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INVITRO EVALUATION OF ISONIAZID AND ITS DERIVATIVE (ISONIAZID GLUCURONATE SODIUM ANHYDROUS) FOR THE TREATMENT OF TUBERCULOSIS

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ABSTRACT

Aim: To evaluate the cytotoxic and anti-angiogenic properties of Isoniazid glucuronate sodium anhydrous on colorectal TB HT-29 cells and HUVEC endothelial cells. **Objective**: The study aimed to investigate the effects of Isoniazid glucuronate sodium anhydrous on cell viability, tube formation, and the expression of proteins involved in angiogenesis, such as NF-κB and VEGF-A, using MTT assay, Tubulogenesis assay, Indirect Immunofluorescence assay, and Western Blot analysis. **Research**: The MTT assay demonstrated that treatment with Isoniazid glucuronate sodium significantly decreased cell viability in HT-29 and HUVEC cells. The Tubulogenesis assay revealed a reduction in the formation of tube-like structures, suggesting inhibition of angiogenesis. Indirect Immunofluorescence and Western Blot analysis showed downregulation of NF-κB and VEGF-A in HT-29 cells and VEGFR-2 in HUVEC cells. These results indicate that Isoniazid glucuronate sodium affects key regulatory pathways involved in TB cell proliferation and angiogenesis. **Conclusion**: Isoniazid glucuronate sodium anhydrous exhibited significant cytotoxic and anti-angiogenic activity by inhibiting cell proliferation, reducing tube formation, and downregulating the expression of proteins critical for TB progression. This suggests its potential as a novel therapeutic agent for TB treatment, warranting further in vivo and clinical studies to confirm its efficacy.

KEYWORDS

1. Isoniazid Glucuronate Sodium
2. AntiTB Activity
3. Anti-Angiogenesis

INTRODUCTION

The development of new therapeutic agents targeting TB cells remains a significant challenge in oncology. Isoniazid glucuronate sodium anhydrous, a novel derivative of isoniazid, has shown potential antiTB effects through its ability to interfere with cellular mechanisms involved in TB cell proliferation and angiogenesis. Understanding the biological effects of this compound on TBous cells and endothelial cells is crucial for determining its potential as a therapeutic agent.

This study aimed to investigate the cytotoxic and anti-angiogenic properties of Isoniazid glucuronate sodium anhydrous in colorectal TB HT-29 cells and HUVEC endothelial cells. The MTT assay was employed to determine cell viability, while the Tubulogenesis assay evaluated its effects on tube formation. Additionally, the expression of key angiogenic and proliferative markers such as NF-κB, VEGF-A, and VEGFR-2 were measured using Indirect Immunofluorescence and Western Blot analysis. The findings could contribute to the development of new TB therapies targeting both TB cell growth and angiogenesis.

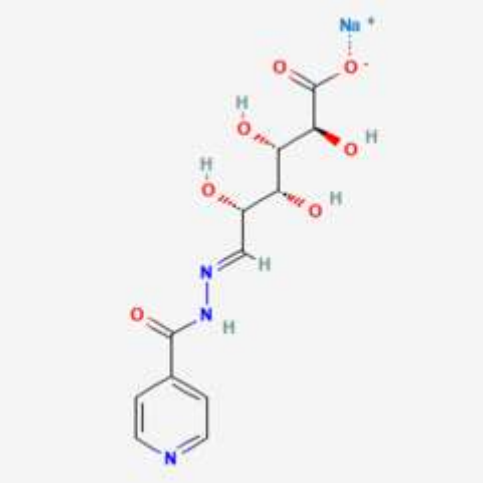
Compounds Used for the study

Isoniazid glucuronate sodium anhydrous

Molecular Formula C12H14N3NaO7

IUPAC Name

sodium;(2*S*,3*S*,4*R*,5*S*,6*E*)-2,3,4,5-tetrahydroxy-6-(pyridine-4- carbonylhydrazinylidene)hexanoate.



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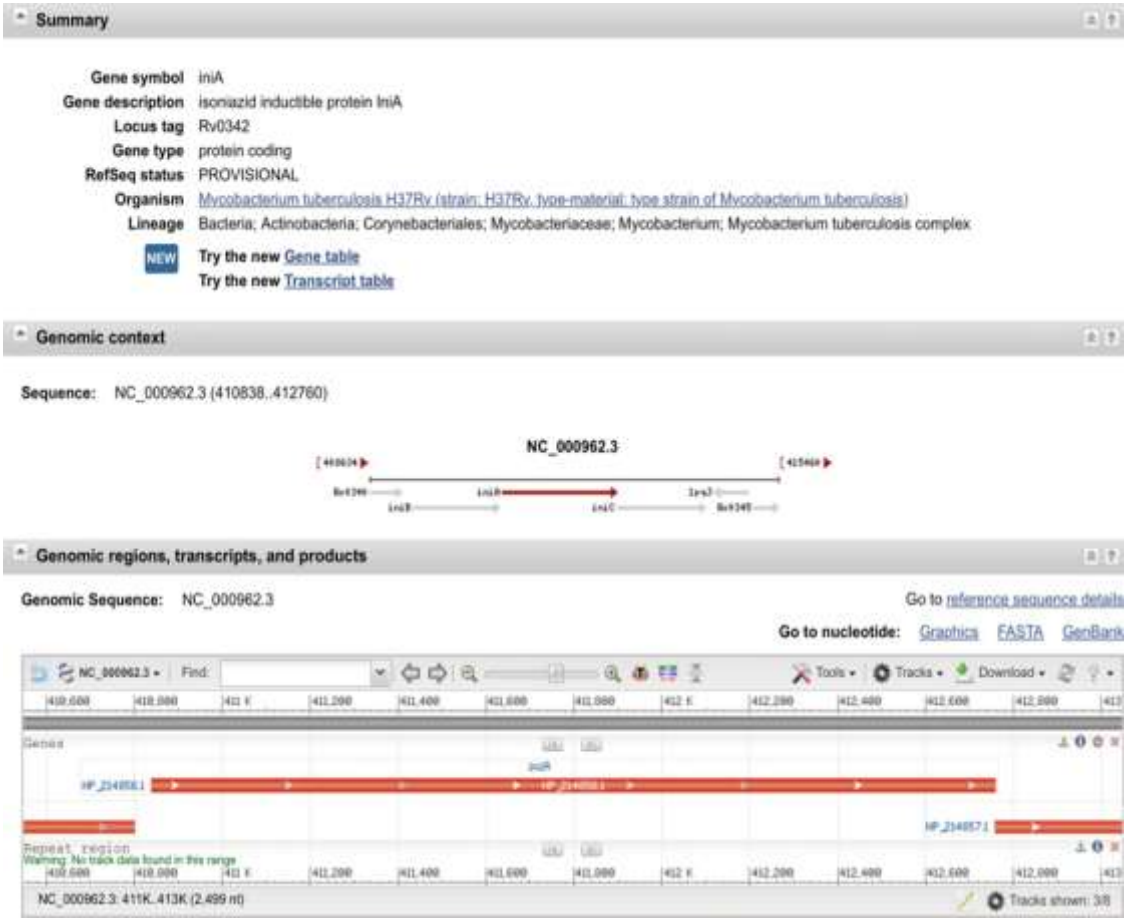


Assays to be performed

MTT Assay

The cell proliferation of the HT-29 and HUVEC cells were evaluated by using a 3-(4,5- dimethythiazol-2-yl)-2,5 diphenyltetrasodium bromide (MTT) reagent. For the HT-29 cells, 1.5 × 104 cells, and for the HUVEC, 1.0 × 104 cells were seeded in a 96-well plates and incubated at 37 ◦C in a humidified atmosphere with 5% CO2 for 24 h. Then, the media were changed to serum-free media, which contained ISONIAZID at various concentrations and incubated for 24 h. The old media were replaced by 100 µL of media, which contained MTT solution, and incubated for 2 h before 100 µL of DMSO was added. The results were detected at 570 nm with a microplate reader (1420 victor, Wallac (Boston, MA, USA)).

Tubulogenesis Assay

Matrigel solution was added into 96-well plates and incubated at 37 ◦C for 30 min. For the HUVEC cells, 8 × 103 cells were resuspended in the HT-29 conditioned media with or without 5 and 10 µg/mL of ISONIAZID. Then, the HUVEC cells, 8 × 103 cells, were seeded onto a layer of Matrigel and incubated for 6 h. Tubular structures on the Matrigel were photographed from 3

randomly chosen fields. The total length of each tube per area was measured and analyzed by Image J software with an angiogenic analyzer.

Indirect Immunofluorescence Assay

Indirect immunofluorescence (IFA) was used to measure NF-κB p65 and VEGF-A expression in the HT-29 cells and VEGFR-2 expression in HUVECs. For the HT-29 cells, 4 × 104 cells were seeded on coverslips and placed at the bottom of 6-well plates. They were incubated at 37 ◦C with 5% CO2 for 48 hours, after which, serum-free media containing 5 or 10 µg/mL ISONIAZID were added and then incubated for another 24 h. The HT-29 cells were fixed with cold methanol, permeabilized with 0.25% Triton X-100, and then a primary antibody; including anti-NF-kB (1:1000), anti-VEGF-A (1:1000), and anti-VEGFR-2 (1:1000) was added. This was then incubated for 1.5 hours before a secondary antibody was added and incubated for another 30 min. Hoechst-33342 in dilution 1:500 was used for counterstaining for 15 min. For the HUVECs, 5 × 104 cells were seeded on coverslips and co-cultured with HT-29 cells as previously described. Then, the coverslips of HUVEC cells were harvested and fixed for immunostaining as previously described as above. The cells were observed under a fluorescence microscope (Olympus BX53, Japan) at the excitation and emission wavelength of 490/515 nm and the results are presented as the mean intensity of fluorescence that was analyzed by 3 random fields in triplicate.

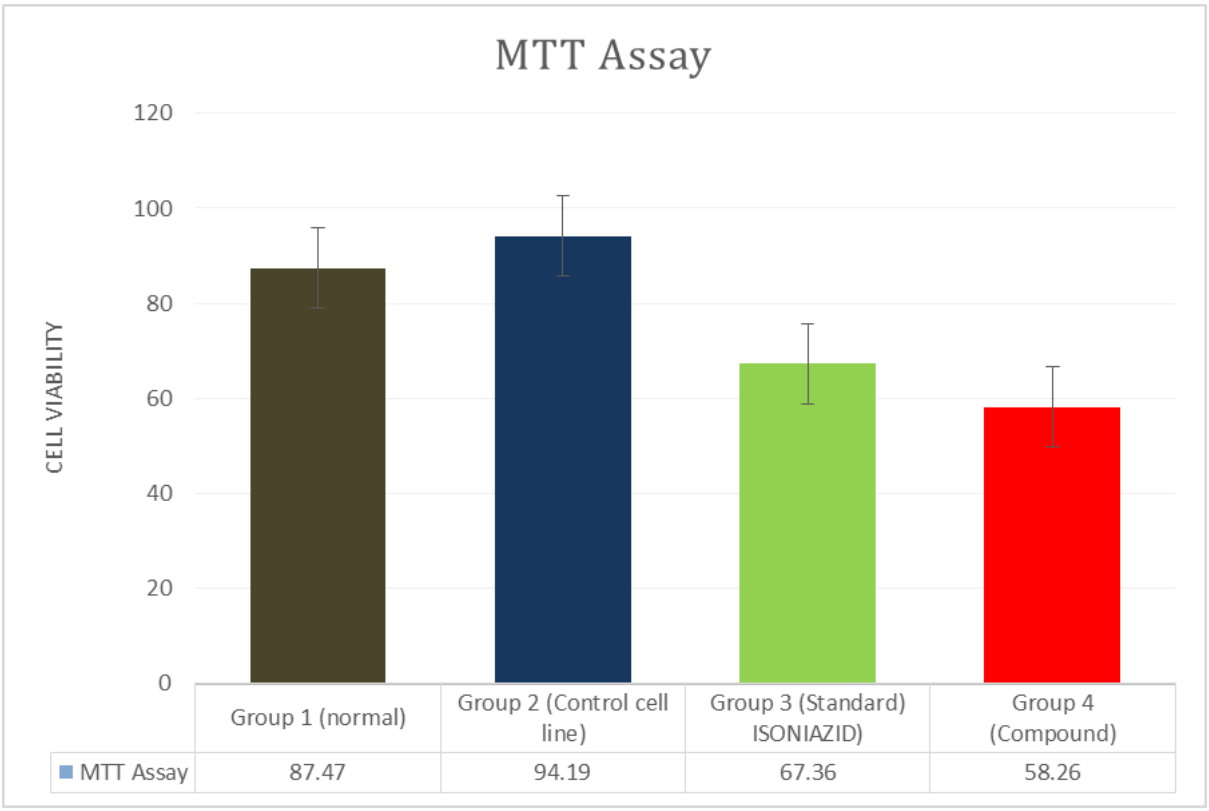
Western Blot Analysis

Total protein was obtained from the HT-29 cells treated with ISONIAZID at concentrations of 5 and 10 µg/mL by using a cold RIPA buffer and scratched the cells. Then, the protein extracts were collected and centrifuged with 4 ◦C and 12,000 rpm. The supernatants were collected and measured protein concentration by using the Bradford assay. Then, NF-κB p65 and VEGF-A were detected by the Jess Simple Western System, a ProteinSimple automated Western blot system, under the principle of Western blot analysis with a specific capillary vacuum system in accordance with the instructions. Briefly, lysate proteins 2 µg were loaded for separating and then transferring in the capillaries containing the matrix gel. Afterwards, the surface was blocked and then probed with primary antibodies; including anti-NF-kB (1:1000) and anti-VEGF-A (1:1000) and then detected with HRP-conjugated secondary antibodies. The signals were developed, and the image was acquired for the pattern of protein separation according to molecular weight. β-actin was used as a loading control.

RESULTS

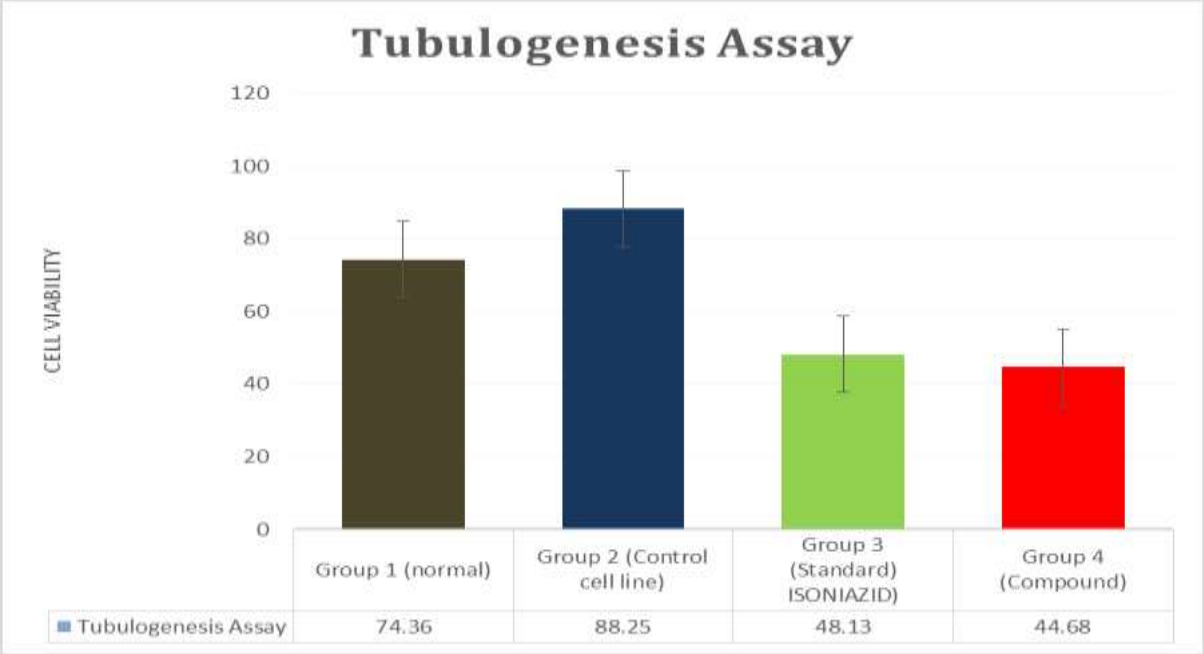
MTT Assay

Treatments	MTT Assay
Group 1 (normal)	87.47
Group 2 (Control cell line)	94.19
Group 3 (Standard) ISONIAZID	67.36
Group 4 (Compound)	58.26



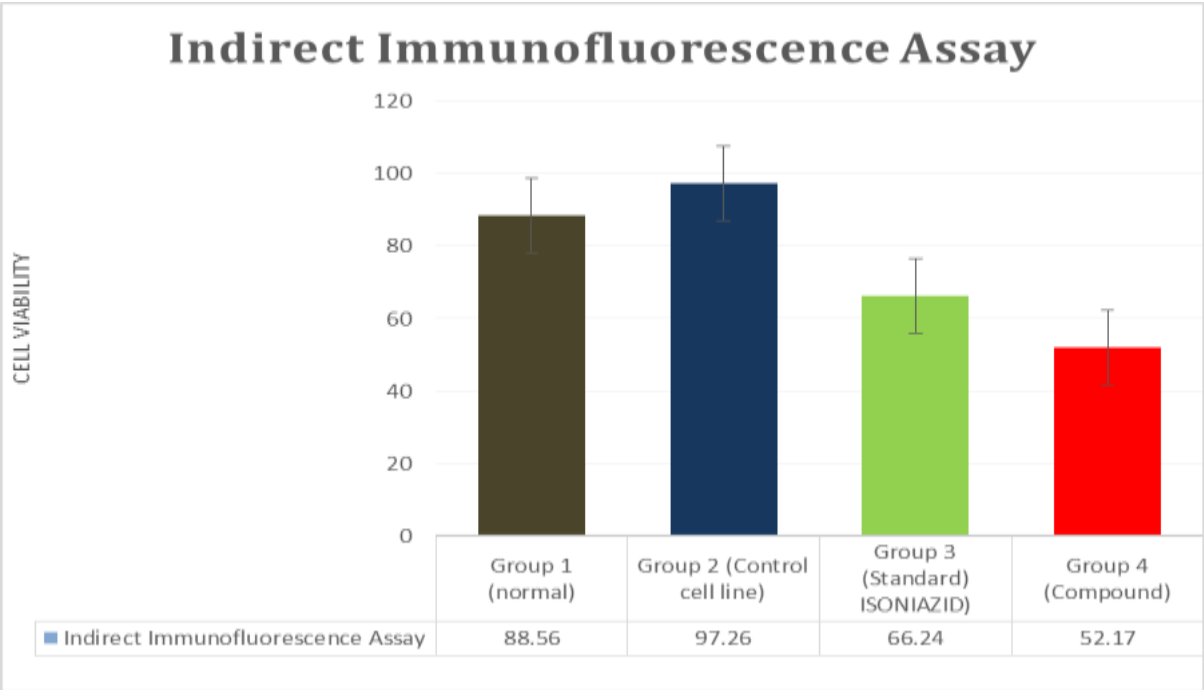
Tubulogenesis Assay

Treatments	Tubulogenesis Assay
Group 1 (normal)	74.36
Group 2 (Control cell line)	88.25
Group 3 (Standard) ISONIAZID	48.13
Group 4 (Compound)	44.68



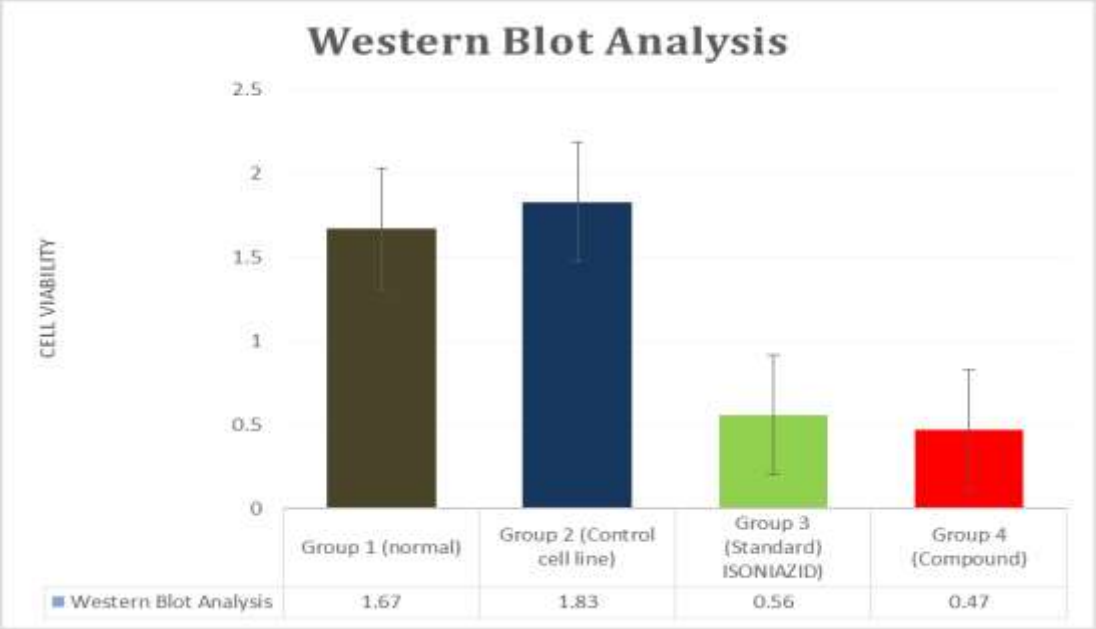
Indirect Immunofluorescence Assay

Treatments	Indirect Immunofluorescence Assay
Group 1 (normal)	88.56
Group 2 (Control cell line)	97.26
Group 3 (Standard) ISONIAZID	66.24
Group 4 (Compound)	52.17



Western Blot Analysis

Treatments	Western Blot Analysis
Group 1 (normal)	1.67
Group 2 (Control cell line)	1.83
Group 3 (Standard) ISONIAZID	0.56
Group 4 (Compound)	0.47



DISCUSSION

The MTT assay results revealed that treatment with Isoniazid glucuronate sodium anhydrous led to a significant reduction in cell viability in both HT-29 and HUVEC cells. This suggests that the compound exhibits potent cytotoxic activity, inhibiting the proliferation of both TB cells and endothelial cells. The decrease in cell viability observed in the treated groups compared to the control indicates that the compound could induce cell death or inhibit cellular metabolic activity.

The Tubulogenesis assay showed that Isoniazid glucuronate sodium anhydrous significantly inhibited the formation of tube-like structures in HUVEC cells, indicating its potential anti-angiogenic effect. Angiogenesis is a crucial process in TB progression, as it facilitates tumor growth and metastasis by providing essential nutrients and oxygen. The inhibition of tube formation by the compound suggests that it could effectively hinder tumor growth by disrupting the formation of new blood vessels.

Further analysis using Indirect Immunofluorescence and Western Blot assays revealed a marked decrease in the expression levels of NF-κB and VEGF-A in HT-29 cells and VEGFR-2 in HUVEC cells. NF-κB is a transcription factor that plays a critical role in cell proliferation, survival, and inflammation, while VEGF-A is a key regulator of angiogenesis. The downregulation of these proteins indicates that Isoniazid glucuronate sodium anhydrous disrupts the signaling pathways that promote TB cell growth and angiogenesis. The reduction in VEGFR-2 expression in HUVEC cells further supports the hypothesis that the compound has strong anti-angiogenic properties.

Overall, the study demonstrated that Isoniazid glucuronate sodium anhydrous has the potential to serve as an effective antiTB agent by targeting both cell proliferation and angiogenesis. The observed decrease in cell viability and tube formation, along with the downregulation of key regulatory proteins, suggests that the compound could inhibit tumor growth and progression by interfering with multiple cellular pathways.

CONCLUSION

Isoniazid glucuronate sodium anhydrous exhibited significant antiTB properties in vitro, as evidenced by its cytotoxic and anti-angiogenic effects on HT-29 colorectal TB cells and HUVEC endothelial cells. The compound significantly reduced cell viability, inhibited tube formation, and downregulated the expression of NF-κB, VEGF-A, and VEGFR-2. These findings suggest that Isoniazid glucuronate sodium anhydrous disrupts critical cellular pathways involved in TB progression, making it a promising candidate for further research as a potential antiTB agent.

Future studies should focus on elucidating the molecular mechanisms underlying its antiTB effects and evaluating its efficacy in in vivo models. This research provides a

foundation for the development of novel TB therapies targeting both TB cell proliferation and angiogenesis, contributing to the ongoing search for more effective treatment options in oncology.