescence (MFI ratio) and NO-Cbl ID_{50} values (Pearson r = –0.92, $p = 0.0779$). Specifically, cell lines with higher TCII-R expression were more sensitive to NO-Cbl-induced growth inhibition.

3.4 TCII Overexpression Sensitizes Cancer Cells to NO-Cbl

To directly test the functional consequence of increased TCII expression, SRB dose–response assays were performed in wild-type and TCII-transfected OVCAR-3, WM9 and DU145 cells (Fig. 4). Overexpression of TCII increased sensitivity to NO-Cbl, as evidenced by a substantial reduction in ID_{50} values across cell lines.

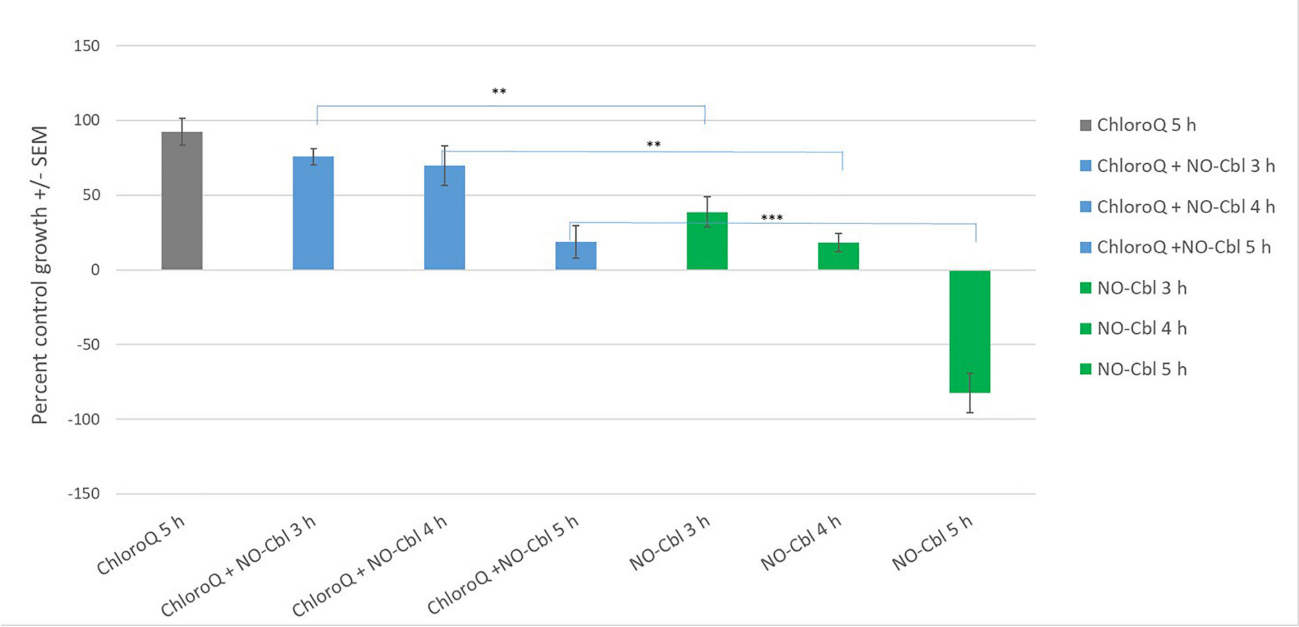

Fig. 2. Chloroquine protects OVCAR-3 cells from NO-Cbl–mediated cytotoxicity. Percent control growth (mean ± SEM) was determined using the SRB assay after 96 hours. OVCAR-3 cells were treated with NO-Cbl alone (NO-Cbl 3 h, 4 h, 5 h) or pretreated with chloroquine (CQ) for 30 minutes prior to pulsed exposure to NO-Cbl (CQ+NO-Cbl 3 h, 4 h, 5 h). Additional controls included CQ alone (CQ 5 h). Chloroquine pretreatment resulted in a significant, time-dependent protection against NO-Cbl-induced cell death at all tested time points (**$p < 0.001$; ***$p < 0.0001$ unpaired *t*-test). Negative percent control growth values indicate cell death. Error bars represent SEM. Statistical significance was calculated between each NO-Cbl and the corresponding CQ+NO-Cbl group for each pulse duration. NO-Cbl, Nitrosylcobalamin.

4. Discussion

This study demonstrates that nitrosylcobalamin (NO-Cbl) leverages the native cobalamin transport and processing machinery—particularly transcobalamin II (TCII) and its receptor (TCII-R/CD320)—to achieve potent and selective antitumor activity, confirming the "Trojan horse" hypothesis. Various components of the cobalamin uptake and utilization pathway, long understood for its essentiality in normal cell function and disease [9,24] is revealed here as a mechanistic determinant for NO-Cbl sensitivity in tumor cells.

Our first line of evidence comes from antibody-mediated blockade of TCII-R (Fig. 1), where we observed a robust, dilution-dependent inhibition of cell proliferation in NIH-OVCAR-3 and MCF-7 cell lines. WM9 cells were unresponsive which is consistent with non-TCII mechanisms associated with cobalamin uptake by melanoma [25]. These findings are consistent with earlier studies demonstrating that antibody or genetic disruption of the cobalamin uptake machinery inhibits tumor cell growth and DNA synthesis [9], and further support the notion that tumor dependence on TCII-mediated B_{12} import can be therapeutically exploited. Notably, our findings expand on work by others that show TCII-R blockade limits vitamin B_{12} utilization [26,27] by demonstrating the same vulnerability with an engineered cobalamin analog.

Secondly, our lysosomal alkalinization experiments (Fig. 2) demonstrate the effects of chloroquine pretreatment, by neutralizing the acidic environment of the lysosome, to protect OVCAR-3 cells from NO-Cbl-induced cytotoxicity at all pulse durations. For example, NO-Cbl alone caused a pulse-duration-dependent decrease in cell growth (to –82.5% at 5 hours), while chloroquine plus NO-Cbl resulted in protection (69.8–92.4% growth across all time points, $p < 0.001$). These results confirm that lysosomal acidification is needed for NO-Cbl activation and cytotoxicity, supporting classical models of cobalamin metabolism where lysosomal processing is required to liberate B_{12} from its transport protein [28].

Third, flow cytometric analysis (Fig. 3) revealed a negative correlation between TCII-R expression (measured as MFI ratio) and NO-Cbl ID_{50} across four cell lines (Pearson r = –0.92). Jurkat cells (high TCII-R) were most sensitive (ID_{50} = 7.5 µM), while WM9 (lowest TCII-R) were most resistant (ID_{50} = 22 µM). This aligns with recent findings in both clinical and preclinical studies, where CD320 (TCII-R) expression is upregulated in proliferative tissues and in a wide variety of cancers, correlating with cobalamin demand and malignant potential [7,29,30].

Finally, our TCII transfection experiments (Fig. 4) show that expression of TCII in OVCAR-3, WM9, and DU145 cells reduced the NO-Cbl ID_{50} (the concentration

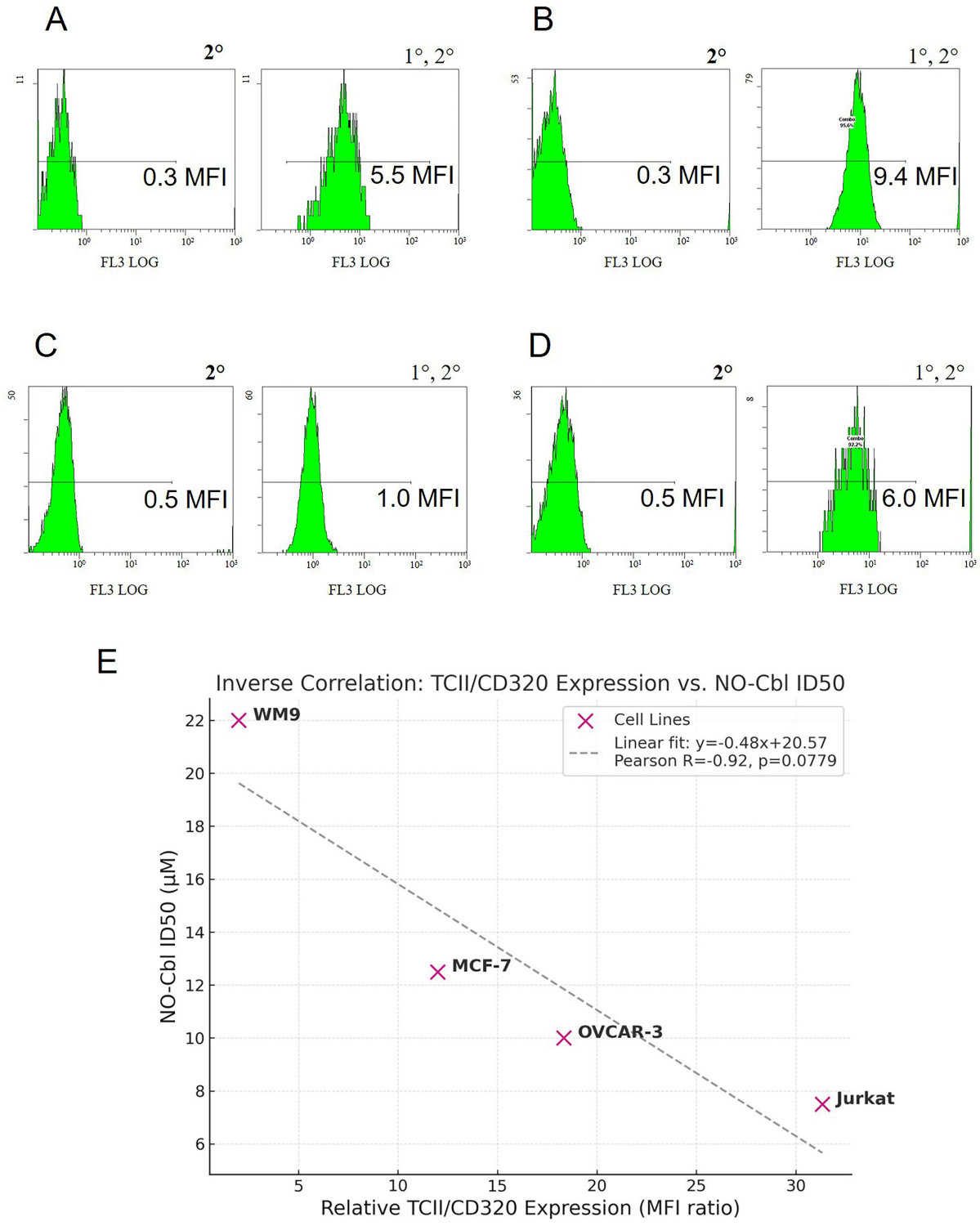

Fig. 3. Relationship between relative TCII-R (CD320) expression and NO-Cbl sensitivity in human cancer cell lines. (A–D) Representative flow cytometry histograms for OVCAR-3 (A), Jurkat (B), WM9 (C) and MCF-7 (D) cells stained with secondary antibody only (left) or with both anti-CD320 primary and secondary antibody (right). (E) Correlation between relative TCII-R expression and NO-Cbl ID_{50} values for the four cell lines. Pearson's r = –0.92, p = 0.0779. Higher TCII-R relative fluorescence is associated with greater NO-Cbl sensitivity. Relative MFI was calculated as the mean fluorescence intensity (MFI) of the CD320-stained sample divided by the MFI of the secondary-only control.

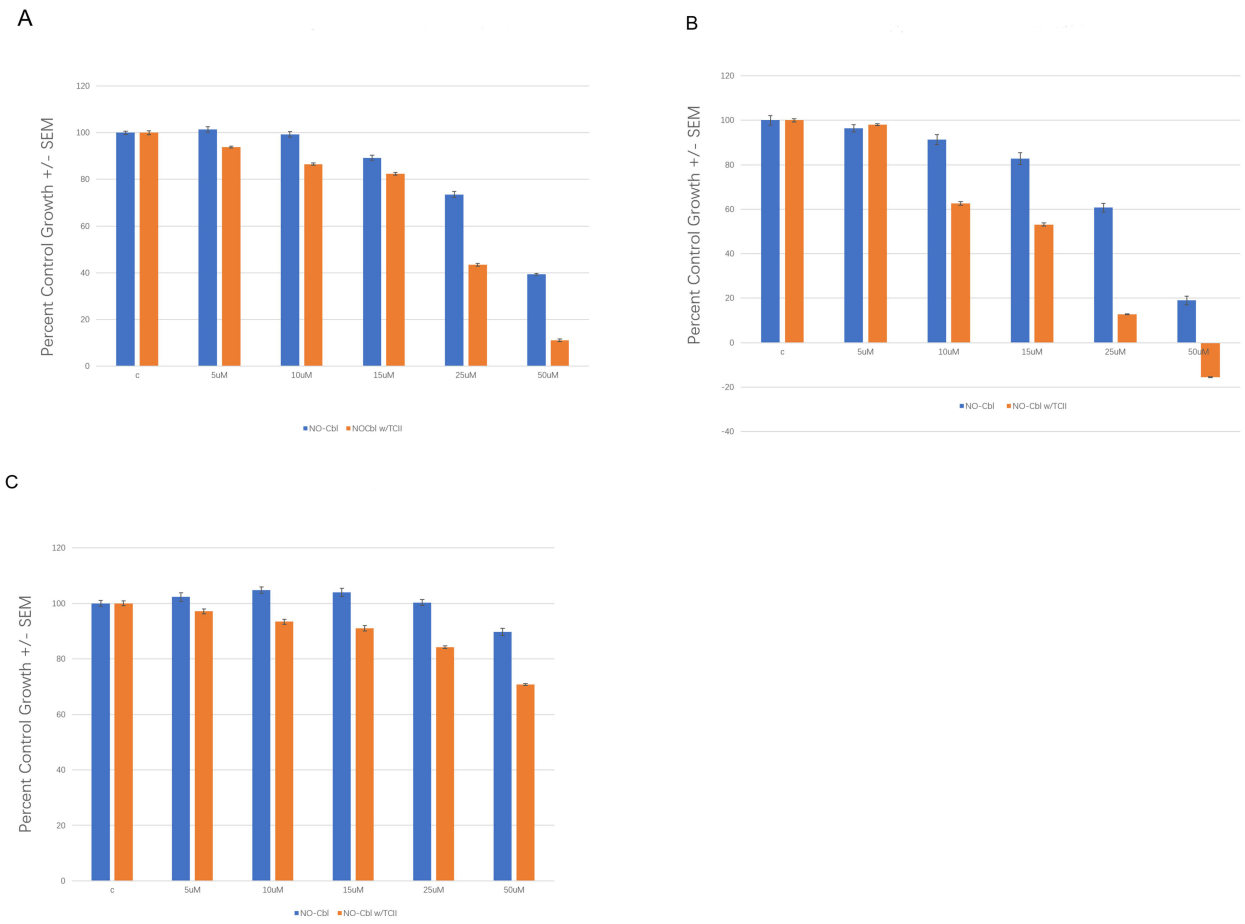

Fig. 4. Sensitization to NO-Cbl by TCII Overexpression in Human Cancer Cell Lines. An SRB assay dose–response using NO-Cbl was performed in TCII transfected cells compared to wild type. (A) OVCAR-3. (B) WM9. (C) DU145.

needed to inhibit cell growth by 50%). This is in agreement with animal and tissue studies that show TCII upregulation in tumors and in endothelial cells within the tumor microenvironment [14].

Taken together, our data support a model in which NO-Cbl exploits the cobalamin pathway to selectively target tumor cells. This "Trojan horse" mechanism requires that an upper-axial (β-position) modification preserve TCII (and IF) recognition and downstream trafficking/processing, consistent with functional studies showing β-position substitutions do not disrupt TCII or IF binding and intracellular conversion [31–34]. In line with this requirement, our spectral and chemical analyses confirm NO-Cbl synthesis [35], and our biological data demonstrate antitumor activity with intratumoral NO release in cobalamin-avid tumors [18]. Independent work using radiolabeled B12 conjugates for tumor imaging and targeted delivery further supports the translational premise of physiologically trafficked cobalamin-based platforms [27,36–42].

When considering clinical translation, it is important to note that normal human plasma cobalamin concentrations are in the nanomolar to picomolar range and rarely exceed ~10 µM even with high-dose parenteral therapy. While our prior murine xenograft study [18] demonstrated no histologic evidence of toxicity after 54 days of daily dosing at 100 mg/kg, and our long-term canine case series [43] tracked up to 61 months of daily NO-Cbl treatment without clinically significant adverse effects, these supraphysiologic exposures may not fully predict human tolerance. Careful, stepwise dose-escalation studies will therefore be required to define the maximum tolerated dose (MTD) and confirm safety under physiological conditions.

5. Conclusion

Our work helps elucidate the mechanism of NO-Cbl action in cancer suggesting new opportunities for biomarker-driven, precision therapies using vitamin B_{12} analogs. Future directions include the exploration of biomarkers of cobalamin pathway activity to guide patient selection, combination approaches with lysosome-targeting agents, and investigation of resistance mechanisms. Future clinical development should incorporate careful dose-finding studies to establish safety parameters in humans, recognizing that physiological plasma cobalamin levels dif-

fer substantially from those achieved in experimental systems. These steps will be essential to translate the favorable preclinical profile into a safe and effective therapeutic strategy.

Availability of Data and Materials

The data sets used and analyzed during the current study are available from the corresponding author upon request.

Author Contributions

JAB designed the research study, conducted experiments, and performed statistical analysis. JAB and AMS wrote and edited the manuscript, analyzed the data and created the figures. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and have agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

JAB is employed by Nitric Oxide Services, LLC. AMS is affiliated with the Bauer Research Foundation, Inc. However, the judgments in data interpretation and manuscript writing were not influenced by these affiliations. Both authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

ChatGPT 4o was used as an editing tool to create a more concise structure. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

[1] Minot GR, Murphy WP. Landmark article (JAMA 1926). Treatment of pernicious anemia by a special diet. By George R. Minot and William P. Murphy. JAMA. 1983; 250: 3328–3335. https://doi.org/10.1001/jama.250.24.3328.

[2] KACZKA E, WOLF DE, FOLKERS K. Identification of crystalline vitamin B12a. Journal of the American Chemical Society. 1949; 71: 1514. https://doi.org/10.1021/ja01172a527.

[3] KACZKA EA, WOLF DE, KUEHL FA, Jr, FOLKERS K. Vitamin B12: reactions of cyano-cobalamin and related compounds. Science (New York, N.Y.). 1950; 112: 354–355. https://doi.org/10.1126/science.112.2909.354-a.

[4] O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010; 2: 299–316. https://doi.org/10.3390/nu2030299.

[5] Linnell JC, Matthews DM. Cobalamin metabolism and its clinical aspects. Clinical Science (London, England: 1979). 1984; 66: 113–121. https://doi.org/10.1042/cs0660113.

[6] Locasale JW. Serine, glycine and one-carbon units: cancer metabolism in full circle. Nature Reviews. Cancer. 2013; 13: 572–583. https://doi.org/10.1038/nrc3557.

[7] Zhang S, Jiang Z, Wang P, Jiang W, Ding W, Zhong L. TCBIR/CD320: a potential therapeutic target upregulated in endothelial cells and associated with immune cell infiltration in liver hepatocellular carcinoma. Discover Oncology. 2024; 15: 255. https://doi.org/10.1007/s12672-024-01122-w.

[8] Kim YI. Folate and cancer: a tale of Dr. Jekyll and Mr. Hyde? The American Journal of Clinical Nutrition. 2018; 107: 139–142. https://doi.org/10.1093/ajcn/nqx076.

[9] Seetharam B, Yammani RR. Cobalamin transport proteins and their cell-surface receptors. Expert Reviews in Molecular Medicine. 2003; 5: 1–18. https://doi.org/10.1017/S1462399403006422.

[10] Quadros EV, Sequeira JM. Cellular uptake of cobalamin: transcobalamin and the TCblR/CD320 receptor. Biochimie. 2013; 95: 1008–1018. https://doi.org/10.1016/j.biochi.2013.02.004.

[11] Seetharam B. Receptor-mediated endocytosis of cobalamin (vitamin B12). Annual Review of Nutrition. 1999; 19: 173–195. https://doi.org/10.1146/annurev.nutr.19.1.173.

[12] Carmel R, Neely SM, Francis RB, Jr. Human umbilical vein endothelial cells secrete transcobalamin II. Blood. 1990; 75: 251–254.

[13] Jiang W, Nakayama Y, Sequeira JM, Quadros EV. Characterizing monoclonal antibodies to antigenic domains of TCblR/CD320, the receptor for cellular uptake of transcobalamin-bound cobalamin. Drug Delivery. 2011; 18: 74–78. https://doi.org/10.3109/10717544.2010.509745.

[14] Lacombe V, Lenaers G, Urbanski G. Diagnostic and Therapeutic Perspectives Associated to Cobalamin-Dependent Metabolism and Transcobalamins' Synthesis in Solid Cancers. Nutrients. 2022; 14: 2058. https://doi.org/10.3390/nu14102058.

[15] Lyon P, Strippoli V, Fang B, Cimmino L. B Vitamins and One-Carbon Metabolism: Implications in Human Health and Disease. Nutrients. 2020; 12: 2867. https://doi.org/10.3390/nu12092867.

[16] Bauer JA. Synthesis, characterization and nitric oxide release profile of nitrosylcobalamin: a potential chemotherapeutic

agent. Anti-cancer Drugs. 1998; 9: 239–244. https://doi.org/10.1097/00001813-199803000-00006.

[17] Sysel AM, Bauer JA. Evaluation of the Binding Affinity of Nitrosylcobalamin to Intrinsic Factor as a Predictive Model for Cobalamin Binding Protein Interactions: A Comparative Study with Hydroxocobalamin. Frontiers in Bioscience (Elite Edition). 2025; 17: 26810. https://doi.org/10.31083/FBE26810.

[18] Bauer JA, Morrison BH, Grane RW, Jacobs BS, Dabney S, Gamero AM, *et al*. Effects of interferon beta on transcobalamin II-receptor expression and antitumor activity of nitrosylcobalamin. Journal of the National Cancer Institute. 2002; 94: 1010–1019. https://doi.org/10.1093/jnci/94.13.1010.

[19] Quadros EV, Rothenberg SP, Pan YC, Stein S. Purification and molecular characterization of human transcobalamin II. The Journal of Biological Chemistry. 1986; 261: 15455–15460.

[20] Quadros EV, Rothenberg SP, McLoughlin P. Characterization of monoclonal antibodies to epitopes of human transcobalamin II. Biochemical and Biophysical Research Communications. 1996; 222: 149–154. https://doi.org/10.1006/bbrc.1996.0713.

[21] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al*. New colorimetric cytotoxicity assay for anticancer-drug screening. Journal of the National Cancer Institute. 1990; 82: 1107–1112. https://doi.org/10.1093/jnci/82.13.1107.

[22] Bauer JA, Sysel AM, Heston AJ, Dunphy MJ. Standardized sulforhodamine b colorimetric cell proliferation assay for anticancer activity screening in educational and research laboratories. MethodsX. 2025; 15: 103469. https://doi.org/10.1016/j.mex.2025.103469.

[23] Bose S, Feix J, Seetharam S, Seetharam B. Dimerization of transcobalamin II receptor. Requirement of a structurally ordered lipid bilayer. The Journal of Biological Chemistry. 1996; 271: 11718–11725. https://doi.org/10.1074/jbc.271.20.11718.

[24] Selhub J. Folate, vitamin B12 and vitamin B6 and one carbon metabolism. The Journal of Nutrition, Health & Aging. 2002; 6: 39–42.

[25] Waibel R, Treichler H, Schaefer NG, van Staveren DR, Mundwiler S, Kunze S, *et al*. New derivatives of vitamin B12 show preferential targeting of tumors. Cancer Research. 2008; 68: 2904–2911. https://doi.org/10.1158/0008-5472.CAN-07-6771.

[26] Pluvinage JV, Ngo T, Fouassier C, McDonagh M, Holmes BB, Bartley CM, *et al*. Transcobalamin receptor antibodies in autoimmune vitamin B12 central deficiency. Science Translational Medicine. 2024; 16: eadl3758. https://doi.org/10.1126/scitranslmed.adl3758.

[27] Quadros EV, Nakayama Y, Sequeira JM. Saporin Conjugated Monoclonal Antibody to the Transcobalamin Receptor TCblR/*CD*320 Is Effective in Targeting and Destroying Cancer Cells. Journal of Cancer Therapy. 2013; 4: 1074–1081. https://doi.org/10.4236/jct.2013.46122.

[28] Zhao H, Ruberu K, Li H, Garner B. Perturbation of neuronal cobalamin transport by lysosomal enzyme inhibition. Bioscience Reports. 2014; 34: e00092. https://doi.org/10.1042/BSR20130130.

[29] Sysel AM, Valli VE, Bauer JA. Immunohistochemical quantification of the cobalamin transport protein, cell surface receptor and Ki-67 in naturally occurring canine and feline malignant tumors and in adjacent normal tissues. Oncotarget. 2015; 6: 2331–2348. https://doi.org/10.18632/oncotarget.3206.

[30] Sysel AM, Valli VE, Nagle RB, Bauer JA. Immunohistochemical quantification of the vitamin B12 transport protein (TCII), cell surface receptor (TCII-R) and Ki-67 in human tumor xenografts. Anticancer Research. 2013; 33: 4203–4212.

[31] Kolhouse JF, Allen RH. Absorption, plasma transport, and cellular retention of cobalamin analogues in the rabbit. Evidence for the existence of multiple mechanisms that prevent the absorption and tissue dissemination of naturally occurring cobalamin analogues. The Journal of Clinical Investigation. 1977; 60: 1381–1392. https://doi.org/10.1172/JCI108899.

[32] Allen RH, Seetharam B, Allen NC, Podell ER, Alpers DH. Correction of cobalamin malabsorption in pancreatic insufficiency with a cobalamin analogue that binds with high affinity to R protein but not to intrinsic factor. In vivo evidence that a failure to partially degrade R protein is responsible for cobalamin malabsorption in pancreatic insufficiency. The Journal of Clinical Investigation. 1978; 61: 1628–1634. https://doi.org/10.1172/JCI109083.

[33] McLean GR, Pathare PM, Wilbur DS, Morgan AC, Woodhouse CS, Schrader JW, *et al*. Cobalamin analogues modulate the growth of leukemia cells in vitro. Cancer Research. 1997; 57: 4015–4022.

[34] Hogenkamp HP, Collins DA, Live D, Benson LM, Naylor S. Synthesis and characterization of nido-carborane-cobalamin conjugates. Nuclear Medicine and Biology. 2000; 27: 89–92. https://doi.org/10.1016/s0969-8051(99)00081-5.

[35] Dunphy MJ, Sysel AM, Lupica JA, Griffith K, Sherrod T, Bauer JA. A Stability-Indicating HPLC Method for the Determination of Nitrosylcobalamin (NO-Cbl), a Novel Vitamin B_{12} Analog. Chromatographia. 2014; 77: 571–589. https://doi.org/10.1007/s10337-014-2645-5.

[36] Collins DA, Hogenkamp HP, O'Connor MK, Naylor S, Benson LM, Hardyman TJ, *et al*. Biodistribution of radiolabeled adenosylcobalamin in patients diagnosed with various malignancies. Mayo Clinic Proceedings. 2000; 75: 568–580. https://doi.org/10.4065/75.6.568.

[37] Gendron LN, Zites DC, LaRochelle EPM, Gunn JR, Pogue BW, Shell TA, *et al*. Tumor targeting vitamin B12 derivatives for X-ray induced treatment of pancreatic adenocarcinoma. Photodiagnosis and Photodynamic Therapy. 2020; 30: 101637. https://doi.org/10.1016/j.pdpdt.2019.101637.

[38] Collins DA. Imaging Cobalamin Uptake within Malignant Breast Tumors In Vivo. Molecular Imaging and Biology. 2019; 21: 356–367. https://doi.org/10.1007/s11307-018-1232-9.

[39] Collins DA, Hogenkamp HP. Transcobalamin II receptor imaging via radiolabeled diethylene-triaminepentaacetate cobalamin analogs. Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine. 1997; 38: 717–723.

[40] Collins DA, Hogenkamp HP, Gebhard MW. Tumor imaging via indium 111-labeled DTPA-adenosylcobalamin. Mayo Clinic Proceedings. 1999; 74: 687–691. https://doi.org/10.4065/74.7.687.

[41] Ikotun OF, Marquez BV, Fazen CH, Kahkoska AR, Doyle RP, Lapi SE. Investigation of a vitamin B12 conjugate as a PET imaging probe. ChemMedChem. 2014; 9: 1244–1251. https://doi.org/10.1002/cmdc.201400048.

[42] Sah BR, Schibli R, Waibel R, von Boehmer L, Bläuenstein P, Nexo E, *et al*. Tumor imaging in patients with advanced tumors using a new (99m) Tc-radiolabeled vitamin B12 derivative. Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine. 2014; 55: 43–49. https://doi.org/10.2967/jnumed.113.122499.

[43] Bauer JA, Frye G, Bahr A, Gieg J, Brofman P. Anti-tumor effects of nitrosylcobalamin against spontaneous tumors in dogs. Investigational New Drugs. 2010; 28: 694–702. https://doi.org/10.1007/s10637-009-9282-0.