SDIR Scientific Days

ABSTRACTS

Novel perspective of anticancer metal-based drugs: Characteristics of heterometallic complexes and their potential applications

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Background: Platinum-based drugs are widely used in medical treatments, but their effectiveness is limited by toxicity. The mechanism of action is well understood, while the side effects are thought to arise from interactions between platinum (a soft acid) and biomolecules containing sulfur as a donor atom (soft bases), such as thiols and thioethers. One potential approach to overcome these limitations is to design novel heterometallic complexes with metal centers that have different coordination geometries, kinetic properties, affinities, and reactivities towards biologically relevant nucleophiles.
Material and Methods: The novel heterometallic complexes: [{cis-PtCl(NH3)(μ-pyrazine)ZnCl(terpy)}](ClO4)2 (Pt-L1-Zn), [{cis-PtCl(NH3)(μ-4,4'-bipyridyl)ZnCl(terpy)}](ClO4)2 (Pt-L2-Zn), [{ZnCl(terpy)(μ-pyrazine)CuCl(terpy)}](ClO4)2 (Zn-L1-Cu), [{ZnCl(terpy)(μ-4,4'-bipyridyl)CuCl(terpy)}](ClO4)2 (Zn-L2-Cu) (terpy = 2,2':6',2''-terpyridine, L1 = pyrazine, L2 = 4,4'-bipyridyl) were synthesized and characterized by elemental analyses, UV–Vis, IR and 1H NMR spectroscopy. Cell viability was assessed using the MTT assay.
Results: The study of interactions between the complexes and biomolecules under physiological conditions revealed that heterometallic complexes with two different metal centers significantly influence the reactivity order and coordination modes of biomolecules. Cytotoxicity assays showed that all complexes substantially reduced cell viability in human colorectal cancer cells (HCT-116) and human breast cancer cells (MDA-MB-231). Notably, these complexes exhibited significant cytotoxic effects, with improved efficacy against HCT-116 cells compared to cisplatin, especially after 72 hours.
Conclusions: The differing reactivities of the metal centers result in various coordination modes of biomolecules and increased cytotoxicity. Additionally, the nature of the linker between the metal centers significantly impacts reactivity.

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Keywords: copper(II); heterometallic complexes; platinum(II); zinc(II);

Application of numerical models in the behavior of cancer cells

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Background: The aim of this paper is to present diverse numerical models that were applied to model the behavior of cancer cells on various levels.
Material and Methods: A numerical model based on reaction-diffusion equations was applied to analyze the behavior of cancer cells over time on tissue level and the effect of prescribed drug therapy on the tumor was also considered [1]. A similar numerical model was used to model the behavior of cancer cells at the cellular level, *in-vitro* following drug treatment [2]. Another model was applied to predict the behavior of cancer cells after electroporation treatment [3]. The numerical simulations of the WNT signalling process in cells are presented in [4].
Results: In all considered numerical models, the results of numerical simulations are compared with experimental findings. The proposed method for modeling the behavior of cancer cells at the tissue level was tested using patient-specific data that was used to calculate tumour volumes at specific moments in time. The comparison and good agreement of numerical results with the clinical data showed the efficiency and accuracy of the proposed method. In the case of other mentioned numerical models, the experimental data was used to estimate the values of parameters of the model. The analysis of the parameter changes for different treatments helped better elucidate the effect of the considered treatments have on some aspects of the behavior of cells, i.e. decrease cell proliferation, intensify cell death, and/or increase oxygen consumption, among others.
Conclusions: The main benefit of using numerical simulations is that they enable a quantitative description of the behavior of cells, with a set of parameters, that then makes it possible to analyze how the applied treatments influence the change of these parameters and hence the behavior of cells. Also, numerical models can be used to predict the behavior of cells in other conditions and estimate the number of viable cells after other treatments that were not studied experimentally, thus reducing the time and money needed to perform many experiments.

Keywords: cancer cell progression; finite element method; multi-scale models; parameter estimation; WNT signaling

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Molecular markers of migration and invasion in colorectal cancer cell lines and tissues

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Background: The aim of this paper is to present investigation of migratory/invasive potential of two different colorectal cancer cell lines HCT-116 and SW-480, and expression of markers of epithelial-mesenchymal transition (EMT), responsible for cell behavior allowing an epithelial cell to gain greater motility as a key point in triggering metastasis [1].
Material and Methods: individual cell migration of colorectal cancer cell lines was detected via Transwell and RTCA assays. For the determination of collective cell migration, we used Scratch assay, and migratory potential was tracked within 12 and 24 h [2, 3]. Detection and quantification of proteins responsible for EMT: promigratory (vimentin, N-cadherin and nuclear β-catenin), antimigratory (E-cadherin and cytoplasmic β-catenin), and markers of invasion (Snail, Slug, MMP-7 and MMP-9) was done, were performed using immunofluorescence and flow cytometry [1].
Results: HCT-116 cells showed strong migratory potential detected in both individual and collective migration. Furthermore, low expression of E-cadherin and β-catenin was observed in intercellular connections, as well as high expression of N-cadherin, vimentin, MMP-7 and MMP-9. Also, these cells are characterized by elevated expression of the regulatory markers: nuclear β-catenin, Slug and Snail. On the other side, we observed a weaker migratory potential of SW-480 cells, probably due to a strong expression of antimigratory markers E-cadherin and cytoplasmic β-catenin, which are responsible for establishment of intercellular junctions. Moreover, promigratory markers vimentin, N-cadherin and Snail were observed as highly expressed. Lower concentrations of MMP-7, MMP-9, and nuclear β-catenin were detected in SW-480 cells, indicating that these are clearly important markers responsible for the migratory/invasive potential of these cells.
Conclusions: Based on the obtained results, it can be concluded that these two cell lines are completely different in terms of migratory and invasive potential. This can be explained by the origin of the investigated cells. Namely, HCT-116 is an aggressive cell line isolated from stage IV colorectal carcinoma, poorly differentiated and resistant to chemotherapeutics. These cells are characterized with advanced EMT that enables it to have an epithelial-like phenotype and to migrate individually. As for SW-480 cells, they are less aggressive, isolated from stage II colorectal carcinoma, well differentiated with less advanced EMT. Stronger intercellular connections between these cells enable them to form clusters and to retain the collective type of migration. Based on everything presented, we can conclude that these two cell lines will have completely different responses to therapeutic treatments in terms of cell behavior in migration and invasion processes.

Keywords: cancer cell migration, EMT, invasion, colorectal cancer, protein expression

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Detection of mutations in *JAK2*, *MPL* and *NPM1* genes in myeloproliferative neoplasms

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Background: Myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell disorders, characterized by the abnormal proliferation of one or more lineages and an increased number of mature cells (erythrocytes, leukocytes, and platelets) in the blood. The genetic basis of MPN includes the *JAK2* (Janus kinase 2), *MPL* (myeloproliferative leukemia virus oncogene), *NPM1* (nucleophosmin 1), *CALR* (calreticulin) and *FLT3* (fms-like tyrosine kinase 3) gene mutations.
Material and methods: *JAK2 V617F, MPL W515L, MPL W515K, NPM1* type A, B and D, *CALR* type I and II, and *FLT3* ITD mutation testing was performed on whole blood samples of patients with MPN, using the real-time PCR technique for *JAK2, MPL* and *NPM1* mutations and gel electrophoresis for CALR and FLT3 mutations.
Results: *JAK2* V617F mutation was detected in 78 (58,21%), *MPL* W515L in 12 (8,96%), *MPL* W515K in 4 (2,99%), *NPM1* type A in 3 (2,24%) and *FLT3* ITD in 19 (14,2%) of 134 samples. *CALR* type I mutation was detected in 6 (15,0%) and *CALR* type II in 1 (2,5%) of 40 samples.
Conclusion: The real-time PCR technique provides the possibility of monitoring the amplification of the target gene at any time, as well as a faster and more precise software interpretation of the results compared to gel electrophoresis-however, it is not applicable for the detection of *CALR* and *FLT3* mutations. Determining the exact mutation present in patients with MPN is crucial for assessing the risk of developing severe disease or complications, as well as providing adequate personalized therapy.

Keywords: electrophoresis, *JAK2,* Myeloproliferative, *MPL*, *NPM1*, Real-Time PCR

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Colorectal cancer screening: great opportunities and new challenges

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Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related death in Western countries. Evidence from several studies have shown that CRC screening is effective and cost-effective in average-risk population. The aim of population-based screening is to discover asymptomatic disease, thus favoring its detection at early stages and, consequently, enabling its adequate treatment. Implementation of organized programs is recommended because they include a structure responsible for service delivery, quality assurance and evaluation.
Recommended CRC screening strategies fall into two broad categories: stool tests that primarily detect cancer, which include detection of occult blood or exfoliated DNA; and structural exams, that are effective in detecting both cancer and premalignant lesions, which include flexible sigmoidoscopy, colonoscopy, endoscopic capsule, and CT-colonography. Among these strategies, testing for occult blood in stool using the fecal immunochemical test (FIT) is predominant in Europe, Canada and Australia, while colonoscopy is the dominant screening modality in the US.
The COLONPREV study, an ongoing pragmatic multicenter, nation-wide, randomized controlled trial, will allow us to evaluate the efficacy of once-only colonoscopy and biennial FIT with respect to the reduction of CRC-related mortality in average-risk individuals in a programmatic screening setting. This study, along with two additional ongoing projects –the NORDICC and the CONFIRM studies–, may contribute to demonstrating the usefulness of these approaches, as well as establishing the most cost-effective strategy in a population-based scenario.
Finally, it is important to recognize that current screening strategies are limited by low sensitivity for advanced adenomas, a high false-positive rate resulting in unnecessary colonoscopies, and insufficient compliance. The use of biomarkers, others than blood in feces, may overcome these limitations. In that sense, genetic and epigenetic changes can constitute non-invasive biomarkers if they are measurable in different biological fluids. In that sense, exfoliation of neoplastic cells in feces is a continuous process in patients with CRC, whereas cancer cells and other tumor-associated markers may reach systemic circulation facilitated by the angiogenic process.

Keywords: colorectal neoplasms, screening, prevention

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Lung cancer in Vojvodina: progress and priorities

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Background: Lung cancer (LC) is the most frequently diagnosed cancer and the leading cause of cancer mortality in both sexes in Serbia and worldwide. The aim of this presentation was to describe LC incidence and mortality in Vojvodina, Serbia, and to analyze Institute for Pulmonary Diseases (IPBV) LC hospital registry data from 2010 to 2020.
Material and methods: Institute for Public Health of Vojvodina and Serbian cancer registry data for trend analysis, and IPBV LC hospital registry data for patient characteristics from 2011 to 2020 were used.
Results: A decrease in LC mortality rates among males in Vojvodina was observed, from 73.7 per 100 000 in 2010 to 48.1 per 100 000 in 2021. The average LC incidence showed differences by Districts of Vojvodina. The highest LC incidence for males was reported in Central Banat (80.2/100 000), and for females in North Bačka District (38.1/100 000). The highest LC mortality among males was found in North Banat (70.4/100 000), while for females in North Bačka District (26.0/100 000).
According to the IPBV LC hospital registry from a total of 12,055 LC patients, 30.4% were females. The percentage of female LC patients significantly ($p<0.001$) increased from 26.9% (306/1139) in 2011 to 35.9% (379/1056) in 2020. Male LC patients were older compared to females (64.56± 8.4 vs. 63.43 ± 9.0 years; $p <0.001$). Most of the patients at the time of LC confirmation were active smokers (61.9%), without significant differences by gender (62.1% vs. 61.4%, respectively, $p=0.466$).
The most common histological LC type was adenocarcinoma (41.8%), followed by squamous cell carcinoma (30.0%) and SCLC (15.4%). A significantly ($p<0.001$) higher percentages of adenocarcinoma (49.9% vs. 38.4%), SCLC (17.6% vs. 14.5%) and carcinoid tumors (1.1% vs. 0.4%) were observed in females compared to males, while a percentage of squamous cell carcinoma was significantly higher in males than in females (34.8% vs. 19.0%, respectively; $p<0.001$).
During the study period, the total number of patients with adenocarcinoma, squamous cell carcinoma and SCLC decreased in males, while among females the linear trends for adenocarcinoma and squamous cell carcinoma is increased.
Conclusion: LC mortality rates among males showed a decreasing trend, while among females incidence rates are still increasing. Due to increasing trends in the incidence and mortality of LC among women, it is essential to reduce the prevalence of smoking in the female population and to promote LC screening in Serbia.

Key words: lung cancer, rates, gender, histological types, Vojvodina

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Detection of KRAS, NRAS and BRAF mutations in oncology patients

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The aim of this study was to present method and statistical results of RAS and BRAF gene mutation testing in CRC and melanoma patients, respectively, from the Autonomous Province of Vojvodina, Republic of Serbia. DNA mutations in related genes were analyzed from FFPET samples by Real-Time PCR. RAS mutation was detected in 1073 of 2161 patients (49.65%). The most common mutations of KRAS gene was in exon 2 with slightly increased incidence in women. Mutation rates in KRAS exons 2, 3 and 4 were 41.2%, 4.8% and 4,7%, respectively. Mutation rates in NRAS exons 2, 3 and 4 were 3.9%, 4.1% and 2,4%, respectively. BRAF mutation was detected in 226 of 474 patients (47.68%). The biggest challenges of RAS/RAF molecular testing are: the availability and quality of tissue samples and reagents for molecular tests, as well as the speed of testing to obtain valid findings in a timely manner. The validation of detection methods and the communication of clinicians/therapists with laboratory personnel who perform these and other tests are of great importance, as well as the exchange of experience with other health centers and laboratories.

Keywords: KRAS; NRAS; BRAF; mutation, colorectal cancer; melanoma.

In vitro investigation of the antiproliferative effects of newly synthesized compounds

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In practice, finding new drugs is very challenging. Despite advances in the understanding of biological systems and the development of state-of-the-art technologies, the process is still long and expensive. These drugs are tested at different stages, all with the goal of bringing that drug to the market and to be intended for the treatment of future patients. Medicines are first tested *in vitro*. Cell lines form the backbone of cancer research. These individual groups of cells, typically collected from patients' tumor samples and cultured to grow indefinitely in the laboratory, enable everything from basic genetic research to drug discovery. The NCI-60 human tumor cell line panel has proved to be a useful tool for the global cancer research community in the search for novel chemotherapeutics. The publicly available cell line characterization and compound screening data from the NCI-60 assay have significantly contributed to the understanding of cellular mechanisms targeted by new oncology agents. Signature sensitivity/resistance patterns generated for a given chemotherapeutic agent against the NCI-60 panel provide target information and the insights into the mechanism of action associated with the tested agent. We hope that one day it will become possible to quickly design cheap, more specific, effective, non-toxic and personalized drugs.

Keywords: Antiproliferative effect, Cell Lines, Cytotoxicity, *In Vitro*.

Potential of epigenetic therapies in the management of colon cancer: the impact of antioxidant pretreatment on vorinostat *in vitro* cytotoxicity

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Background: Vorinostat is a histone deacetylases (HDAC) inhibitor that promotes apoptosis of malignant cells by several mechanisms. Multiple studies have failed to show efficacy of vorinostat as a monotherapy against solid tumors, but it has shown a great potential to act synergistically with various chemotherapeutics. Conflicting results were obtained regarding the role of oxidative stress in antitumor effects of HDAC inhibitors, and therefore the aim of our study was to analyze the influence of antioxidants on cytotoxic activity of vorinostat towards colon cancer cells.
Material and Methods: Human colon adenocarcinoma HT-29 cells were used to assess the cytotoxicity of vorinostat, alone or in combination with antioxidant agents N-acetyl-cysteine (NAC) and α-tocopherol (TOC), using the colorimetric MTT assay. Multiple drug effects were examined by calculating the combination index (CI) as described by Chou and Talalay, using CompuSyn software. CI<1 is evidence for synergism, whereas CI>1 is evidence of antagonism.
Results: Vorinostat exhibited a modest cytotoxic activity against HT-29 cells, in a concentration dependent manner. IC50 value of vorinostat was 5.1 μM, while the clinically relevant concentrations are between 1 and 2 μM. In combination studies, HT-29 cells were treated with 1 μM and 2 μM vorinostat, 30 minutes after being treated with 10 mM NAC and 3 μM TOC that displayed negligible antiproliferative effects. Both NAC and TOC managed to sensitize cells towards the activity of vorinostat, especially in a concentration of 2 μM. Calculated CIs of 0.1981 and 0.0803 for NAC, and CIs of 0.3566 and 0.0774 for TOC, in combination with 1 μM and 2 μM vorinostat respectively, suggest that their synergistic effects in concentrations that can be achieved in vivo. The effect of NAC pretreatment was more pronounced than that of TOC for a lower concentration of vorinostat, while it was similar for a higher concentration of vorinostat.
Conclusion: The response to vorinostat could be improved by combining it with antioxidants. The mechanisms responsible for this synergistic effect should be investigated in more depth.

Key words: solid tumor; epigenetics; histone deacetylase; combination index.

Detection of *EGFR* mutations in lung cancer – the role of tissue and liquid biopsy

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Over the past two decades, the identification of oncogenic genomic alterations in non-small cell lung cancer (NSCLC) and the development of targeted therapies designed to block the oncogenic driver has enabled personalized treatment and improved outcomes. Activating mutations in the gene encoding the epidermal growth factor receptor (EGFR) tyrosine kinase are present in about 15% of Caucasian and 50% of Asian NSCLC patients. The most common activating *EGFR* mutations are deletions of exon 19 and L858R point mutation within exon 21, representing 90% of all *EGFR* mutations. Despite the success of EGFR tyrosine kinase inhibitors (TKIs) in the first line treatment setting in NSCLC patients with *EGFR* mutations, acquired resistance inevitably occurs and can be caused through EGFR-dependent or EGFR-independent mechanisms. Since the initial therapeutic choice depends on the genetic profiling of tumours, tissue biopsy has remained the gold standard for molecular analysis. Commercial real-time polymerase chain reaction (PCR) assays were developed, followed by the clinical application of next generation sequencing (NGS) panels. Given that many patients with NSCLC are diagnosed by either a cytology specimen or a small tissue biopsy, the appropriate management of specimens is critical in attempting to maximize the amount of diagnostic, prognostic, and predictive information obtained from molecular testing. Several recommendations state that cell blocks, smears, or other cytological preparations, which contain sufficient tumor cells can be suitable specimens for lung cancer biomarker

molecular testing, including *EGFR* testing. In cases in which the tumour is inaccessible or tissue sample is inadequate, liquid biopsy represents an alternative source for investigating genetic alterations in cancer. Liquid biopsy is the analysis of tumour-derived material (e.g cell-free tumour DNA, ctDNA) from body fluids. Liquid biopsy is a minimally invasive procedure which can be serially repeated to monitor disease evolution, including the development of resistance, and can demonstrate tumour heterogeneity. However, this procedure has several limitations, such as unknown potential of tumour cells to shed ctDNA, lower test sensitivity and inability to monitor histological transformation. The International Association for the Study of Lung Cancer (IASLC) highlights the complementary nature of tissue and liquid biopsies in the therapeutic decision-making for advanced NSCLC.

Keywords: Biomarkers, Biopsy, Cell-Free Nucleic Acids, EGFR protein, Lung Neoplasms

Genetic and epigenetic factors in cancerogenesis

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Basic and translational cancer research have advanced at an impressive pace in recent years. Molecular mechanisms of cancer initiation, promotion and progression have been identified and described in detail, directing the discovery and development of innovative cancer therapeutic agents. Cancerogenesis is the multistage clonal process of mutation accumulation, enabling the progression into a more malignant stage. The genetic mutations that contribute to the transformation of healthy cells into cancerous cells have been the subject of extensive research. The molecular aberrations that lead to cancer development are often characterized by gain-of-function (GOF) and loss-of-function (LOF) mutations in a variety of oncogenes and tumor suppressor genes. One of the key examples is the LOF mutation of p53, after which the mutant p53 serves as a dominant negative inhibitor of wild-type p53. Numerous computational biology and bioinformatics groups developed the tools to analyze these large datasets and distinguish mutations in the genes that contribute to tumor progression (cancer driver mutations) from those that are neutral (passenger mutations). Mutations in cancer-driver genes qualitatively or quantitatively alter the function of genes and proteins, consequently affecting the cellular processes in which these proteins participate. Oncogene mutations are linked with differences in patient survival, clinical outcomes, metastatic or recurrent tumors, and serve as predictors of tumor responsiveness to anti-cancer drugs. The complexity of cancer biology can be explained as the interplay between genetic and epigenetic abnormalities that are mutually beneficial in order to drive cancer initiation and progression. The main objective of epigenetic therapy in the era of personalized precision medicine is to detect cancer biomarkers to improve risk assessment, diagnosis, and targeted treatment interventions. The emergence of next-generation sequencing technology and artificial intelligence has advanced our understanding of carcinogenesis, enabling the multi-omics analyzes of the genome, transcriptome, and epigenetic panels to guide the further discoveries and developments in precision medicine.

Key words: carcinogenesis; genetics; epigenomics; precision medicine

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Lung cancer screening in Vojvodina, is it beginning of achieving the goal?

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Lung cancer (LC) is one of the leading public health problems in the Republic of Serbia. More than 6,500 new cases of lung cancer are diagnosed annually. Unfortunately, almost a similar number of patients die each year from this deadly disease. Five-year survival from lung cancer in the world is about 20%. At the time of diagnosis, about 70% of patients with non-small cell lung cancer have advanced disease (stages IIIB, IIIC and IV). The introduction of the National Program for screening and early detection of lung cancer would enable diagnosis in the earlier stages of the disease, which would increase the possibilities of radical treatment, improve overall survival and reduce overall mortality. There are several current guidelines for lung cancer screening. Most guidelines recommend the use of low-dose computed tomography (LDCT) for the population at a high risk for lung cancer, such as smokers and individuals over 50 years old. The first lung cancer screening pilot program in Serbia, Vojvodina started in September 2020 at the Institute for Lung Diseases of Vojvodina, and from March 2023 in the general hospital Subotica. Subjects in this program were people without symptoms, aged 50-74 years with a history of smoking. Active smokers > 30 cigarettes/year or ex-smokers who have stopped smoking within the previous 10 years. The selection of respondents included doctors from the Novi Sad Health Center, the Subotica Health Center, and the LDCT imaging was performed at the Institute for Pulmonary Diseases of Vojvodina and general hospital Subotica. During the three-year period, the program faced difficulties and challenges that needed to be overcome in order to improve and increase the number of subjects screened. During that period, a total of 6,589 LDCT scans were performed on 4,005 people who showed up for screening. Positive results for pulmonary RADS score were found in 9.7% (389/4005). The detection rate of lung cancer was 2.1% (86/4005). More than 50% of lung cancers detected in screening were stage I or II disease. 65% of lung cancer cases are diagnosed in the surgically resectable disease stage (I-IIIA). After analyzing the first screening results, we can conclude that innovative approaches, education and a more recognizable campaign are needed to increase the response rate among participants with a negative baseline LDCT.