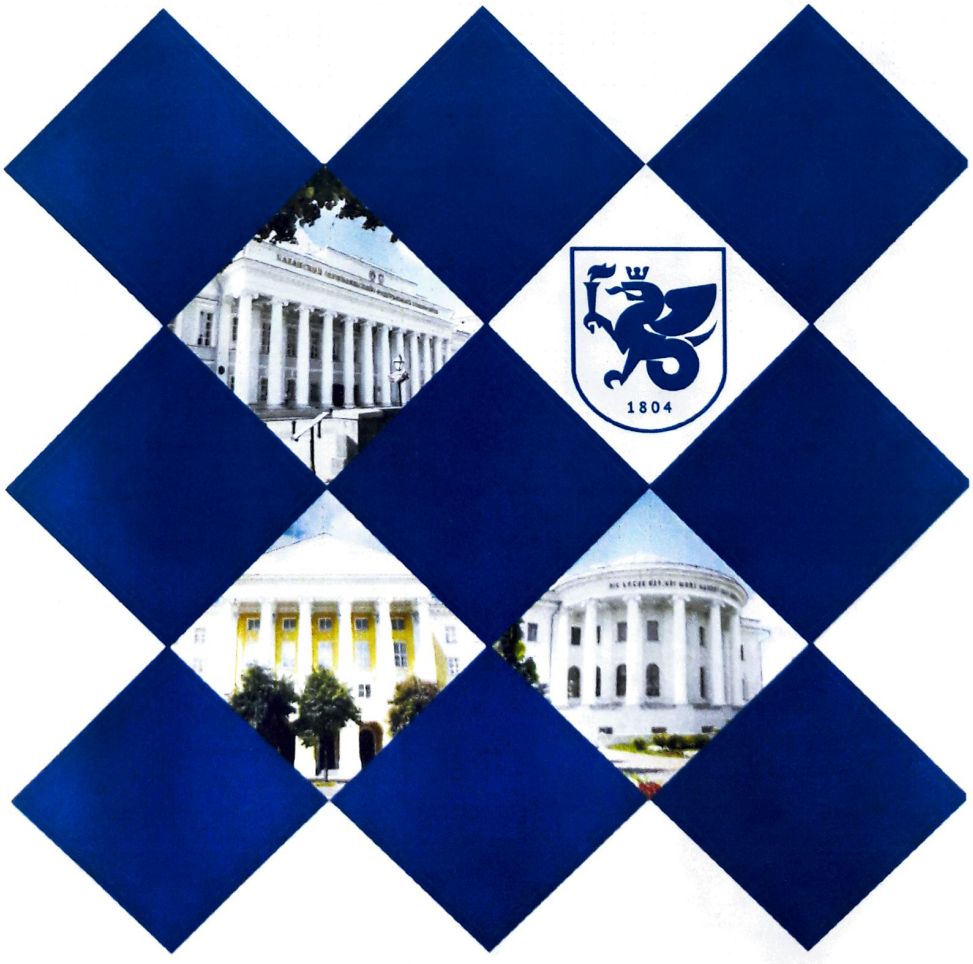
KAZAN FEDERAL UNIVERSITY
Institute of Fundamental Medicine and Biology

Kazan Precision Medicine Workshop

ABSTRACT BOOK



September 10-12
2018

Table of contest

Small molecule modulators of mutant p53 and their activity in genetically modified MCF7 cells

R.N. Mingaleeva[1]*, R.M. Sayarova[1], R.R. Nazyrova[1], M. Baud[2], A.A. Rizvanov[1], R.R. Miftakhova[1], E.R. Bulatov[1]

[1]*Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia*

[2]*University of Southampton, United Kingdom*

Background: In the struggle for obtaining evolutionary advantage cancer cells often acquire *TP53* gene mutations to evade apoptosis. Y220C is the ninth most common mutation for *TP53* gene and is annually observed in about 100,000 new diagnosed cancer cases worldwide. This mutation results in partial denaturation and loss of functions. Selective small molecules can stabilize the mutant protein and restore its impaired transcriptional functions. Aminobenzothiazole MB725 and its derivatives represent a highly promising scaffold for development of potent stabilisers/activators of p53(Y220C) mutant. In this study we explore how MB725 and its analog could effectively restore biological activity of p53(Y220C) mutant.

Methods: As one of the biological models we use breast carcinoma cell line MCF7 (p53wt) and two genetically modified variants, the first one has p53–/– status and the second one is p53-mutant (Y220C). Heterozygous MCF7 p53–/– cell line was obtained using CRISPR/Cas9 gene editing technology. MCF7 p53wt cells were stably transduced with lentivirus encoding *Cas9* gene, and then transfected with plasmid encoding *TP53*-specific sgRNA. TP53 gene region in each population was DNA sequenced to confirm frameshift in proximity of Cas9-induced double-strand break. Cytotoxicity of MB725 and its analogs was assessed using MTS test. Effect of the compounds on cell proliferation and viability was monitored by xCELLigence real time cell analysis. Biological activity of the compounds is being investigated via analysis of p53-dependent gene expression by quantitative real-time reverse transcription PCR, quantitative analysis of alterations in intracellular protein levels by immunoblotting, cytofluorometric analysis of cell cycle and programmed cell death.

Results: According to the results, MB725 preferentially bind to recombinant p53(Y220C) mutant rather than p53wt. The viability of p53wt cells was not significantly changed after treatment with MB725.

Conclusion: These promising results will provide an important scientific groundwork for development of prospective personalized anti-tumor drugs.

Acknowledgments: The study was supported by Grant of the President of Russian Federation MK-4253.2018.4 to E.B., RFBR 18-34-00702 mol_a Grant to R.S. and performed in accordance with Program of Competitive Growth of Kazan Federal University.

Evaluating efficacy of anti-CD19 CAR-T cells against CD19-positive 2D and 3D solid tumor models

Aygul Valiullina[1]*, Regina Sayarova[1], Margarita Zhuravleva[1], Sevindzh Kletukhina[1], Alexey Petukhov[1,2], Emil Bulatov[1], Albert Rizvanov[1]

[1] *Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia*

[2] *Institute of Hematology, Almazov National Medical Research Centre, Saint Petersburg, Russia*

Background: Administration of CAR-T cells demonstrated remarkable success in treatment of hematologic malignancies (HM), however this novel cell therapy is significantly less efficient against solid tumors (ST). Poor clinical peformance of CAR-T therapy in ST is primarily accounted for biological differences between ST and HM. Therefore it is important to develop models simulating *in vivo* conditions for testing effectiveness of CAR-T therapy against ST. In current study we evaluate anti-CD19 CAR-Ts against several 2D monolayer cell cultures and 3D bioprinted solid tumor models.

Methods: We constructed plasmid with 2nd-generation anti-CD19 CAR and also recombinant vector containing *CD19* gene under control of internal ubiquitin C promoter and puromycin resistance gene. T-cells obtained from healthy donor were activated and transduced with lentivirus. CD19-positive cells were generated by transduction of MDA231, MDA468, A431, H522 solid tumor cell lines with CD19_p2a_PuroR recombinant lentiviral vector and further incubation with puromycin to allow selection. After that anti-CD19 CAR-T cells were applied onto CD19-positive tumor cell 2D monolayer or 3D constructs bioprinted using hydrogel composition. Efficacy of anti-CD19 CAR-T cells was assessed using vitality assay, fluorescent and confocal microscopy.

Results: According to the results, anti-CD19 CAR-Ts were succesfully used to fight CD19-positive cancer cells in 2D monolayer cell cultures and 3D bioprinted solid tumor models.

Conclusion: We propose that reported approach is suitable for screening and evaluating CAR-Ts against 2D and 3D solid tumor models.

Acknowledgments: Work performed in accordance with Program of Competitive Growth of Kazan Federal University.