

Razvoj protitumorskih celičnih terapij



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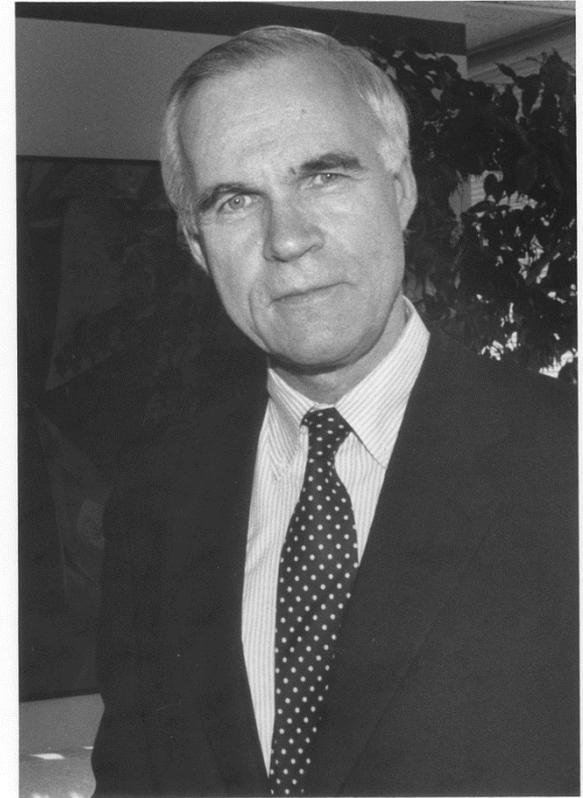
William Coley (1862 – 1936) – oče tumorske imunoterapije

- V 18. in 19. stoletju so ob kirurškem zdravljenju raka puščali odprte kirurške rane in namerno ustvarjali gnojne rane, ga bi gnojenje uničilo tumor
- Leta 1891 je ameriški kirurg William Coley poročal o zdravljenju neoperabilnih bolnikov z rakom s toksičnim pripravkom *Streptococcus pyogenes* – zaradi poročil o bolnikih z rakom, ki so ozdraveli po streptokokni okužbi kirurške rane (erizipel).
- Do leta 1963 so to zdravljenje uporabljali za zdravljenje sarkomov (po Coley-u 51% uspešnost, kasnejše analize – 20% uspešnost, l. 1963 FDA zavrne terapijo kot neuspešno in z veliko neželjenimi učinki.



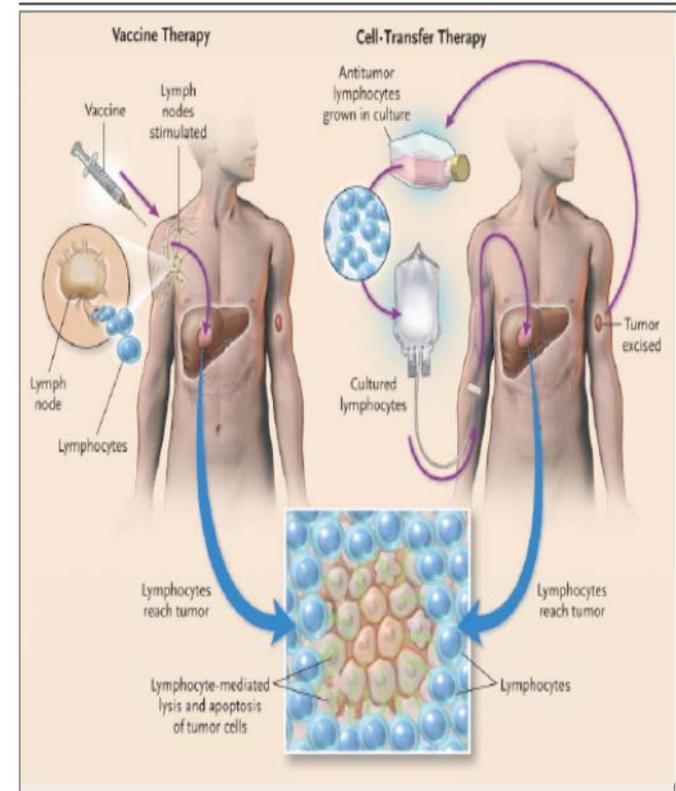
Lloyd John Old (1933 – 2011) – oče tumorske imunologije

- Vodja raziskav na „Memorial Sloan Kettering Cancer Center“
- S sodelavci je uvedel bacillus Calmette-Guérin (BCG) v imunoterapijo tumorjev
- odkril je povezavo med glavnim histokompatibilnim kompleksom (MHC) in levkemijo
- odkril je povezavo med virusom Epstein-Barr (EBV) in nazofaringealnim karcinomom
- odkril je dejavnik tumorske nekroze (TNF)
- z odkritjem TL, Lyt (CD8) antigenov je opredelil koncept diferenciacijskih antigenov celične površine
- neodvisno od dveh drugih skupin odkril p53



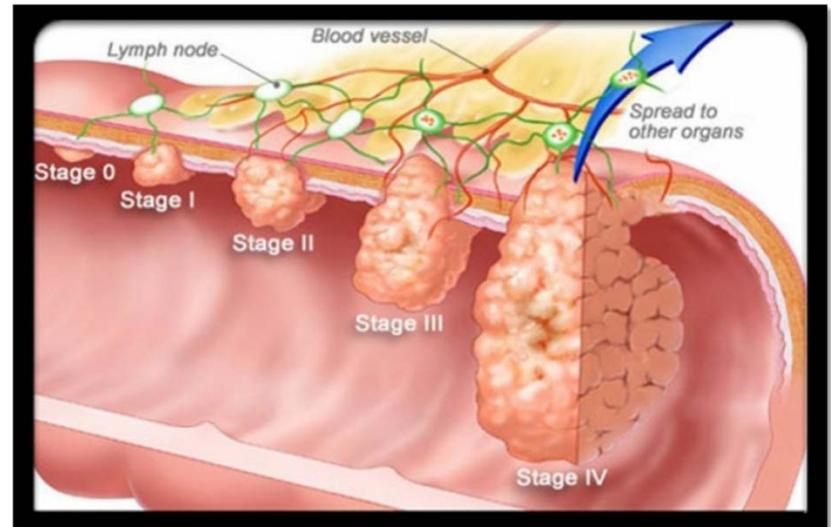
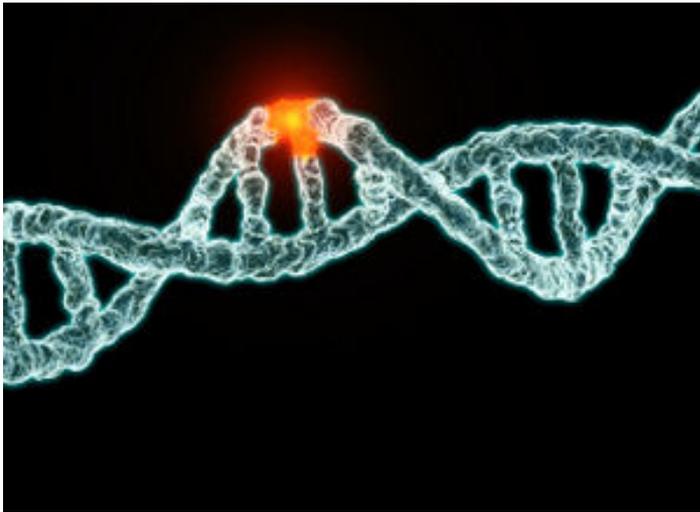
Steven A. Rosenberg (1940)

- Predstojnik kirurgije na Nacionalnem inštitutu za raka v Bethesda v Marylandu
- Začetnik razvoja tumorske imunoterapije s citokini (IL-2) in adoptivne imunoterapije s tumor-infiltrirajočimi limfociti T (TIL).



Kaj je rak?

Rak je splošno ime za obsežno skupino različnih bolezni, katerih glavna značilnost je nenadzorovana razrast spremenjenih, rakastih celic, ki pa nastanejo zaradi nepopravljive spremembe v celičnem jedru, mutacije genov



Zdravljenje: kirurško, obsevalno, citostatsko, tarčno, imunološko

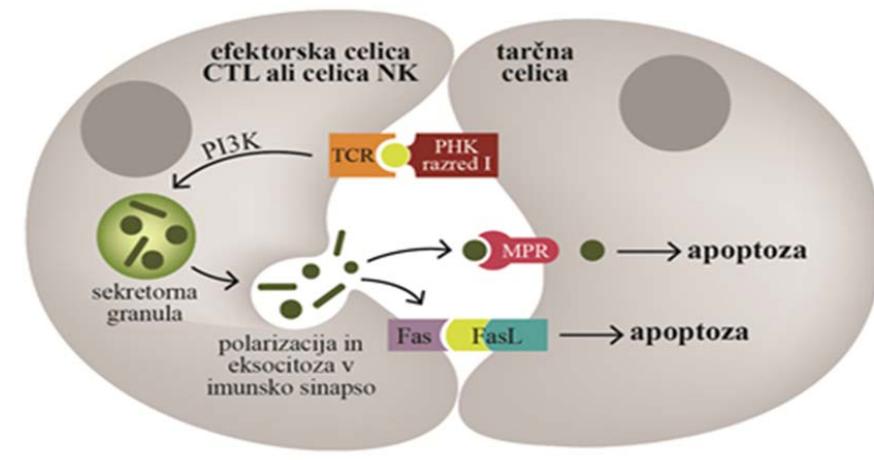
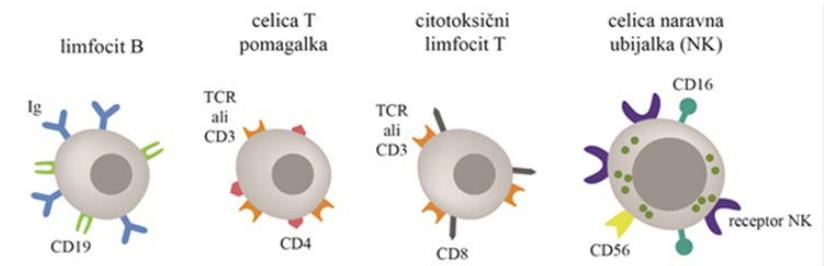
Odvisno od vrste in razširjenosti raka



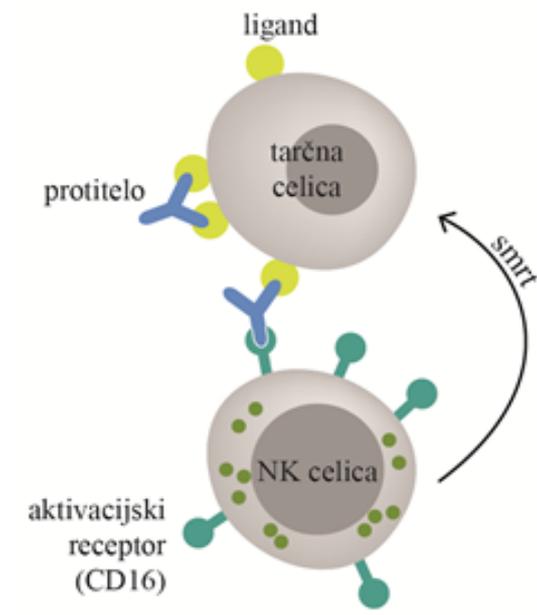
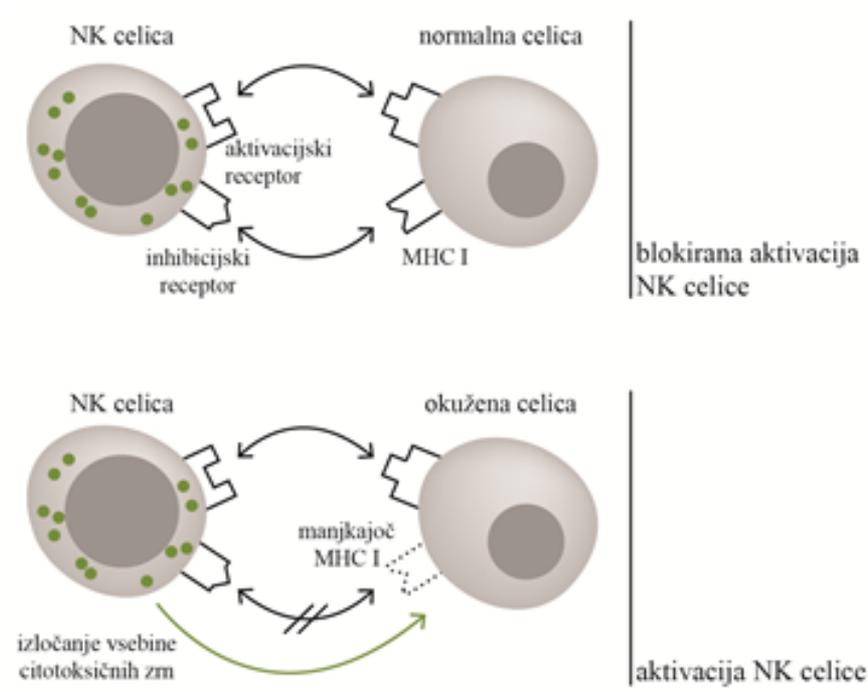
Teorija: rakaste celice nenehno nastajajo, 99,999...% jih normalno uniči imunski sistem

Protitumorski imunski odzivi

- Vsaka tumorska celica vsebuje spremenjene (mutirane) beljakovine, ki ji omogočajo nekontrolirano rast
- Imunski sistem ima mehanizme za odkrivanje ne-lastnih celičnih beljakovin (virusnih, spremenjenih tumorskih) in citotoksično uničevanje celic s spremenjenimi beljakovinami



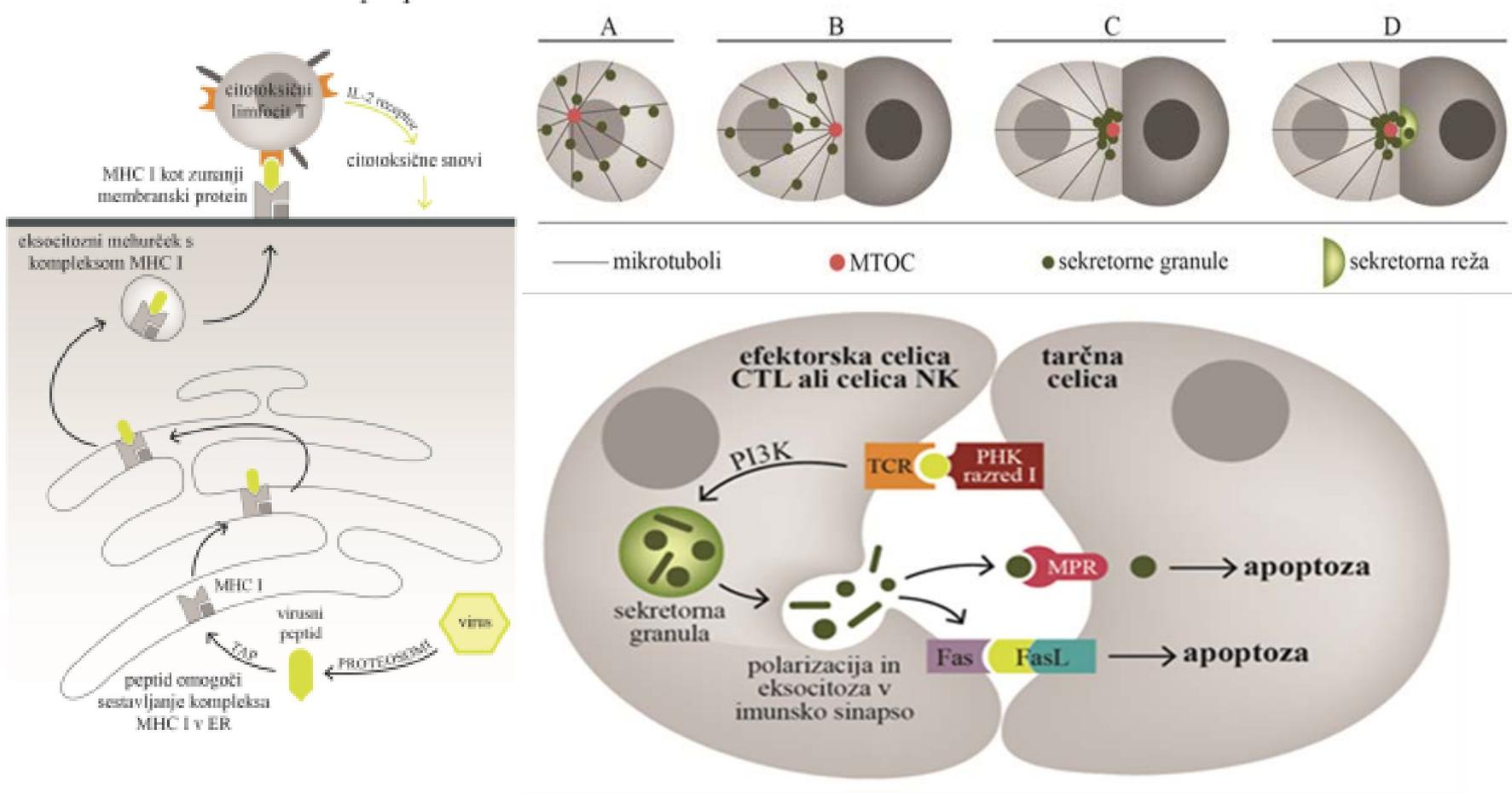
Celice NK



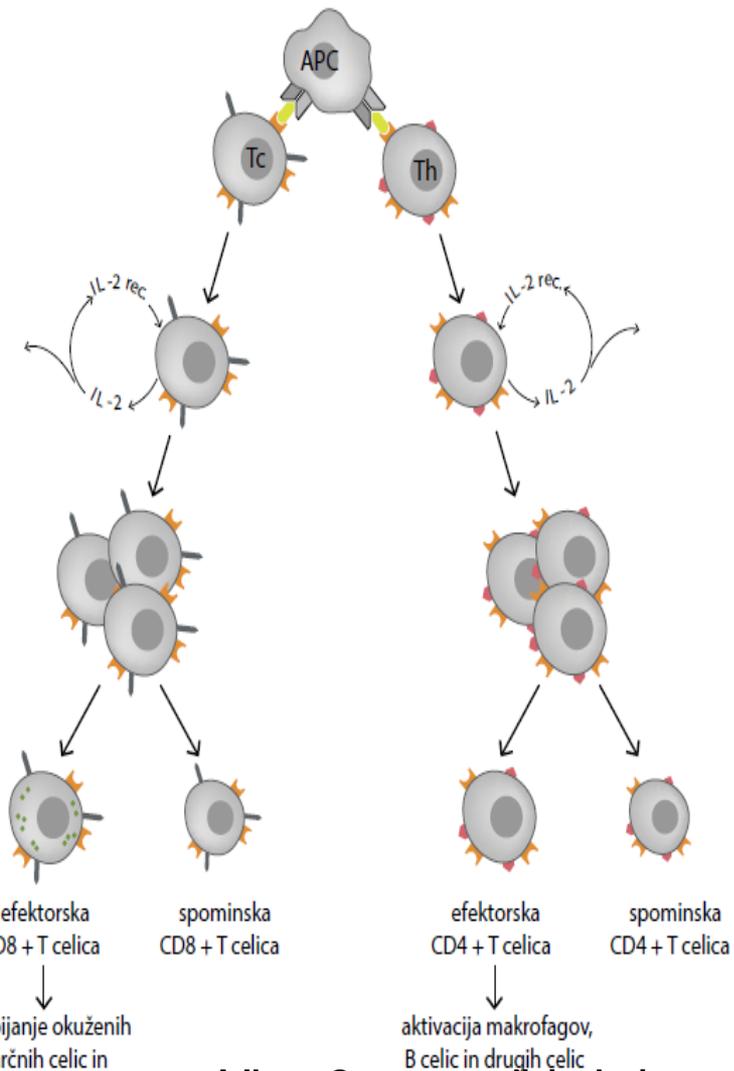
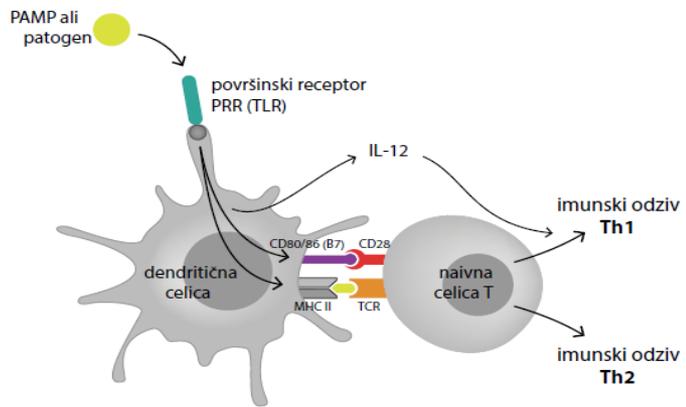
- Inhibitorni receptorji celic NK prepoznajo molekule MHC razreda I v kompleksu s peptidom, kar zagotavlja, da celice NK ne napadejo zdravih gostiteljskih celic
- Celice NK se aktivirajo ob stiku z okuženimi celicami, pri katerih je izražanje molekul MHC razreda I zmanjšano.

MHC I in citotoksični napad na okužene celice

Nastanek molekule MHC I in prepoznavna okužene celice



Klonska ekspanzija

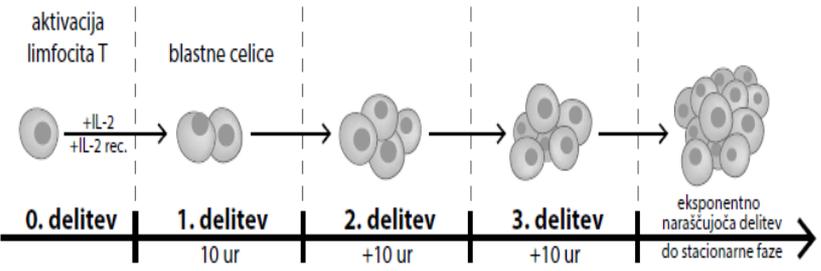


prepoznavanje antigena

aktivacija

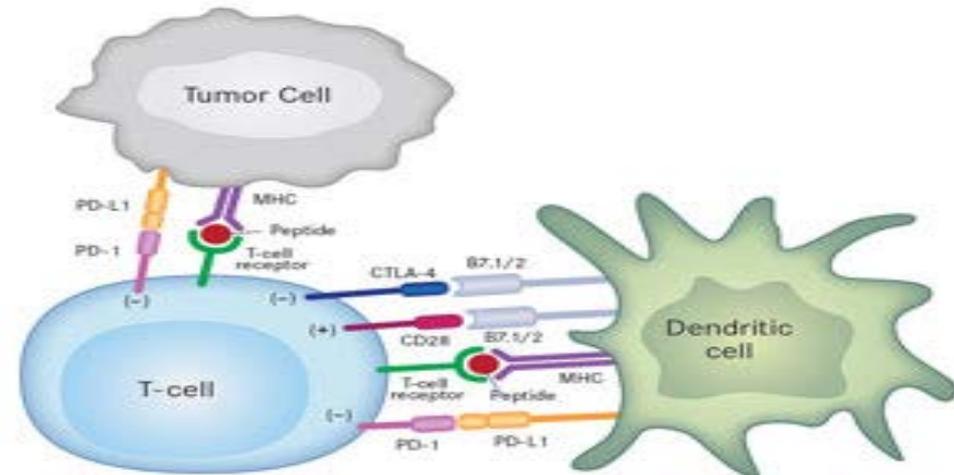
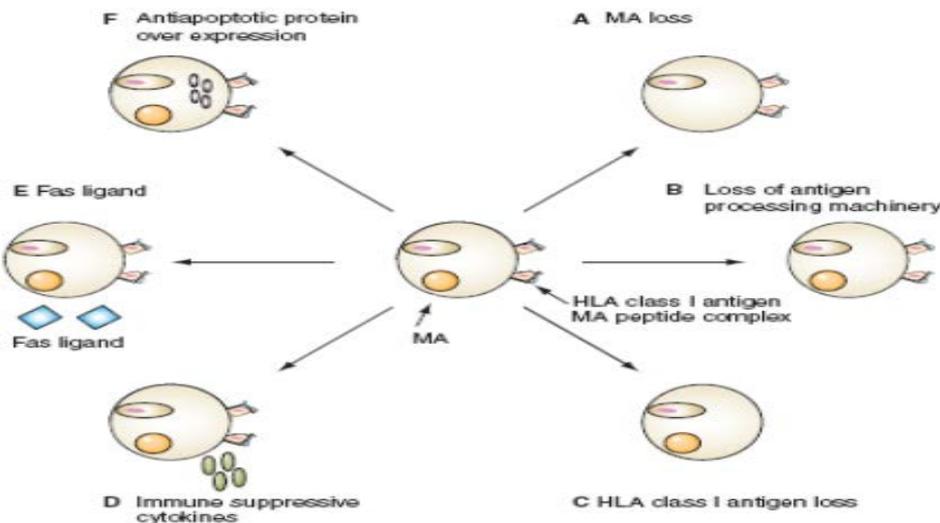
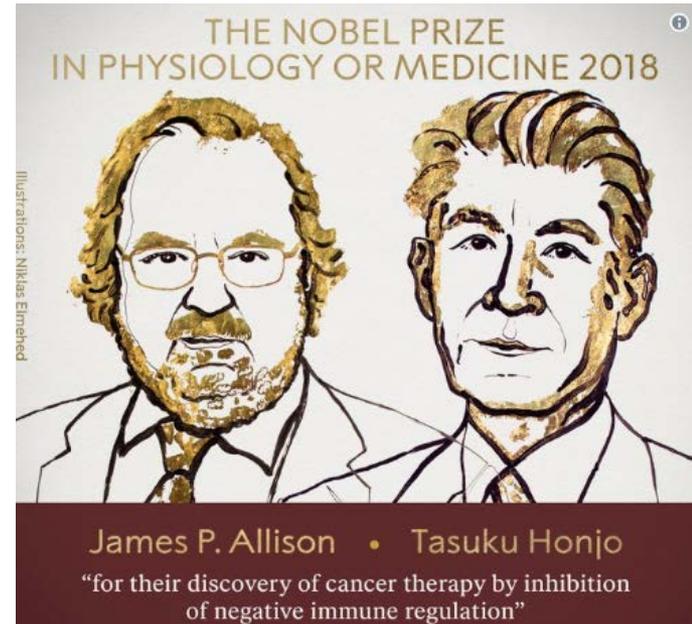
klonska ekspanzija in diferenciacija

diferencirane celice



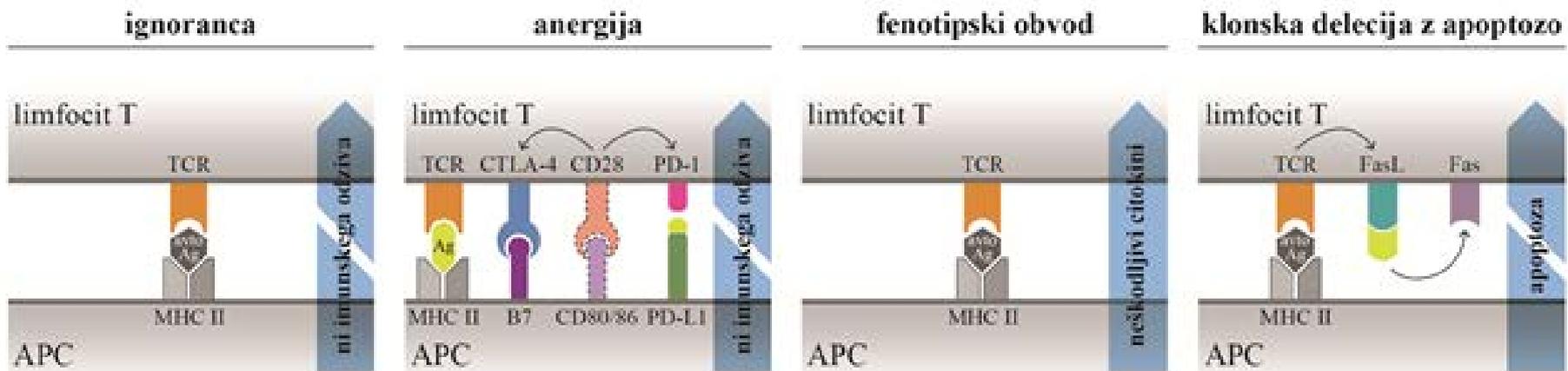
Mehanizmi, s katerimi se tumor izogne imunskemu odzivu

- Tumorske celice neučinkovito predstavljajo tumorske antigene
- Indukcija anergije ali delecije tumorsko specifičnih limfocitov T
- Imunoregulatorni limfociti T CD4+CD25+
- **Imunoregulatorne nadzorne točke (CTLA 4, B7-H1, B7-H4) – normalno služijo imunoregulaciji ob (kroničnem) vnetju**
- Izločanje immunosupresivnih citokinov, imunostimulacija tumorjev



Mehanizmi periferne tolerance

- mehanizmi intrinzične periferne celične T tolerance: ignoranca, anergija, apoptoza, fenotipski obvod;
- mehanizmi ekstrinzične periferne T celične tolerance: tolerogene dendritične celice, regulatorne celice T





Multifocal gastric adenocarcinoma in a patient with LRBA deficiency

Nina Bratanić¹, Jernej Kovac², Katka Pohar³, Katarina Trebušak Podkrajšek^{2,4}, Aljož Ihan³, Tadej Battelino^{1,4} and Magdalena Avbelj Stefanija^{1*}

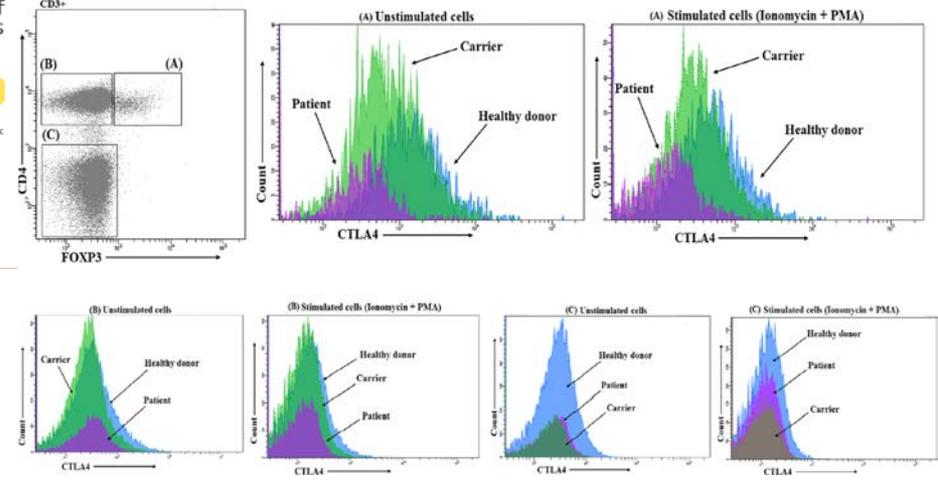
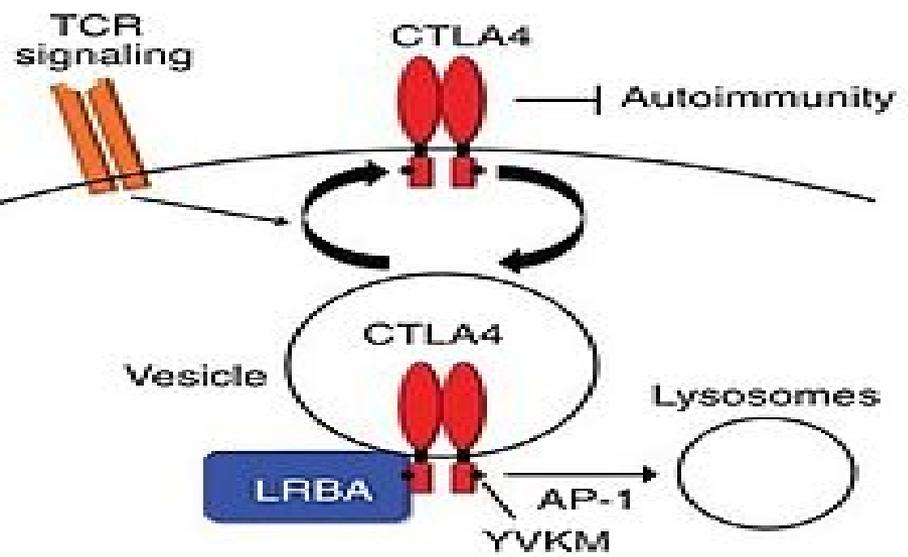
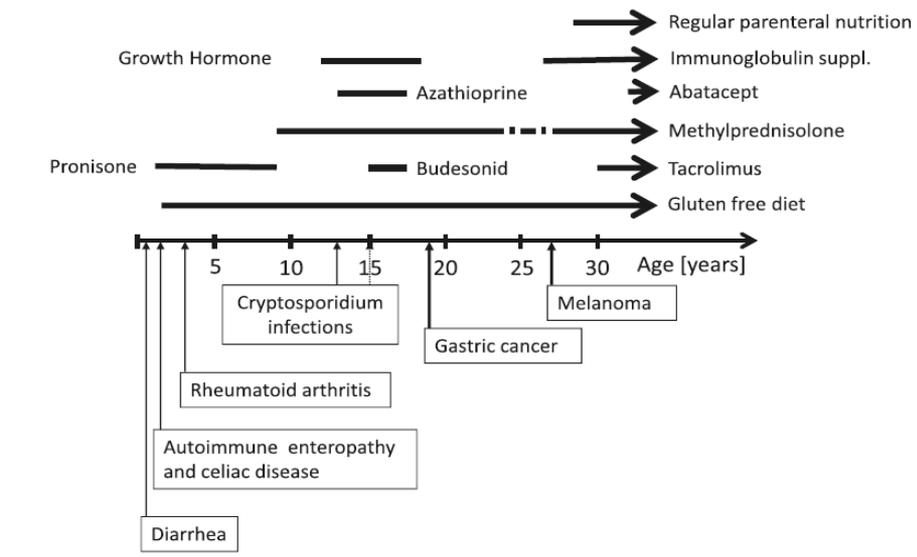
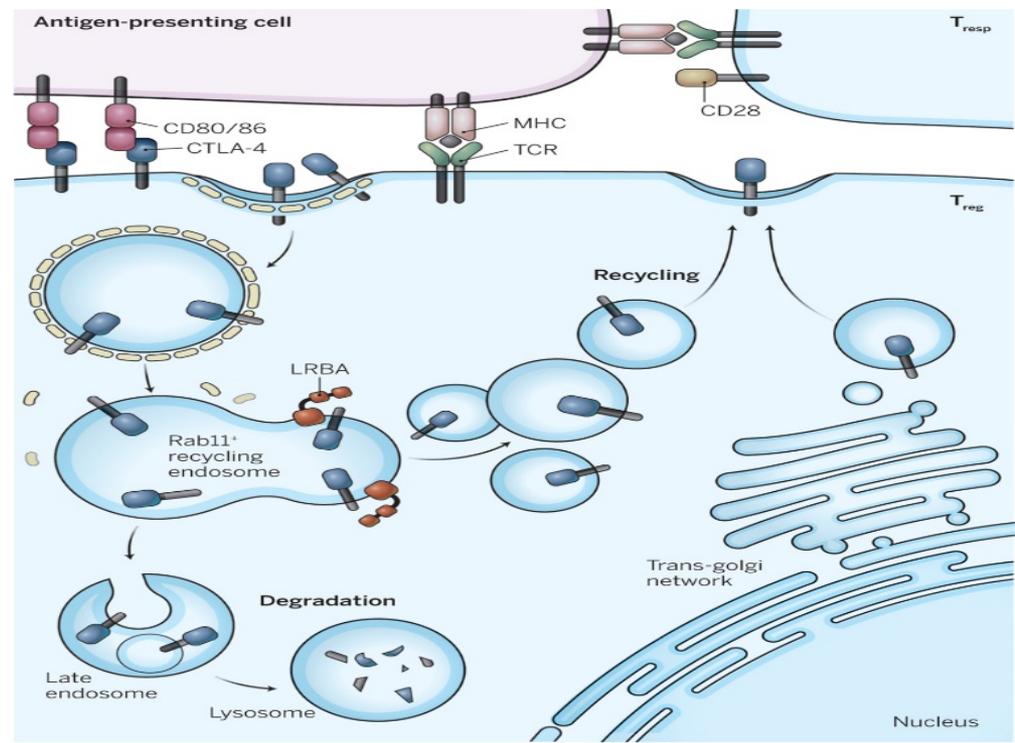
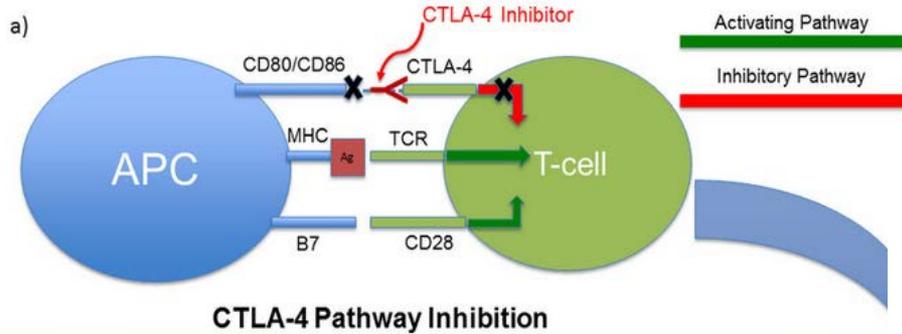


Fig. 2 Total and mobilized CTLA4 levels in CD3+ cells. Unstimulated and stimulated FOXP3+ CD4+ T cells (A), FoxP3 negative CD4 T cell (B), and CD4 negative T cells (C) from LRBA deficient patient (violet heterozygous carrier (yellow) and healthy donor (blue). Legend: PM – phorbol 12-myristate 13-acetate

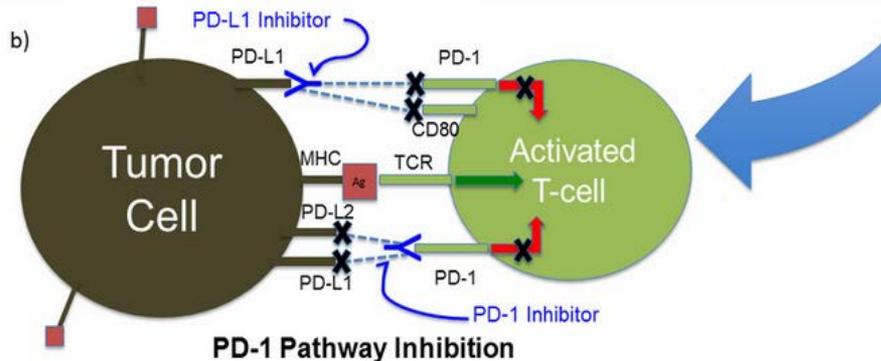


CTLA-4 in PD-L1/PD-1 pot za izogibanje adaptivnemu imunskemu odzivu

Discovery Med 2018;12:101



CTLA-4 Pathway Inhibition



PD-1 Pathway Inhibition

Therapeutic	Lead company	Antibody type	Affinity/ K_D^*	Reference	Cancer Type	Antibody	Year
					Melanoma	Ipilimum ab	2011
					Melanoma	Nivolumab	2014
					Melanoma	Pembrolizum ab	2014
					Non-small cell lung cancer	Nivolumab	2015
					Non-small cell lung cancer	Pembrolizum ab	2015
					Melanoma (BRAF wild-type)	Ipilimum ab+ nivolumab	2015
					Melanoma (adjuvant)	Ipilimum ab	2015
					Renal cell carcinoma	Nivolumab	2015
					Hodgkin lymphoma	Nivolumab	2016
					Urothelial carcinoma	Atezolizumab	2016
					Head and neck squamous cell carcinoma	Nivolumab	2016
					Head and neck squamous cell carcinoma	Pembrolizum ab	2016
					Melanoma (any BRAF status)	Ipilimum ab+ nivolumab	2016
					Non-small cell lung cancer	Atezolizumab	2016
					Hodgkin lymphoma	Pembrolizum ab	2017
					Merkel cell carcinoma	Avelumab	2017
					Urothelial carcinoma	Avelumab	2017
					Urothelial carcinoma	Durvalumab	2017
					Urothelial carcinoma	Nivolumab	2017
					Urothelial carcinoma	Pembrolizum ab	2017
					MSI-high or MMR-deficient solid tumors of any histology	Pembrolizum ab	2017
					MSI-high, MMR-deficient metastatic colorectal cancer	Nivolumab	2017
					Pediatric melanoma	Ipilimum ab	2017
					Hepatocellular carcinoma	Nivolumab	2017
					Gastric and gastroesophageal carcinoma	Pembrolizum ab	2017
					Non-small cell lung cancer	Durvalumab	2018
					Renal cell carcinoma	Ipilimum ab+ nivolumab	2018

Therapeutic	Lead company	Antibody type	Affinity/ K_D^*	Reference
Anti-PDL1				
MPDL3280A	Roche	Engineered IgG1 (no ADCC)	0.4nM	Herbst, et al. ASCO 2013
MEDI-4736	AstraZeneca	Modified IgG1 (no ADCC)	Not available	Stewart, et al. Cancer Res 2011
BMS-936559	Bristol-Myers Squibb	IgG4	Not available	Brahmer, et al. NEJM 2012
Anti-PD1				
Nivolumab	Bristol-Myers Squibb	IgG4	2.6nM	Brahmer, et al. J Clin Oncol 2010
MK3475 (pembrolizumab)	Merck & Co	IgG4 (humanised)	29pM	Patnaik, et al. J Clin Oncol 2012
AMP-224	GlaxoSmithKline	PD-L2 IgG1 Fc fusion	Not available	Smothers, et al. Ann Oncol 2013



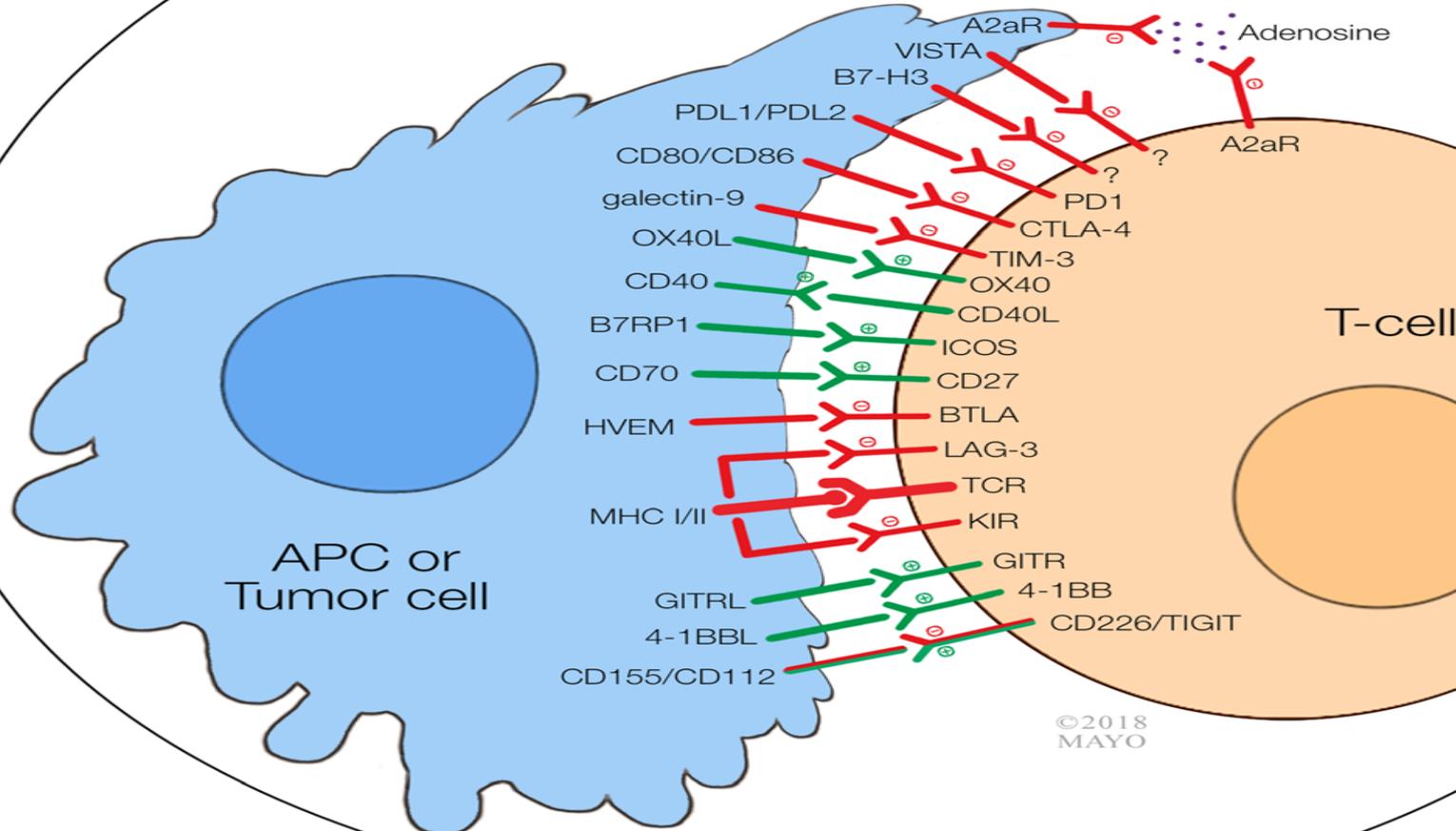
Next generation of immune checkpoint therapy in cancer: new developments and challenges

Julian A. Marin-Acevedo¹, Bhagirathbhai Dholaria^{2,3}, Aixa E. Soyano², Keith L. Knutson⁴, Saranya Chumsri² and Yanyan Lou^{2*}

Other potential pathways

IL10
IDO
TGF- β

CD47
TLR
Arginase



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MAYO

■ INHIBITORY PATHWAYS
■ STIMULATORY PATHWAYS

Delovanje imunoterapij

Stimulacija obstoječe imunosti:

1. Vnetna : citokinska th, BCG instilacija (rak mehurja), onkolitični virusi
2. Limfocitna: celice LAK, celice TIL

Tarčno uničevanje tumorjev

- 1. cepiva proti onkogenim virusom, tumorska cepiva (dendritične celice, mRNK), CAR-T, CAR-NK
- 2. Monoklonska protitelesa, inhibicija kontrolnih točk, *ex vivo* ekspanzirani specifični limfociti T – adoptivna celična T terapija

Prve ideje cepiv: cepiva iz tumorskih celic

- Avtologna celična cepiva - oslabiljene tumorske celice bolnika:
 - celoten antigenski repertoar, specifičen za bolnikov tumor
 - polivalentni protitumorski imunski odziv
 - variabilna imunogenost tumorjev med bolniki
- Alogenska celična cepiva - celične linije iz tumorskih celic bolnikov:
 - standardizirana, široko dostopna
 - uporabna pri večjem številu bolnikov
 - Canvaxin, Melacin

Cepiva z dendritičnimi celicami

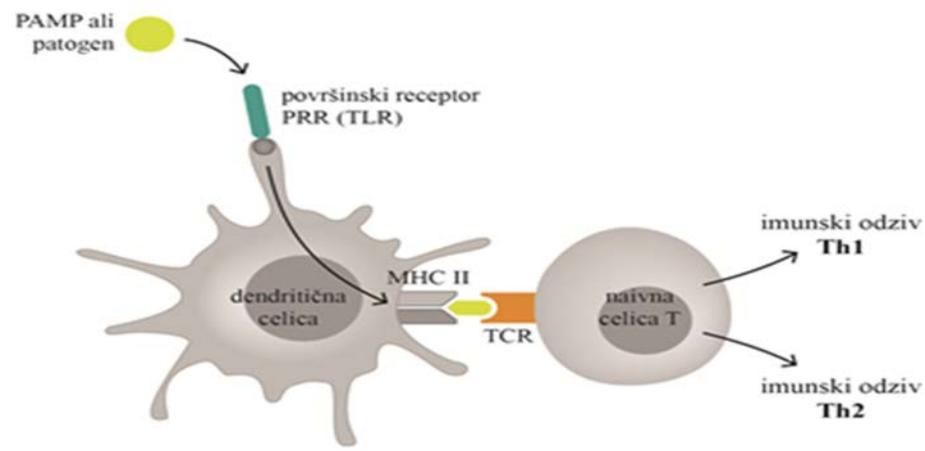
DC so antigen predstavitvene celice, ključne za navzkrižno aktivacijo naivnih CD8+ limfocitov T

Zrele DC: CD34+ prekurzorji iz kostnega mozga ali periferne krvi + citokini

Nezrele visoko fagocitne DC: periferni monociti + GM-CSF in IL-4

“Loading” s tumorskimi antigeni:

- peptidi, proteini, eksosomi, heat shock proteini, apoptotske celice, celični lizati
- transfekcija s cDNA ali mRNA
- fuzija DC s tumorsko celico

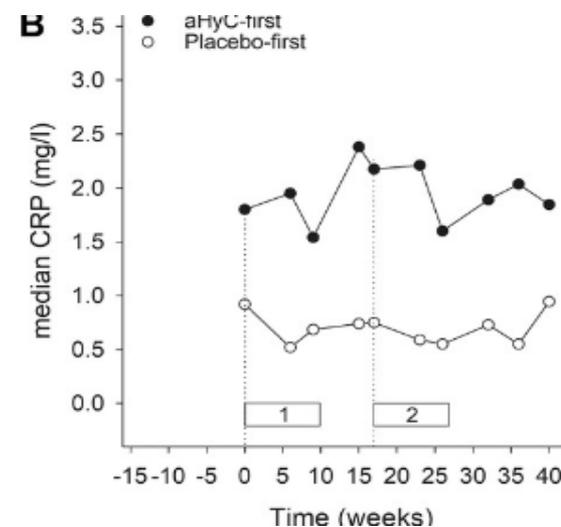
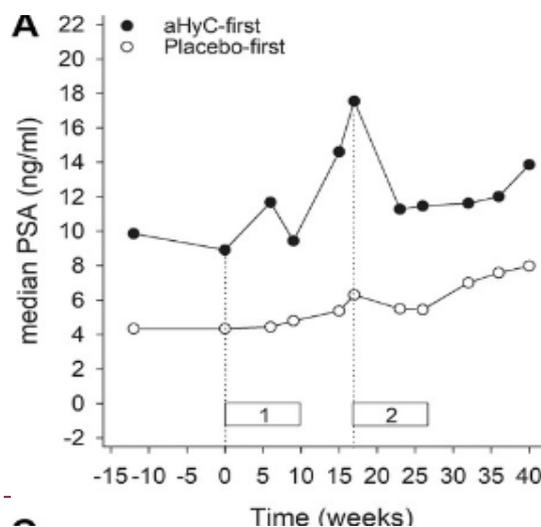
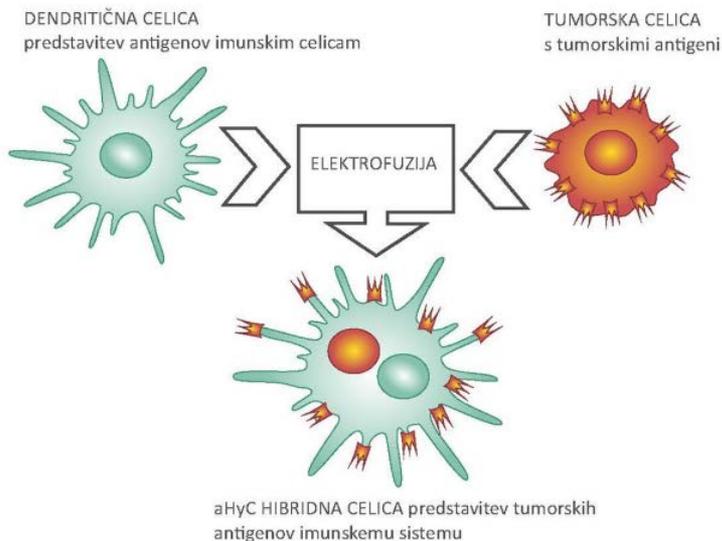


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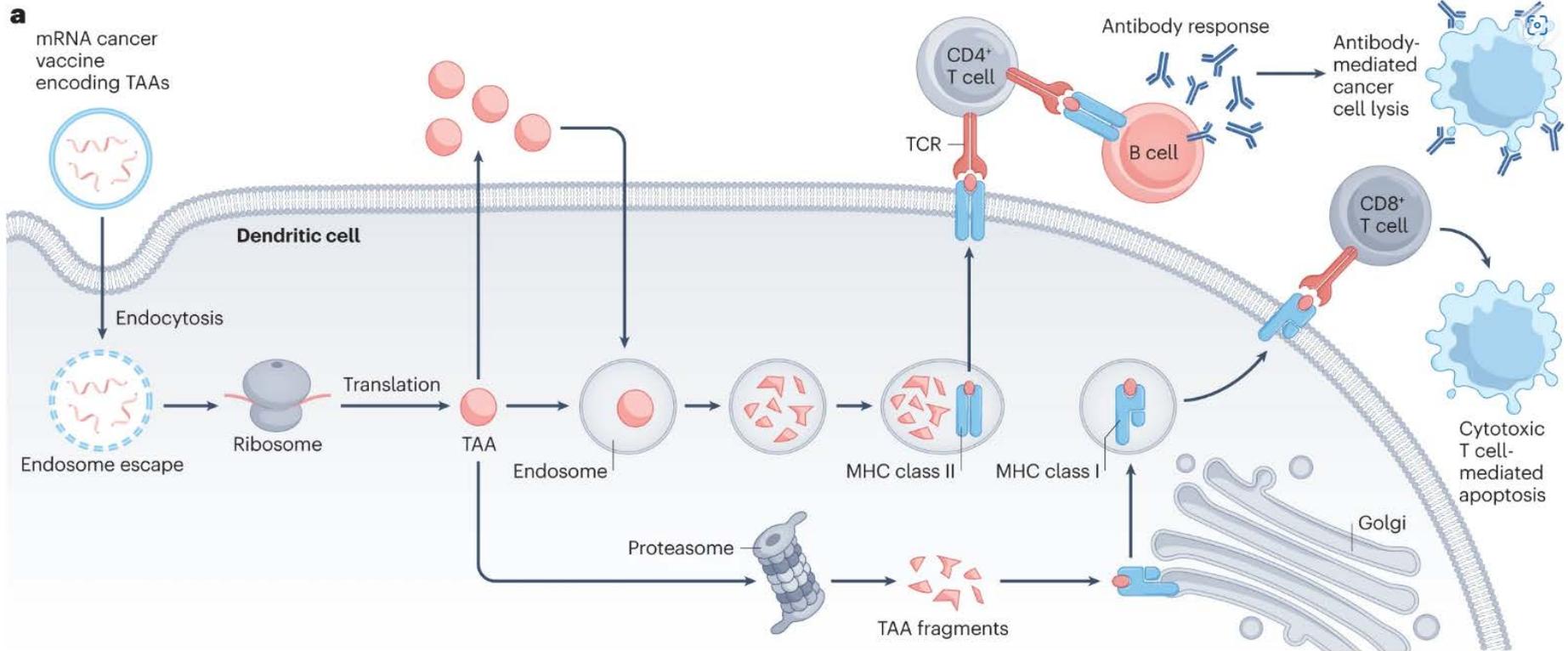
Survival of castration-resistant prostate cancer patients treated with dendritic–tumor cell hybridomas is negatively correlated with changes in peripheral blood CD56^{bright}CD16⁻ natural killer cells

Helena Haque Chowdhury, Simon Hawlina, Mateja Gabrijel, Saša Trkov Bobnar, Marko Kreft, Gordan Lenart, Marko Cukjati, Andreja Nataša Kopitar, Nataša Kejžar, Alojz Ihan ... [See all authors](#) ▾

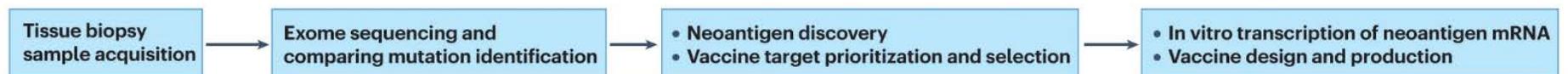
First published: 26 August 2021 | <https://doi.org/10.1002/ctm2.505> | Citations: 2



mRNK protitumorsko cepivo

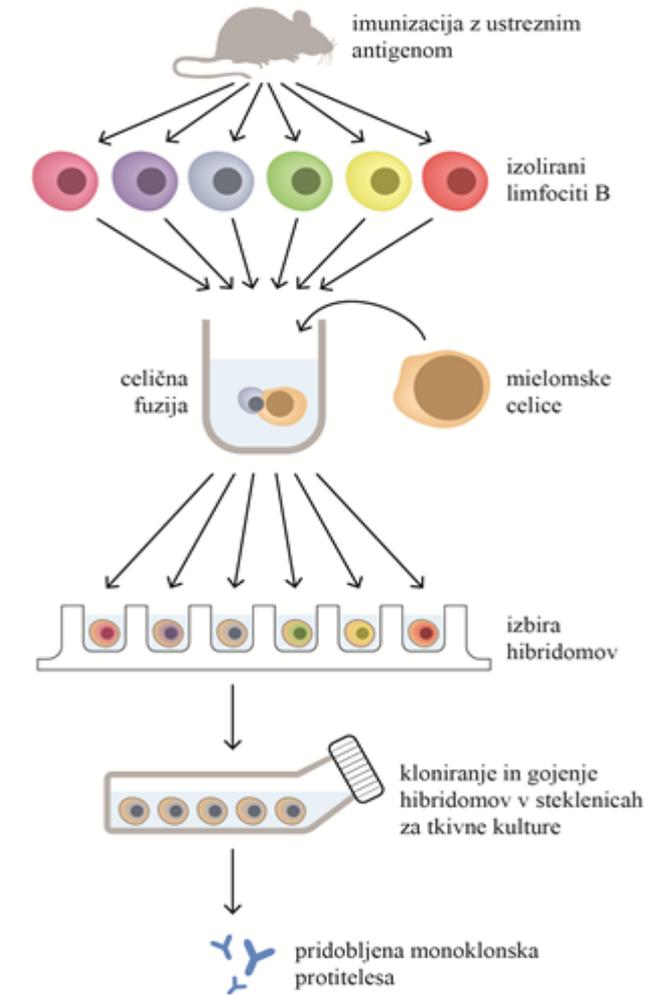


b Designing a neoantigen mRNA cancer vaccine



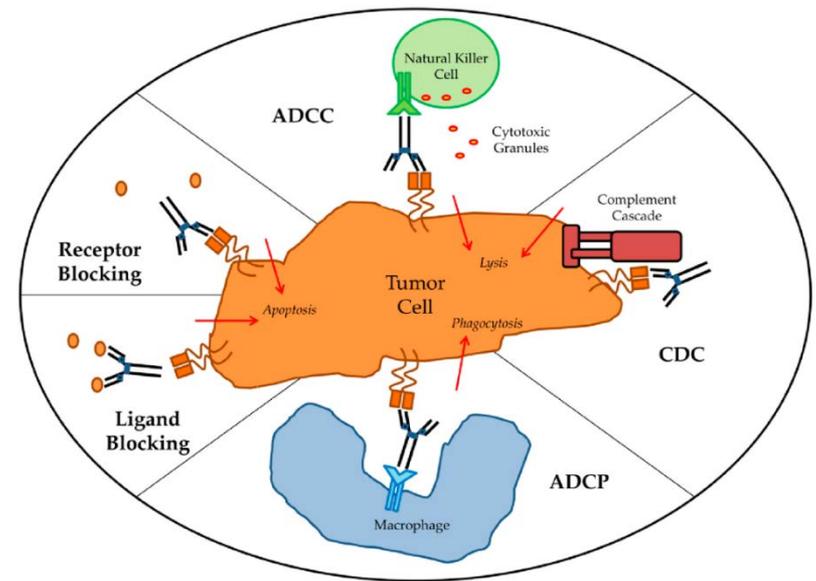
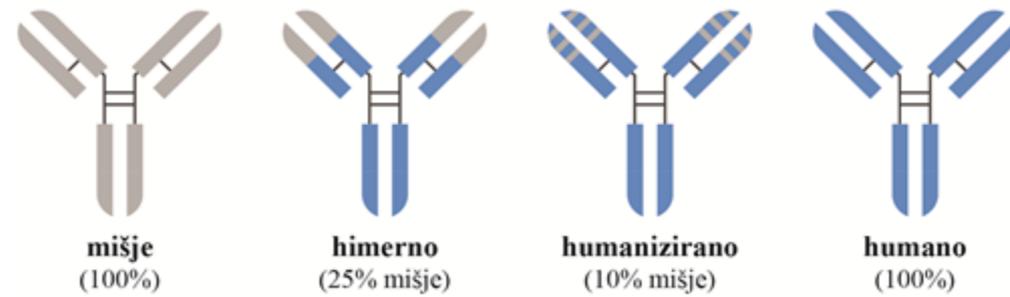
Tarčne imunoterapije je omogočil izum priprave monoklonskih protiteles

- L.1975 sta Georges J.F. Köhler in Cesar Milstein iznašla način za proizvodnjo monoklonskih protiteles
- Cepljenje miši s človeškimi proteini in zlitje limfocitov B z mielomskimi (malignimi) celicami. Pri tem nastanejo združene, hibridomske celice, ki tvorijo protitelesa (zaradi Ig genov limfocitov B) in se neprestano razmnožujejo (zaradi genov tumorskih celic). Za to odkritje sta leta 1984 prejela Nobelovo nagrado za medicino.

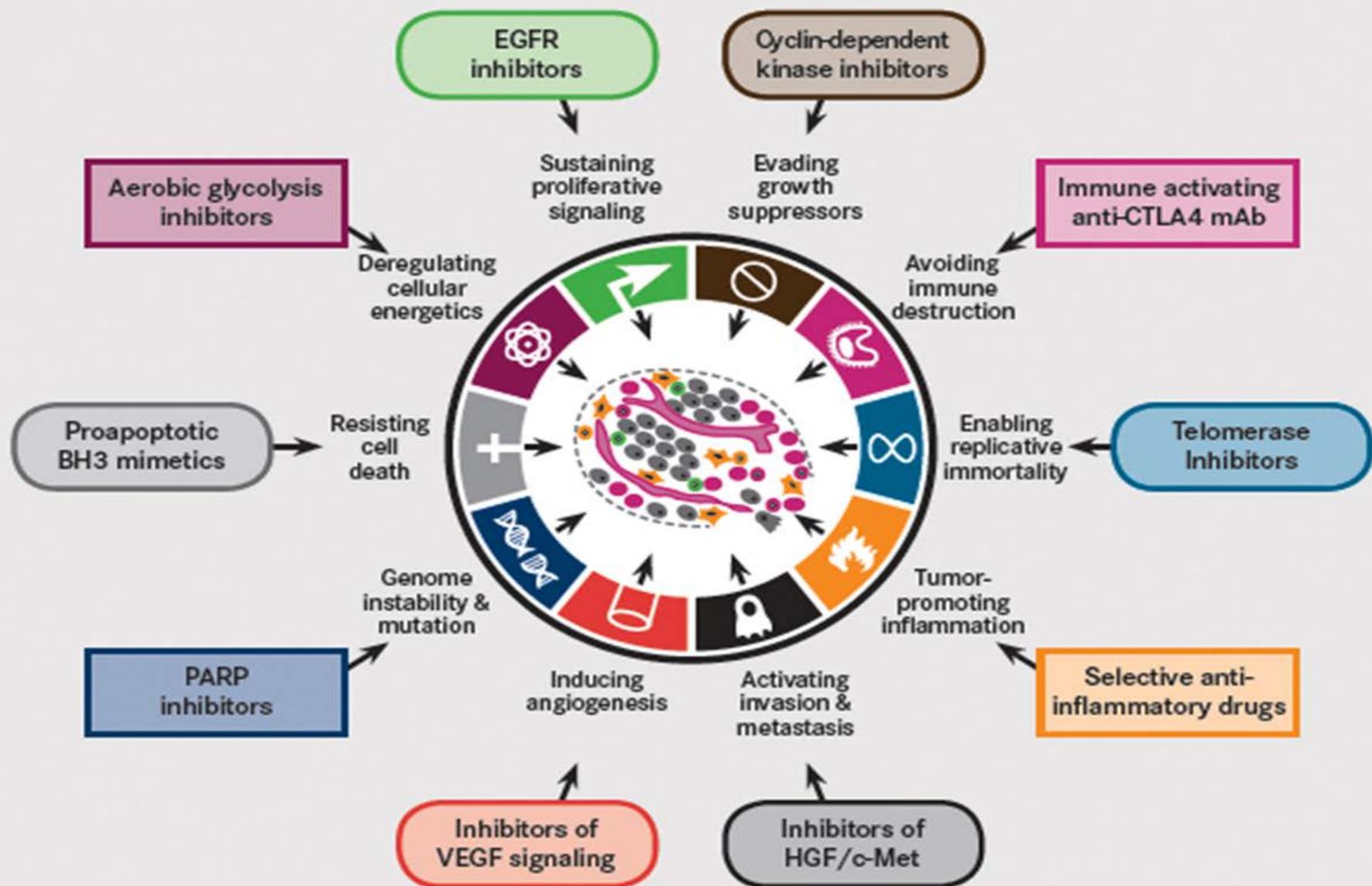


Monoklonska protitelesa kot tarčna zdravila

- Monoklonska protitelesa so lahko izjemno učinkovita tarčna zdravila
- Po aplikaciji v telo se vežejo na določen protein, ki opravlja pomembno funkcijo. Z vezavo bodisi inaktivirajo ciljno funkcijo (na primer aktivacijo skozi receptor CTLA-4 in PD-L1/PD-1) bodisi uničijo populacijo celic, ki ima na površini tarčni protein za monoklonsko protitelo (na primer tumorsko celico CD20 – B limfocit)



Name	Antigen	Format	Indications (Year of First Approval) ¹
Antibody–Drug Conjugates (ADCs)			
Gemtuzumab ozogamicin	CD33	Humanized ADC	Acute myeloid leukemia (2000)
Brentuximab vedotin	CD30	Chimeric ADC	Hodgkin’s lymphoma and Anaplastic large-cell lymphoma (2011)
Trastuzumab emtansine	HER2	Humanized ADC	Breast cancer (2013)
Inotuzumab ozogamicin	CD22	Humanized ADC	Acute lymphoblastic leukemia (2017)
Polatuzumab vedotin	CD79B	Humanized ADC	B-Cell Lymphoma (2019)
Enfortumab vedotin	Nectin-4	Human ADC	Bladder cancer (2019)
Trastuzumab deruxtecan	HER2	Humanized ADC	Breast cancer (2019)
Sacituzumab govitecan	TROP2	Humanized ADC	Triple negative breast cancer (2020)
Moxetumomab pasudotox	CD22	Mouse ADC	Hairy-cell leukemia (2018)
Ibritumomab tiuxetan	CD20	Mouse IgG1-Y90 or In111	Non-Hodgkin’s lymphoma (2002)
Iodine (I131) tositumomab	CD20	Mouse IgG2-I131	Non-Hodgkin’s lymphoma (2003)
Blinatumomab	CD19, CD3	Mouse BiTE	Acute lymphoblastic leukemia (2014)



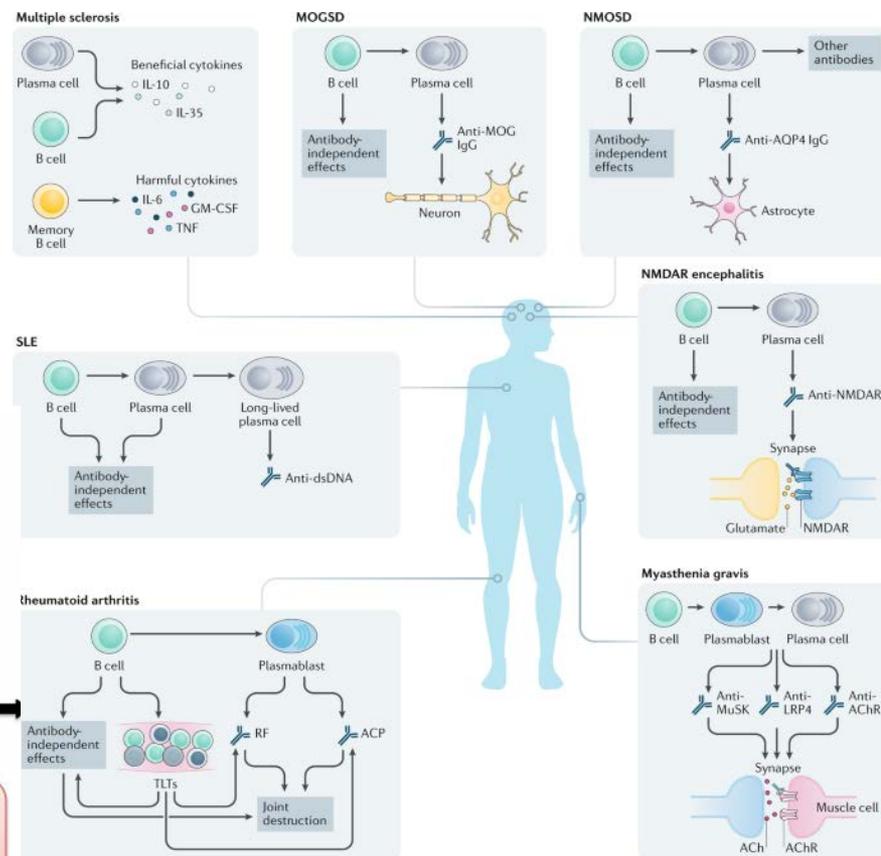
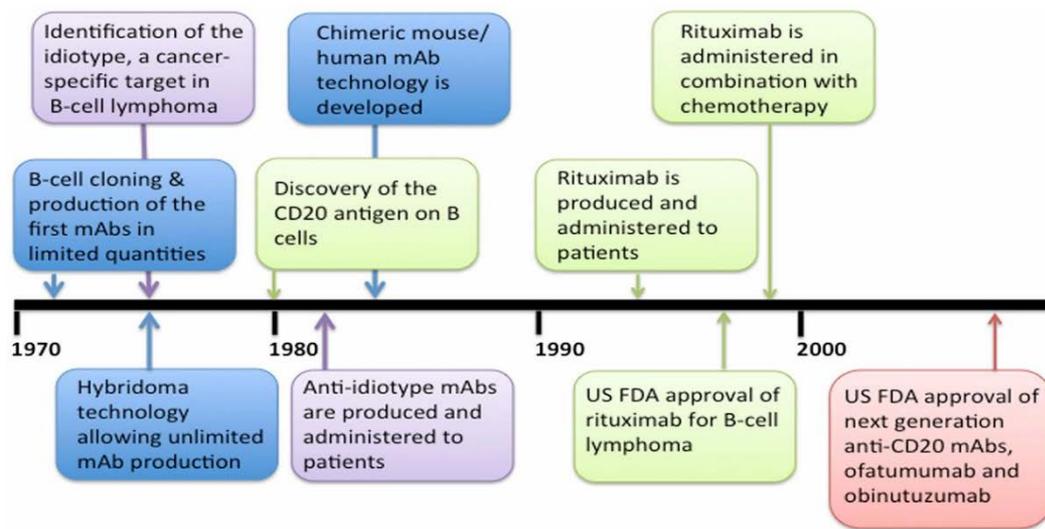
This figure illustrates some of the many approaches employed in developing therapeutics targeted to the known and emerging hallmarks of cancer.

EGFR indicates epidermal growth factor receptor; CTLA4, cytotoxic T lymphocyte-associated antigen 4; mAb, monoclonal antibody; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor; PARP, poly-(ADP ribose) polymerase.

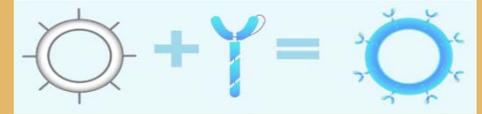
Protitelesa proti CD20 (limfocitom B)

- Pripravki anti-CD20 se vežejo na CD20, ki je na limfocitih B (na normalnih in maligno spremenjenih). S tem uničujejo limfocite B, zato jih uporabljamo za zdravljenje levkemij in limfomov, zraslih iz limfocitov B.

Zaradi hkratnega uničenja zdravih, netumorskih limfocitov B je začasno preprečen tudi bolnikov protitelesni imunski odziv, zato lahko zdravilo uporabimo tudi kot imunosupresiv.



Celice CAR-T (ang. Chimeric antigen receptor T cells)

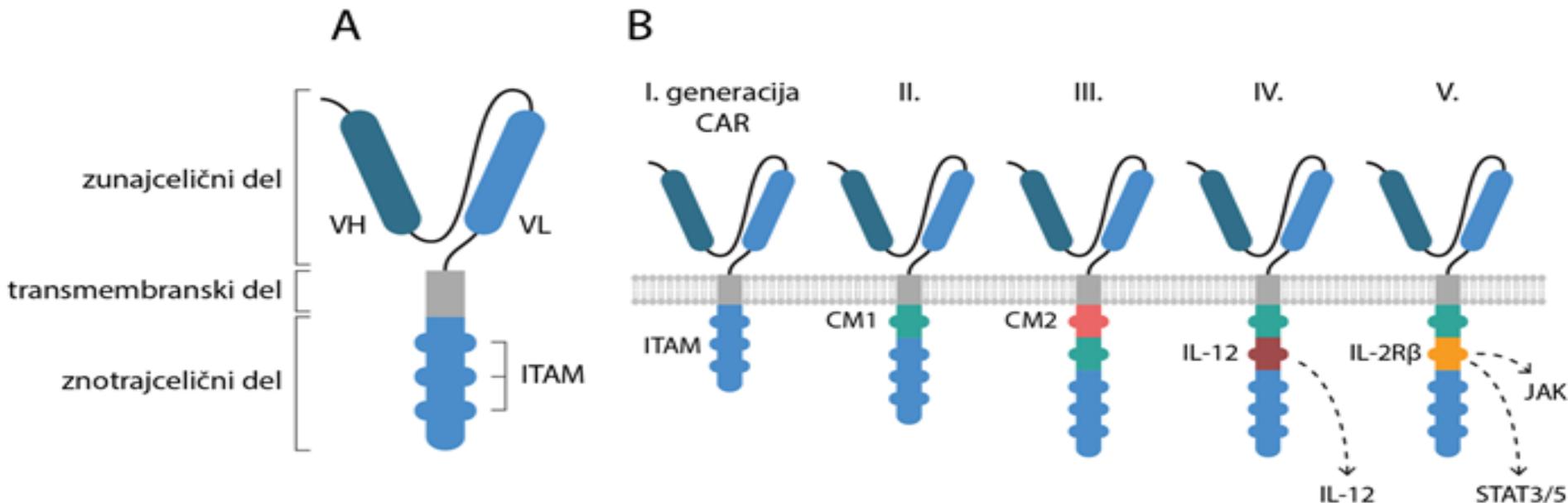


- Bolnikove limfocite T se odvzame iz krvi, jih v inkubatorju okuži z vektorskimi virusi, ki prinesejo v limfocite T nov gen (CAR), ki omogoča prepoznavanje bolnikovega tumorja.
- Tako pripravljene “umetne limfocite T” se v inkubatorju („umetna bezgavka“) dodatno razmnoži (2 tedna) in nato injicira v bolnika z namenom, da celice CAR-T napadejo in uničijo tumor.
- Sintetični receptorji CAR so oblikovani na osnovi enoverižne imunoglobulinske molekule tako, da limfocitom T omogočijo prepoznavo specifičnih tarčnih molekul na površini tumorskih celic. **Za razliko od receptorjev TCR, so receptorji CAR sposobni prepoznave površinskih antigenov neodvisno od molekul pglavitnega histokompatibilnostnega kompleksa (MHC).**

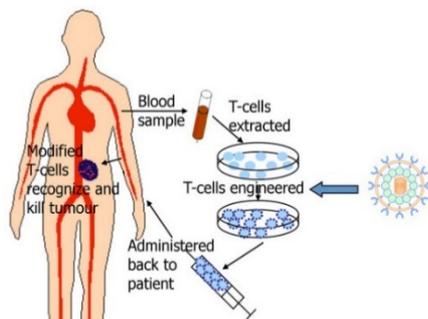


Genska modifikacija T celic

- 1. Uporaba himerno spremenjenih TCR receptorjev – CARs; Eksodomena TCR je spremenjena v fragment BCR – omogoča MHC neodvisno prepoznavo (lahko tumorji prenehajo z izražanjem MHC)
- 2. Uporaba kostimulativnih signalov; povečanje specifičnosti, ON/OFF switch.
- 3. Uporaba himernih receptorjev za izogibanje tumorski supresiji; IL-4/IL-7 himerni receptor, rezistenca na npr. rampamycin
- 4. Utišanje/izražanje novih genov; CCR4 receptor, utišanje endogenega TCR (nove metode CRISPR-CAS9, cinkovi prsti,...)



Zdravljenje z gensko spremenjenimi limfociti T – CAR-T

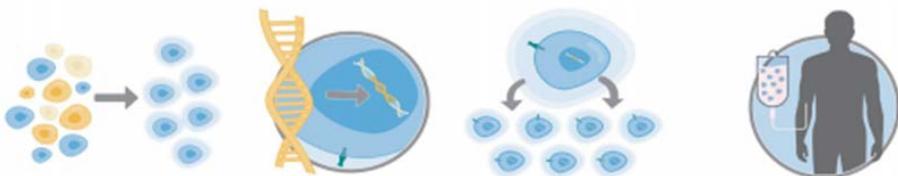


Retrovirusni (lentivirusni)

Vektorski virus – virusi so mikroorganizmi, ki vstopajo v naše celice in nekateri od njih (retrovirusi) vgradijo svoje gene v genom naših celic.

Če v retroviruse vgradimo gen za nov celični receptor (CAR), potem ob okužbi človeške celice retrovirus vgradi v genom človeške celice tudi vstavljen gen CAR.

S tem dobimo modificiran limfocit T, ki ima dodaten receptor za prepoznavo tumorja.

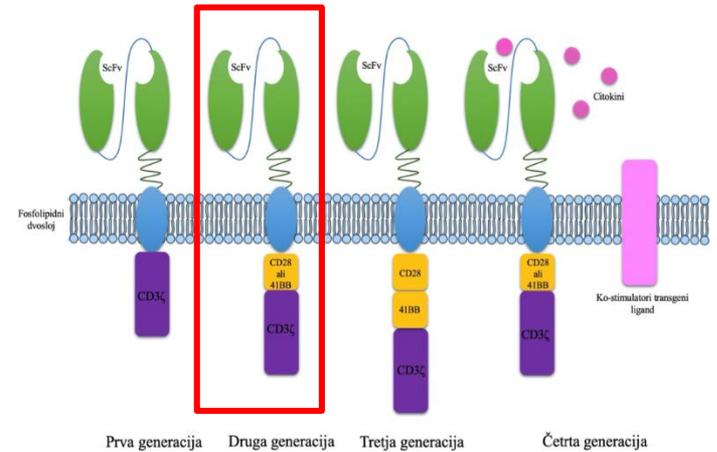
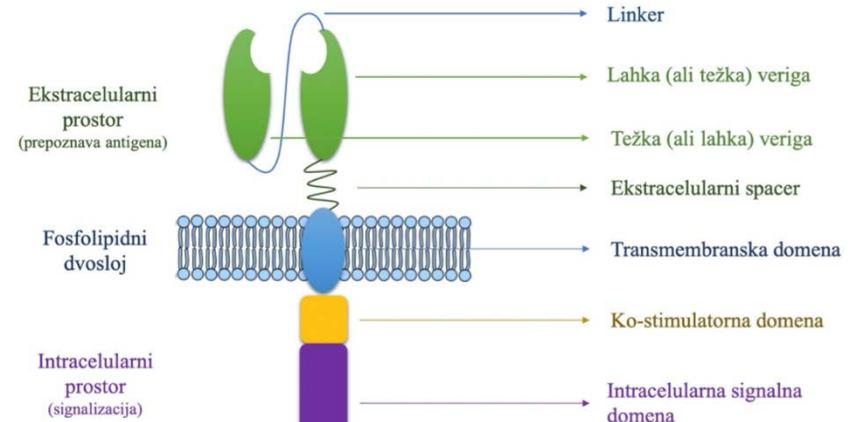
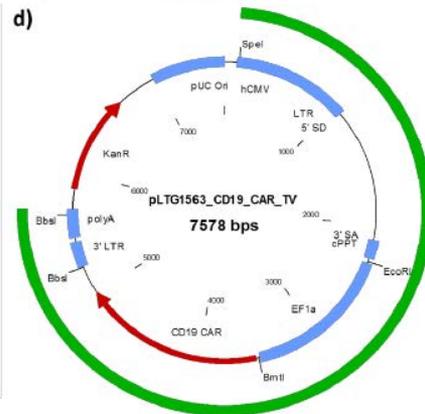
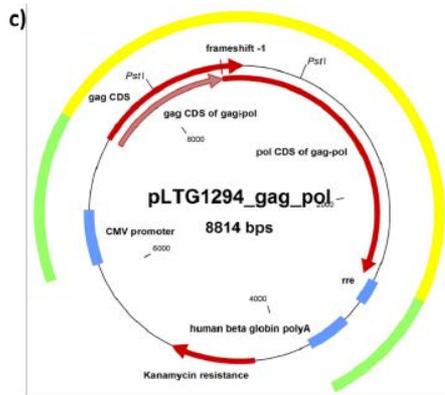
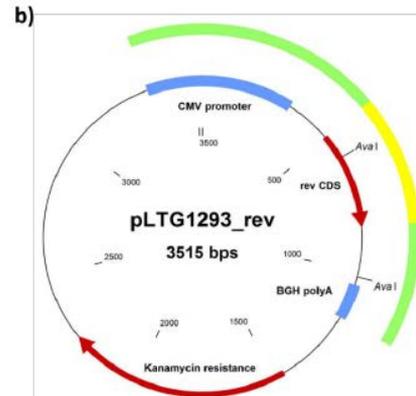
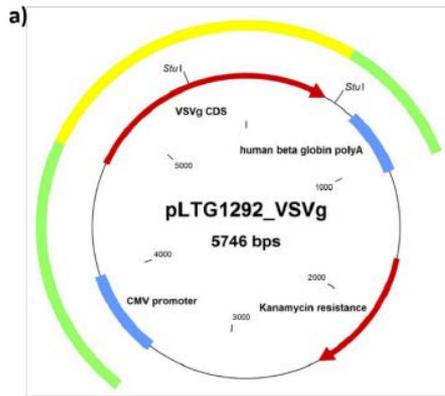


Tumor-specifični antigeni, ki se izražajo na površini krvnih rakov (CD19, CD22, CD123) ali solidnih tumorjev: EGFRvIII, HER2, Mezetelin



Tudi neželjeni učinki: citokinski vihar (cytokine release syndrome CRS), možganski edem, nevrotoksičnost, masovno uničenje aplazija normalnih tarčnih celic (npr limfocitov B).

Vektor aCD19 CAR



Levkoferenza (produkt: PBMC)

Apheresis product
wash/fractionation

**Prodigy – imunomagnetna
selekcija Th in Tc (zaprt sistem)**

COBE 2991, Cell Saver 5,
LOVO, Elutra, Sepax, Prodigy

Dynabeads & MPC,
CliniMACS, Prodigy

Apheresis collection

T-cell selection

Patient

AAPCs, Dynabeads, ExpAct Treg beads,
TransAct beads, Expamer

T-cell activation

infusion

CAR-T cell manufacturing

Retroviral and lentiviral vectors
Transposon/transposase
mRNA electroporation

T-cell cryopreservation

Gene transfer

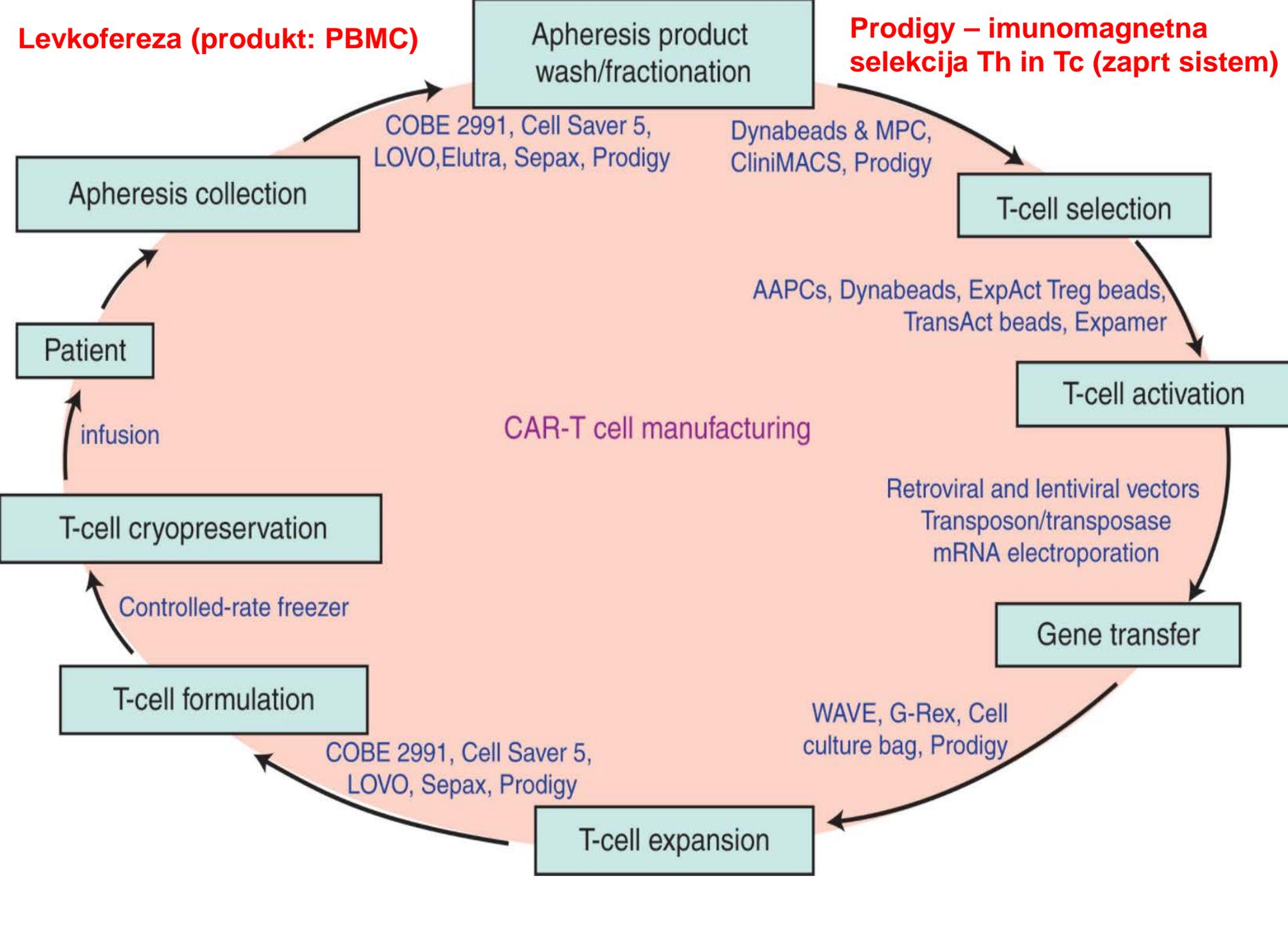
Controlled-rate freezer

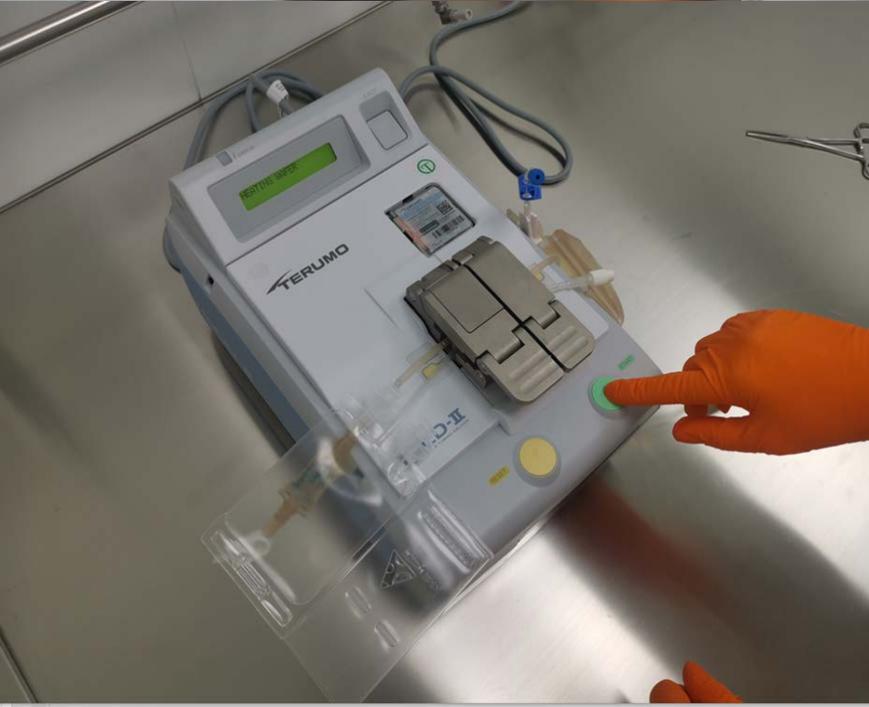
WAVE, G-Rex, Cell
culture bag, Prodigy

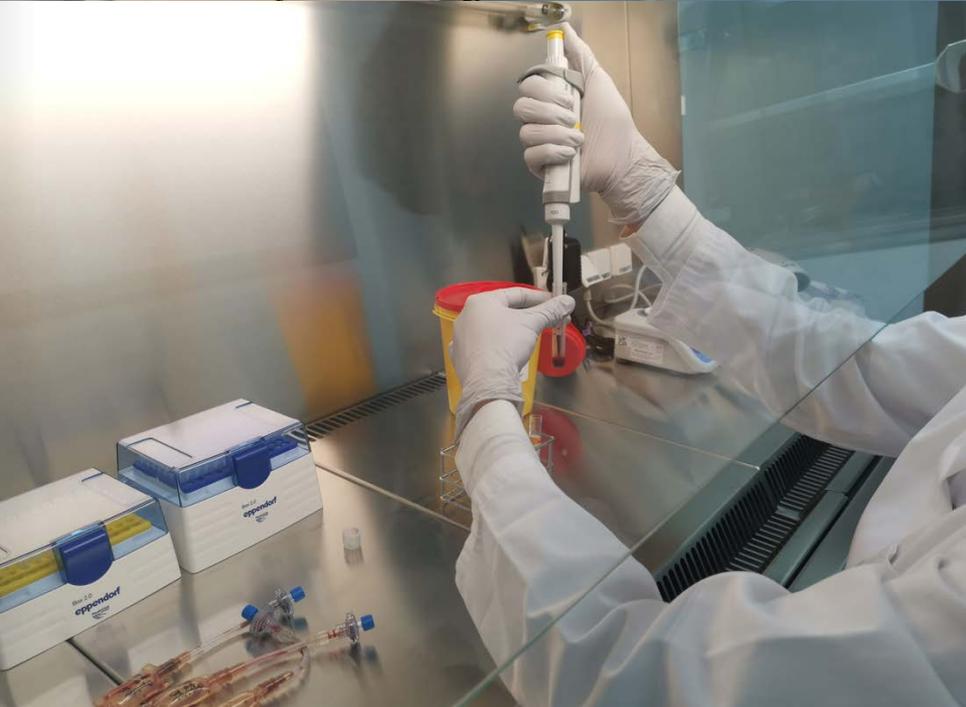
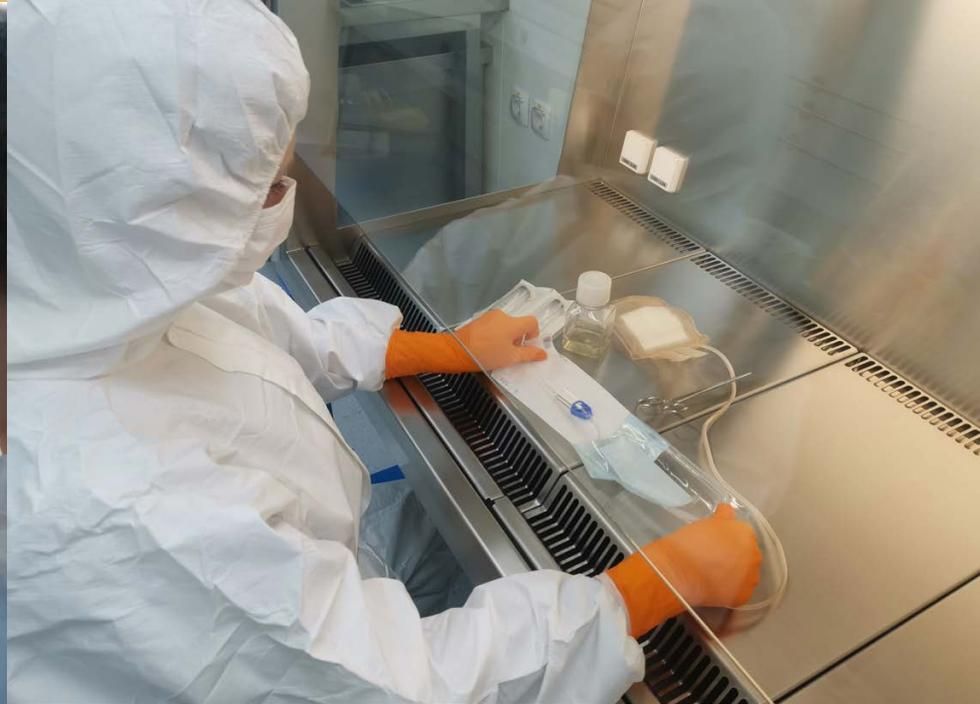
T-cell formulation

T-cell expansion

COBE 2991, Cell Saver 5,
LOVO, Sepax, Prodigy









Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus

Andreas Mackensen ^{1,2,8}, Fabian Müller^{1,2,8}, Dimitrios Mouggiakakos^{1,2,3,8}, Sebastian Böltz ^{2,4}, Artur Wilhelm ^{2,4}, Michael Aigner^{1,2}, Simon Völkl^{1,2}, David Simon ^{2,4}, Arnd Kleyer ^{2,4}, Luis Munoz^{2,4}, Sascha Kretschmann^{1,2}, Soraya Kharboutli^{1,2}, Regina Gary^{1,2}, Hannah Reimann ^{1,2}, Wolf Rösler^{1,2}, Stefan Uderhardt^{2,4}, Holger Bang⁵, Martin Herrmann ^{2,4}, Arif Bülent Ekici ⁶, Christian Buettner⁶, Katharina Maria Habenicht⁷, Thomas H. Winkler ⁷, Gerhard Krönke ^{2,4,8} and Georg Schett ^{2,4,8} 

Systemic lupus erythematosus (SLE) is a life-threatening autoimmune disease characterized by adaptive immune system activation, formation of double-stranded DNA autoantibodies and organ inflammation. Five patients with SLE (four women and one man) with a median (range) age of 22 (6) years, median (range) disease duration of 4 (8) years and active disease (median (range) SLE disease activity index Systemic Lupus Erythematosus Disease Activity Index: 16 (8)) refractory to several immunosuppressive drug treatments were enrolled in a compassionate-use chimeric antigen receptor (CAR) T cell program. Autologous T cells from patients with SLE were transduced with a lentiviral anti-CD19 CAR vector, expanded and reinfused at a dose of 1×10^6 CAR T cells per kg body weight into the patients after lymphodepletion with fludarabine and cyclophosphamide. CAR T cells expanded in vivo, led to deep depletion of B cells, improvement of clinical symptoms and normalization of laboratory parameters including seroconversion of anti-double-stranded DNA antibodies. Remission of SLE according to DORIS criteria was achieved in all five patients after 3 months and the median (range) Systemic Lupus Erythematosus Disease Activity Index score after 3 months was 0 (2). Drug-free remission was maintained during longer follow-up (median (range) of 8 (12) months after CAR T cell administration) and even after the reappearance of B cells, which was observed after a mean (\pm s.d.) of 110 ± 32 d after CAR T cell treatment. Reappearing B cells were naïve and showed non-class-switched B cell receptors. CAR T cell treatment was well tolerated with only mild cytokine-release syndrome. These data suggest that CD19 CAR T cell transfer is feasible, tolerable and highly effective in SLE.

Hvala!!



Univerza v Ljubljani



Hvala!!

Vprašanja??