Opis raziskovalnega dela *(Research work description*)

1. Članica UL (*UL member)*:

Fakulteta za farmacijo (Faculty of pharmacy)

2. Ime, priimek in elektronski naslov mentorja/ice (*Mentor's name, surname and email*):

Martina Gobec, martina.gobec@ffa.uni-lj.si

3. Raziskovalno področje (*Research field*):

Onko-imunologija (oncoimmunology)

4. Opis raziskovalnega dela (Research work description*)*:
Vključuje morebitne dodatne pogoje, ki jih mora izpolnjevati kandidat/ka za mladega raziskovalca/ko, ki niso navedeni v razpisu za mlade raziskovalce *(It includes any additional conditions that the candidate for a young researcher must meet, which are not listed in the call to tender for young researchers.)*.

Slov.: Proteasom je večkatalitični encim in osrednja komponenta ubikvitin-proteasomskega sistema (UPS). Predstavlja ključni regulator znotrajcelične razgradnje beljakovin in posledično uravnava številne celične procese, vključno s proliferacijo, diferenciacijo, procesiranjem antigenov in tvorbo vnetnih molekul. Različne podenote proteasoma (β1, β2, β5, β1i, β2i, β5i) imajo specifične katalitične funkcije, ki edinstveno prispevajo k celični biologiji. Vloga proteasoma v tumorskih in imunskih celicah je dobro raziskana, zaradi česar je proteasom uveljavljena terapevtska tarča pri hematoloških malignih obolenjih. Številni zaviralci proteasoma so trenutno tudi v kliničnih testiranjih za zdravljenje različnih imunskih bolezni.
Čeprav je proteasom v imunskih in tumorskih celicah obsežno raziskan, pa ostaja njegova vloga v trombocitih, zlasti v kontekstu oblikovanja tumorskega mikrookolja, slabo razumljena. Trombociti, majhni delci celic brez jedra, so vse bolj prepoznani kot ključni akterji pri zaščiti rakavih celic pred imunskim sistemom in spodbujanju njihove invazivnosti. Znano je, da lahko trombociti prevzamejo različne tumorsko izločene komponente in posledično spremenijo svoje lastnosti, kar dodatno spodbuja zaviranje imunskega odziva, invazivnost rakavih celic in angiogenezo. Na podlagi tega se poraja vprašanje, ali bi lahko zaradi ključne vloge proteasoma v uravnavanju imunskega sistema z njegovim selektivnim zaviranjem v trombocitih spremenili njihovo funkcionalnost do te mere, da ne bi več nudili zaščite tumorskim celicam.

Kandidat/ka za mladega/o raziskovalca/o bo tako poskušal/a odgovoriti na vprašanje s sistematičnim vrednotenjem vloge posameznih podenot proteasoma v delovanju trombocitov in njihovem vplivu na napredovanje raka dojke. Z uporabo naprednih orodij molekularne biologije, modelov tumorjev, pridobljenih od pacientov, in mikrofluidnih sistemov bo:

- razjasnil/a, kako selektivno zaviranje posameznih podenot proteasoma v trombocitih vpliva na interakcije med tumorji in trombociti ter na oblikovanje tumorskega mikrookolja;
- ocenil/a klinični pomen proteasomsko moduliranih trombocitov na primarnih kulturah raka dojke, pridobljenih iz tumorskih eksplantov bolnic;
- raziskal/a učinke snovi, sproščenih iz trombocitov, na imunski sistem in angiogenezo

Zaželeno je, da ima kandidat/ka znanje angleškega jezika ter predhodno metodološko znanje s področja celične oz. molekularne biologije.

Eng.: The proteasome is a multicatalytic enzyme and a central component of the ubiquitin-proteasome system (UPS). It serves as a key regulator of intracellular protein degradation, thereby controlling numerous cellular processes, including proliferation, differentiation, antigen processing, and the production of inflammatory molecules. Different proteasome subunits (β1, β2, β5, β1i, β2i, β5i) perform specific catalytic functions, uniquely contributing to cellular biology. The role of the proteasome in tumor and immune cells has been well-studied, establishing it as a validated therapeutic target in

hematological malignancies. Numerous proteasome inhibitors are also currently undergoing clinical trials for the treatment of various immune-related diseases.
Although the proteasome is extensively studied in tumor and immune cells, its role in platelets—particularly in the context of shaping the tumor microenvironment (TME)—remains poorly understood. Platelets, small anucleate cell fragments, are increasingly recognized as key players in protecting tumor cells from the immune system and promoting their invasiveness. It is well-documented that platelets can take up various tumor-secreted components, subsequently altering their properties to further suppress immune responses, enhance tumor cell invasiveness, and promote angiogenesis. This raises the question of whether selective inhibition of proteasomes in platelets, given their key role in regulating immune responses, could modify their functionality to the extent that they no longer provide protection to tumor cells.

The candidate will try to answer this question by systematically evaluating the role of individual proteasome subunits in platelet function and their impact on breast cancer progression. Using advanced molecular biology tools, patient-derived tumor models, and microfluidic systems, the candidate will:

- elucidate how selective inhibition of individual proteasome subunits in platelets influences tumor-platelet interactions and the shaping of the TME;
- assess the clinical relevance of proteasome-modulated platelets using primary breast cancer cultures derived from patient tumor explants;
- investigate the effects of platelet-released factors on the immune system and angiogenesis.

It is desirable for the candidate to have knowledge of the English language and prior methodological knowledge in the field of cellular or molecular biology.

5. Priloge, ki jih kandidat priloži k prijavi *(Documents that the candidate submits with the application)*:

☒ **diplomska listina/potrdilo o zaključku študijskega programa** *(diploma certificate for study programme, with which the candidate has enrolled/ will enroll in a doctoral degree programme)*

☒ **priloga k diplomi/ potrdilo o opravljenih obveznostih (***official transcript of all the grades for study programme, with which the candidate has enrolled/will enroll in a doctoral degree programme)*

☒ **potrdilo o do sedaj opravljenih obveznostih z ocenami študijskega programa, s katerim se bo kandidat prijavil na študij** *(official transcript of all the grades the candidate has received so far for the study programme, with which the candidate will enroll to a doctoral degree programme)*

☒ **nagrade** *(awards (e.g. Prešeren Prize of the University of Ljubljana, Prešeren Prize of a University of Ljubljana member and/or another equivalent award))*

☒ **bibliografija** *(bibliography)*

☒ **življenjepis** *(CV)*

☒ **motivacijsko pismo** *(motivation letter)*

☒ **opis dosedanjega sodelovanja pri raziskovalnem delu** *(description of the candidate's research work)*

☐ **osnutek idejne zasnove raziskovalnega dela** *(preliminary research proposal)*

☒ **priporočilno pismo** *(letter of recommendation)*

☐ **druge priloge** *(other attachments)*

Opis raziskovalnega dela *(Research work description*)

1. Članica UL (*UL member)*:

Univerza v Ljubljani, Fakulteta za farmacijo (*University of Ljubljana, Faculty of Pharmacy*)

2. Ime, priimek in elektronski naslov mentorja/ice (*Mentor's name, surname and email*):

Prof. dr. Žiga Jakopin (*ziga.jakopin@ffa.uni-lj.si*)

3. Raziskovalno področje (*Research field*):

Po klasifikaciji ARIS: 1.09 Farmacija (*Pharmacy*) oziroma po klasifikaciji ERC: LS6 Imunost, infekcije in imunoterapija (*Immunity, Infection, and Immunotherapy*)

4. Opis raziskovalnega dela (Research work description*)*:
Vključuje morebitne dodatne pogoje, ki jih mora izpolnjevati kandidat/ka za mladega raziskovalca/ko, ki niso navedeni v razpisu za mlade raziskovalce *(It includes any additional conditions that the candidate for a young researcher must meet, which are not listed in the call to tender for young researchers.)*.

V svetu trenutno vlada akutno pomanjkanje novih adjuvansov in imunoterapevtikov. Adjuvansi povečajo imunogenost cepiv, zaradi česar so njihove ključne komponente. Potrebni so ne le, da povečajo jakost samega odziva ampak tudi, da vplivajo na sam tip odziva, ki bi privedel do najbolj učinkovite zaščitne imunosti proti določenemu patogenu/tumorju. Imunoterapevtiki so sposobni modulacije lastnega imunskega sistema in so uporabni predvsem pri zdravljenju rakavih obolenj, pa tudi vnetnih bolezni in alergij. Napredek v razumevanju prirojene imunosti je odprl nove možnosti za razvoj tovrstnih imunomodulatorjev. Prirojeni imunski sistem sestavljajo imunske celice, ki vsebujejo vrsto receptorjev za prepoznavanje vzorcev (PRR), med drugimi tudi receptorje NOD1. Agonisti PRR stimulirajo te celice in zagotovijo nepogrešljive začetne signale, ki določajo tip, jakost in trajnost imunskega odziva, zato predstavljajo obetavne spojine vodnice za razvoj novih adjuvansov/imunoterapevtikov. Hkratna aktivacija različnih PRR omogoča ojačanje signala. Kovalentna povezava več agonistov PRR pa le še poveča njihovo adjuvantno aktivnost, zato so tudi konjugirani dvojni agonisti postali predmet raziskav. Cilj usposabljanja bo torej raziskati in pripraviti nove imunomodulatorje za ustvarjanje ustreznih imunskih odzivov. *Mladi raziskovalec bo v prvi fazi vključen v razvoj novih agonistov NOD1 in konjugiranih dvojnih agonistov prirojene imunosti. Nato bo z uporabo celičnih testov na primarnih imunskih celicah in celičnih linijah proučil mehanizem delovanja spojin ter imunomodulatorno delovanje sintetiziranih spojin. V sklopu sodelovanja med skupino prof. Jakopina in prof. Slütterja (Leiden University) bomo proučevali ex vivo adjuvantno delovanje sintetiziranih spojin na uveljavljenih modelih, in sicer na mišjih dendritičnih celicah in jih ovrednotili v smislu vpliva na izločanje citokinov, izražanje površinskih označevalcev in sposobnosti indukcije proliferacije T celic CD4 in CD8. Najboljše spojine bodo kot adjuvansi ovrednotene na mišjem modelu vakcinacije. Izbrane spojine bomo kot imunoterapevtike dali ovrednotiti tudi v singenskem mišjem tumorskem modelu. Vzporedno bo mladi raziskovalec pod somentorstvom izr. prof. Janje Zupan (UL FFA, Katedra za klinično biokemijo) ovrednotil agoniste prirojene imunosti tudi na mezenhimskih matičnih/stromalnih celicah (MSC), in sicer s poudarkom na vplivu na polarizacijo, fenotip, sekretorni citokinski profil ter vplivu na interakcije MSC z imunskimi celicami in rakavimi celicami.* *Če povzamemo, mladi raziskovalec bo v okviru doktorske disertacije načrtoval in sintetiziral agoniste NOD1 in konjugirane dvojne agoniste PRR ter vrednotil njihovo biološko aktivnost z različnimi celičnimi testi. Zaželeno je, da ima kandidat za mladega raziskovalca osnovna znanja s področja farmacevtske/organske kemije (načrtovanje, sinteza in analitika spojin) in celične imunologije (biološko vrednotenje spojin z in vitro testi), ki pa jih bo tekom doktorata ponovno osvežil in tudi poglobil.*

Currently, there is an acute shortage of new adjuvants and immunotherapeutics in the world. Adjuvants enhance the immunogenicity of vaccines, making them essential components. They are necessary not only to increase the strength of the immune response but also to influence the specific type of response that would lead to the most effective protective immunity against a particular pathogen or tumor. Immunotherapeutics are capable of modulating the body's immune system and are primarily used for the treatment of cancer, as well as inflammatory diseases and allergies. Advances in understanding innate immunity have opened new opportunities for developing such immunomodulators. The innate immune system consists of immune cells that contain various pattern recognition receptors (PRRs), including NOD1 receptors. PRR agonists stimulate these cells and provide crucial initial signals that determine the type, strength, and duration of the immune response, making them promising lead compounds for the development of new adjuvants and immunotherapeutics. Simultaneous activation of different

PRRs enhances the signal, while covalent binding of multiple PRR agonists further increases their adjuvant activity. As a result, conjugated dual agonists have also become a subject of research. The goal of the training will be to investigate and develop new immunomodulators to generate appropriate immune responses.

In the first phase, the young researcher will be involved in the development of new NOD1 agonists and conjugated dual agonists of innate immunity. Subsequently, using cellular assays on primary immune cells and cell lines, they will study the mechanism of action of these compounds and their immunomodulatory effects. As part of the collaboration between the research groups of Prof. Jakopin and Prof. Slütter (Leiden University), we will investigate the ex vivo adjuvant activity of the synthesized compounds using established models, specifically on murine dendritic cells. These studies will assess their impact on cytokine secretion, the expression of surface markers, and their ability to induce CD4 and CD8 T cell proliferation. The most promising compounds will be evaluated as adjuvants in a murine vaccination model. Selected compounds will also be assessed as immunotherapeutics in a syngeneic murine tumor model. In parallel, the young researcher will evaluate innate immunity agonists on mesenchymal stem/stromal cells (MSCs) under supervision of co-supervisor Assoc. Prof. Janja Zupan (UL FFA, Department of Clinical Biochemistry), focusing on their effects on polarization, phenotype, secretory cytokine profile, and their interactions with immune cells and cancer cells.

In summary, in the scope of doctoral dissertation the young researcher will design and synthesize NOD1 agonists and conjugated dual PRR agonists and then investigate their activity profile in various cell-based assays. It is therefore desirable that the candidate for the young researcher has basic knowledge of medicinal/organic chemistry (design, synthesis and analysis of compounds) as well as cell immunology (i.e., practical skills for conducting biological evaluation in vitro). However, these skills will be refreshed and further deepened during the PhD program.

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