Šifra projekta / Project code J7-4640

Inovativna imunoterapija raka oreko CAR T celic (CARRS)
Innovative CAR T cell cancer immunotherapy (CARRS)

Vodja projekta:
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1. VSEBINSKI OPIS PROJEKTA:

Uporaba imunskega sistema za zdravljenje raka je bila uspešno uporabljena preko zaviralcev kontrolnih točk za preprečevanje izčrpanosti T celic. Drugi **nedavni preboj v imunoterapiji raka** je bil izum gensko spremenjenih T celic, ki izražajo umetni/sintetični **himerni antigenski receptor (CAR)**, ki jih programira, da ciljajo antigene, prisotne na rakavih celicah. CAR ima modularno zgradbo, sestavljeno iz ektodomene, ki veže antigen, vmesnika, transmembranske domene in znotrajcelične signalne domene. Ektodomena je sestavljena iz enoverižnega variabilnega fragmenta (scFv), ki izhaja iz monoklonskega protitelesa, na primer protitelo proti CD19 v trenutno odobrenih FDA in EMA celičnih produktih CAR T. Intracelularna signalna domena običajno vključuje T celično aktivacijsko domeno, ki izhaja iz CD3ζ verige receptorja T celic, kot tudi kostimulatorne domene, kot sta CD28 in 4-1BB. Grupiranje CAR receptorjev, ki ga povzroča vezava ciljnega antigena na površini rakavih celic, sproži prenos signala s fosforilacijo CD3ζ-osnovanimi tirozinskimi aktivacijskimi motivi (ITAM) preko kinaz Lck in ZAP-70, kar vodi do aktivacije T celic in ubijanja tarčne celice s citolizo in sproščanjem citokinov. CAR T celična terapija proti CD19, je bila odobrena za zdravljenje refraktornih in recidivnih pediatričnih ALL in DLBCL. Kljub izjemnemu kliničnemu odzivu zdravljenja s celicami CAR T, **številni izzivi še vedno omejujejo širši klinični prenos/terapevtsko učinkovitost**, vključno z: (i) hudimi neželenimi stranskimi učinki, kot sta citokinski vihar (CRS) in nevrotoksičnost zaradi prekomerne aktivacije T celic, (ii) visoka cena terapije, ki omejuje njeno dostopnost, čeprav bi terapijo lahko ustanovili v lokalni ustanovi z visoko izkušenimi raziskovalci, (iii) nizka proti tumorska aktivnost, (iv) izmikanje antigena in (v) omejena infiltracija CAR T celic v trdne tumorje. Znatna prizadevanja so bila vložena v napredek CAR T celične terapije z uporabo načel sintezne biologije, področja na katerem izkazujemo pomembne napredke. Cilj projekta je pripraviti celice CAR T, da bodo **varnejše in učinkovitejše proti različnim vrstam hematoloških rakov**, ki bi jih lahko **prenesli v klinično okolje za korist bolnikov**.

Augmentation of the immune system for cancer therapy has been successfully applied by checkpoint inhibitors to prevent exhaustion of T cells. The second recent **breakthrough in cancer immunotherapy** has been the invention of genetically engineered T cells that express artificial/synthetic **chimeric antigen receptor (CAR)**, which programs them to target an antigen present on cancer cells. CARs have a modular design consisting of an antigen-binding ectodomain, a hinge, a transmembrane domain and an intracellular signaling domain. The ectodomain consists of a single-chain variable fragment (scFv) derived from a monoclonal antibody, such as anti-CD19 antibody, in the currently FDA and EMA-approved CAR T cell products. The intracellular signaling domain usually comprises T cell activation domain derived from CD3ζ-chain of the T cell receptor as well as costimulatory domains such as CD28 and 4-1BB. Clustering of CARs induced by target antigen binding on the surface of cancer cells initiates signal transduction via phosphorylation of CD3ζ-derived immunoreceptor tyrosine-based activation motifs (ITAM) by kinases Lck and ZAP-70, leading to T cell activation and killing of target cells through cytolysis and cytokine release. CAR T cell therapy targeting CD19 has been approved for treatment of refractory and relapsed pediatric ALL and DLBCL. Despite the remarkable clinical response of CAR T cell therapy, many **challenges still limit wider clinical transition**/therapeutic efficacy, including: (i) severe adverse effects such as cytokine-release syndrome (CRS) and neurotoxicity by excessive T cell activation, (ii) high cost of the therapy which limits its accessibility, although the therapy could be customized and adapted at the local facility with highly experienced researchers, (iii) low anti-tumor activity, (iv) antigen escape and (v) limited infiltration of CAR T cell into solid tumors. Considerable

efforts have been invested to advance CAR T cell therapy by applying principles of synthetic biology, the field where the NIC project group can show a track record of important advances recognized in the field. The objective of this project is to engineer CAR T cells to be safer and more effective against the relevant types of hematologic cancers that could be translated to benefit patients in a clinical setting.

Sodelujoče organizacije:

- **Zavod za transfuzijsko medicino**
- **Univerza v Ljubljani, Medicinska fakulteta, Inštitut za mikrobiologijo in imunologijo**
- **Univerzitetni klinični center Ljubljana, SPS Klinični oddelek za hematologijo**

a. osnovni podatki glede financiranja:

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The project is co-financed by ARRS with 9856 annual hours of price class D for a period of 3 years. Funding starts on October 1, 2022.

b. sestava projektne skupine s povezavami na SICRIS

Na Kemijskem inštitutu v projektni skupini sodelujejo / At the National Institute of Chemistry the project group includes:

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2. faze projekta in njihova realizacija

Specifični cilji so **povečati obseg indikacij** za hematološko CAR T celično terapijo (CD20, CD22 in BCMA in njihove kombinacije, **WP1**), pripraviti **nove tipe humaniziranih regulatorjev za boljši nadzor in varnost terapije** (**WP2**), razviti in testirati **alternativne signalne domene**, ki so učinkovitejše ali odporne na izčrpanost (**WP3**), razvitje **kombinacije s prirojenimi imunskimi signalnimi potmi** za premagovanje tumorskega imunosupresivnega okolja (**WP4**), testiraje izboljšav v **pred kliničnih mišjih modelih** (**WP5**) in analizirati **regulativne poti** za klinični prenos terapij (**WP6**).

The specific goals are to **increase the range of targets**/indications for hematological CAR T cell therapy (CD20, CD22 and BCMA and their combinations, **WP1**), introduce **new types of humanized regulators** to provide **better control and safety of therapy** (**WP2**), develop and test **alternative signaling domains** that may be more effective or resistant to exhaustion (**WP3**), develop **combinations with innate immune signaling pathways** to overcome tumor immunosuppressive environment (**WP4**), test cell-demonstrated enhancements in **preclinical mouse models** (**WP5**) and analyze the quality and **regulatory pathways** towards clinical translation (**WP6**).

3. bibliografske reference, ki izhajajo neposredno iz izvajanja projekta

LAINŠČEK, Duško, GOLOB URBANC, Anja, MIKOLIČ, Veronika, PANTOVIĆ, Jelica, MALENŠEK, Špela, JERALA, Roman. Regulation of CD19 CAR-T cell activation based on an engineered downstream transcription factor. Molecular therapy oncolytics. Jun. 15, 2023, vol. 29, p. 77-90, ISSN 2372-7705, DOI: 10.1016/j.omto.2023.04.005. [COBISS.SI-ID 154300419]

4. logotip ARRS in drugih sofinancerjev

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