the involvement of immune inhibitory cytokines and upregulation of PD-L1 and MIF in BC patients. Non-coding RNAs (miRNAs) are endogenous molecules that have the ability to form a signaling network modulating multiple targets simultaneously. Our group has recently reported the involvement of MALAT-1 in tuning the immunooncogenic profile of BC. Yet, microRNA-30a-5p is a novel miRNA in terms of immunoncology and its involvement in MALAT-1 modulatory network is yet to be explored. The aim of this study was to investigate the involvement of miR-30a-5p in MALAT-1circuit and its impact of PD-L1, MIF, IL-10 and TNF-α in BC.

Methods: BC patients (n=24) were recruited. Bioinformatics analysis was performed. MDA-MB-231 cells were cultured transfected by miR-30a-5p oligonucleotides and MALAT-1 siRNAs using HiPerfect Transfection protocol. RNA was extracted using biazol, reverse transcribed and quantified using qRT-PCR.

Results: MALAT-1 and miR-30a-5p are paradoxically acting mRNAs in BC patients. MALAT-1 is an upregulated oncogenic IncRNA while miR-30a-5p is a downregulated tumor suppressor miRNA in BC patients. In-silico analysis showed a strong binding of miR-30a-5p to MALAT-1. Experimentally, ectopic expression of miR-30a-5p represses MALAT-1 levels while MALAT-1 siRNAs induced miR-30a-5p levels in MDA-MB-231 cells. miR-30a-5p mimics and/or MALAT-1 siRNAs resulted in a marked repression of BC patients (n=9).

Conclusions: MALAT-1/miR-30a-5p are vital players releasing the immune surveillance brakes supported by BC cells. Dual targeting of MALAT-1 and miR-30a-5p is novel immune-oncological therapeutic approach in BC.

Legal entity responsible for the study: German University in Cairo.

Funding: STIFA (Science, Technology & Innovation Funding Authority), grant no. 42354.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2021.08.2036

41P Attenuation of p53 signaling by CDK8/19 sensitizes tumor cells to ionizing radiation

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Background: Responses of tumor cells to therapeutic ionizing radiation include a number of processes largely mediated by the transcription factor p53. To explore the possibility of tumor cell radiosensitization by targeting p53 dependent mechanisms we investigated the role of transcriptional protein kinases CDK8/19 in the balance of cell death vs survival upon irradiation.

Methods: To study this process, real-time PCR, immunoblotting, flow cytometry, clonogenic analysis and beta-galactosidase assays were used.

Results: We demonstrated that HCT116 human colon carcinoma cells (wild type p53) underwent a time- and dose dependent arrest in G2/M phase and senescence after 2-6 Gy single dose γ-irradiation. Virtually no subG1 (apoptotic) events were registered. In contrast, irradiation of the isogenic HCT116p53KO subline (non-functional p53) was lethal. These effects were observed in the mass cell culture and clonogenic assays. Irradiation of HCT116 cells induced p53, p21, PUMA and NOXA at mRNA and protein levels whereas none of these markers was up-regulated in HCT116p53KO. These results implicated p53 mediated processes, particularly p53/p21 dependent cell cycle arrest, in the escape of tumor cells from radiation induced death. To prevent the activation of p53 signaling, HCT116 cells were irradiated in the presence of selective CDK8/19 inhibitors (CDK8/19i). Alone, each of two chemically unrelated CDK8/19i (1 μg) had no effect on cell survival for as long as 14 days. However, in combination with 4 Gy, CDK8/19i potently prevented the accumulation of cells at the G2/M boundary and significantly increased the percentage of dead cells. These events were paralleled by an attenuation of p53 and p21 mRNAs and proteins. In the HCT116p53KO subtype CDK8/19i did not influence the effects of irradiation.

Conclusions: Our results indicate that CDK8/19 inhibition in the irradiated wild type p53 cells is functionally similar to p53 KO. Pharmacological attenuation of p53 responses (HCT116 cells) or genetic inactivation of p53 (HCT116p53KO cells) abrogate the G2/M checkpoint and preclude the repair of irradiation induced damage. Thus, inhibition of CDK8/19 mediated transcriptional reprogramming emerges as a therapeutically attractive approach in tumor radiosensitization.

Legal entity responsible for the study: ITMO University.

Funding: The Russian Foundation of Basic Research (grant No 20-34-90046).

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2021.08.2037

42P Method optimization for the detection of chimerism by real-time PCR and droplet digital PCR

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Background: The objective of the present study was to select combinations of primers for genetic polymorphisms (INDELS) used in the analysis of chimerism associated with post-transplant hematopoietic regeneration as well as to test them under real-time PCR (qPCR) and droplet digital PCR (ddPCR) conditions.

Methods: We searched for genetic polymorphisms, using the EMBL - European Molecular Biology Laboratory - database taking into account certain criteria for selection. There were 85 polymorphisms left at the end of the selection process. For both techniques, we designed primers with the following characteristics: 3° C annealing temperature, amplicon length below 120 bp, at least 3 different nucleotides at the 3’ end between variants and temperature difference between forward and reverse primer of maximum 1° C. Depending on the distribution of genotypes, assays were designed for one or both alleles. Human blood samples from subjects without hematological diseases were obtained in accordance with the Helsinki declaration and genotyped. A novel genotyping protocol, based on 2 reference genes and one allele per polymorphism, was developed. Samples bearing different genotypes were serially diluted to simulate various chimerism levels.

Results: In general, for ddPCR increasing primer concentration determines the increase of the amplitude difference between the negative and the positive droplets without affecting the allele specificity. At the same time, the decrease of the annealing extension temperature determined the same phenomenon. Some of the assays showed no differences between 35 and 45 cycles. The new protocol allows the use of smaller sample volumes as compared to traditional genotyping, by reducing reaction number and template quantity. Simulated and obtained chimerism levels showed good correlation between 50% and 1.5% (R²=0.998), the limit of quantification corresponding to 50 molecules.

Conclusions: The differences between positive and negative samples are more clearly determined in the case of ddPCR, which confirms that this method is more accurate than the qPCR method. In this context we believe that the ddPCR is a valuable technique for early detection in hematopoietic malignancies. Ioana M. Lambescu and Victor S. Ionescu – equal contribution.

Legal entity responsible for the study: V.B. Cismasu.

Funding: National Program d 1N/ 2019/ PN19.29.01.03.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2021.08.2038

43P The effect of quercetin and doxorubicin treatment on the proliferative capacity of tumour cells

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Background: Quercetin is a flavonoid with antioxidant properties, commonly used in tumour pathologies due to anticancer effects. It is a natural polyphenolic compound that has anti-mutagenic and anti-proliferative effects, it has a strong oxidizing capacity and has an important role in regulating cell signaling, cell cycle and apoptosis, all these beneficial effects being demonstrated by several in vitro and in vivo studies. Quercetin is frequently studied mainly due to its potential as a chemopreventive agent being demonstrated its ability to inhibit the initiation and development of tumour cells through various mechanisms. The aim of this study was to investigate the role of quercetin in cell cycle arrest of Walker 256 carcinosarcoma and to highlight its beneficial effects when it is used in tumour chemoprevention.

Methods: The study was performed using three groups of Wistar rats with Walker 256 carcinosarcoma: a control group comprising animals with untreated tumours, the second group comprising animals with tumours treated with doxorubicin and the third group consisting of animals who received daily oral quercetin until tumour development (2 weeks) and then followed by doxorubicin treatment. The effect of quercetin and doxorubicin treatment on the studied tumour cells was evaluated by assessing cell cycle progression using flow cytometry analysis.

Results: Propidium iodide staining of DNA measured by flow cytometry, showed the aggressive nature of Walker 256 carcinosarcoma, the tumour presenting a proliferative fraction rate of 44.37% (7 days) and 48.46% (14 days) and increased values of the S-phase (42.60%, respectively 48.45%). Compared to the control group, the results showed a decrease of the S-phase fraction (28.48% at 7 days, 25.78% at 14 days) and the arrest of the cells in the G0 / G1 phase in the quercetin experimental group. Consequently, a decrease in the proliferative capacity of the cancer cells was observed in the same group (33.28% at 7 days, 30.82% at 14 days).
Conclusions: The results of the study showed that oral administration of quercetin can inhibit the ability of tumour cells to proliferate suggesting the possibility to use it chemopreventive agent in oncology.

Legal entity responsible for the study: The authors.

Funding: has not received any funding.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2021.08.2039

44P Value of comprehensive genomic profiling in pre-screening patients for NTRK fusion in STARTRK2 trial: single centre experience

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Background: Comprehensive genomic profiling (CGP) by next-generation sequencing (NGS) is increasingly used as a pre-screening tool for clinical trials. The aim of this project was to retrospectively determine the scope of alterations identified by CGP that could render patients suitable for alternative early phase clinical trials of genomically-matched (GM) / immunotherapy (IO) or ‘off-label’ drug use.

Methods: Patients were pre-screened for the STARTRK2 study (Roche sponsored study of Entrectinib, NCT02568267) at The Christie NHS Foundation Trust using FoundationOne Dx. Testing is validated for NTRK, ROS1 and ALK fusion testing but all pathogenic alterations are reported on a clinical trial specific Foundation Medicine (FM) report. Results were scrutinised for actionable alterations that could direct patients to alternative clinical trials or off label drug use.

Results: A total of 269 patients with were consented since FM testing was introduced in the trial in Jan’2019. FM yielded results in 229 patients (85.2%), mean age was 54, 58.4% were male and 45.8% had 1-2 prior systemic lines. Most prevalent tumour subtypes were head and neck (21.6%) and sarcomas (7.1%). Most prevalent alterations occurred in: TP53 (12.6%), APC (8.4%) and KRAS (4.6%). Most prevalent actionable alterations occurred in: TP53 (12.6%), APC (8.4%) and KRAS (4.6%). MSI-High was 1.5%. No patients had NTRK/ROS1 fusions, 1 non-small cell lung cancer patient had ALK fusion. 104 (45.4%) patients were potentially eligible for matched clinical trials (101 for GM and 3 for IO) and 61 (26.6%) patients could have been considered for off-label drug use. The most prevalent actionable alterations found across common and rare disease types were PI3KCA (10%), ERBB2 (6.1%), PTEN (3.1%), tumour mutation burden >10 mut/Kb (2.6%) and HRAS (1.7%). The following alterations occurred in <1%: AKT1, KRAS G12C, BRAF V600E, BRCa, FGFR3 and IDH1.

Conclusions: Our results highlight the relevance of CGP in identifying patients for GM or IO within clinical trials or off-label drug use. The retrospective nature of this work and the fact that FM results provided within STARTRK2 are not intended for clinical use precluded implementing these recommendations. NTRK fusions were not detected in our cohort which highlights the rarity of this event in our population.

Legal entity responsible for the study: The authors.

Funding: F. Hoffmann-La Roche AG.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2021.08.2040

45P Enhanced 5-aminolaevulinic acid (ALA)-based photodynamic therapy (PDT) efficacy by hormones in uterine sarcoma cells via upregulation of proporphyrinogen oxidase

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Background: Photodynamic therapy (PDT) is an FDA approved cancer treatment modality for various cancers. In principle, PDT induces a photochemical reaction with exogenous photosensitizer (PS) in cancer cells upon light irradiation with oxygen, which generates reactive oxygen species leading to cancer cell death. Studies showed that hormones enhanced accumulation of photosensitive protoporphyrin IX (PpIX) generated by ALA PS in human endometrial cells, implying the increased PpIX potentially boost up ALA-based PDT effect in hormonal dependent cancers. Yet the underlying mechanism remains unexplored.

Methods: Uterine sarcoma cells were cultured with fluctuated 17β-estradiol (E2) and progesterone (P) levels as human menstrual cycle, then treated with exogenous Hexyl-ALA compared with normal cultured cells. PpIX localization and accumulation in the cells were determined by confocal microscopy and flow cytometry respectively. Hexyl-ALA-PDT effect was evaluated by MTX assay with sulforhodamine (SR) inhibitor of proporphyrinogen oxidase (PPOX) - a limiting factor of PpIX in the heme pathway. The PPOX expression in cells was quantified by flow cytometry.

Results: PpIX localized in mitochondria. PpIX accumulation increased 30% with E2 and P in a time-dependent manner compared with hexyl-ALA (30 µM) only. At 4hr, the phototoxicity increased from 20% to 60% at hexyl-ALA (30 µM, 21/cm2) compared with hormones respectively. Addition of Sul further enhanced the phototoxicity to 70%. PPOX level also enhanced by hormones; implying the hormonal microenvironment boost up Hexyl-ALA-PDT effect in the cells via up-regulation of PPOX.

Conclusions: This study showed that hormones played a vital role in enhancing hexyl-ALA PDT effect via up-regulation of PPOX. The simulated hormonal microenvironment culture model is suitable to study hexyl-ALA-PDT effect in hormonal dependent cancers.

Legal entity responsible for the study: The authors.

Funding: Hexyl-ALA was provided by Photoceura ASA. This study was fully supported by a grant from Research Grants Council (RGC) of the Hong Kong Special Administrative Region, China (project no.: UGC/FDS17/M06/19).

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2021.08.2042

46P Environmental scan of molecular profiling in Canada

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Background: Colorectal Cancer Canada (CCC) developed a pan-tumour, pan-Canadian survey that was sent to healthcare professionals that aimed to conduct a high-level environmental scan of current molecular profiling practices at cancer care institutions across Canada.

Methods: The survey was disseminated in August 2020 using the free web-based software Google Forms. Questions were developed by members of CCC in collaboration with a scientific advisory committee. Questions were written to include all cancer types and were optional to answer. Data was collected in Excel format and analyzed using descriptive statistics in November 2020. Data was reported in aggregate only and pooled by province. This research was funded by sponsorships granted to CCC’s Get Personal program.

Results: The survey sample consisted of 42 healthcare professionals within 26 different institutions, with at least one representative from each province. According to the survey results, turnaround times (TAT) for predictive biomarkers vary depending on the type of biomarker, testing modality, and the availability of testing in-house, with an average TAT of 3.26 weeks for NGS. 69% of respondents reported that molecular profiling is used to make patient care decisions mostly for patients with metastatic disease, and 59.5% of respondents reported that be done only if there is a Health Canada approved therapy associated with that drug. 86% of respondents reported that medical oncologists are ordering molecular profiling for their patients; however, 80% of respondents suggested pathologists should be the ones ordering molecular profiling for patients. As reported by respondents, the main challenges to molecular profiling included: inadequacy and insufficiency of samples (78%), inadequate reimbursement for the test (66%), unavailability of a certain test (53.7%), and long TATs (48.8%).

Conclusions: The lack of standardized guidelines and coordination between centres prevent the administration of precision oncology care in Canada; therefore, in addition to generating knowledge of current profiling practices, this research could potentially influence the health of Canadians on a broader scale, by informing decision-making, influencing standardization of cancer care, and enabling capacity-building in patient-driven research.

Legal entity responsible for the study: M. El Bizri.

Funding: Argen, Bayer, Pfizer, Eli Lilly, Taiho Pharmaceutical, Bristol Myers Squibb, Janssen-Johnson & Johnson.

Disclosure: M. El Bizri: Financial Interests, Institutional, Other: Funding from most major pharmaceutical companies in Canada who are engaged in oncology.

https://doi.org/10.1016/j.annonc.2021.08.2041