TARTU ÜLIKOOL
molekulaar- ja rakubioloogia instituut

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Vähiuuringute uued suunad maailmas ja Eestis: bioloogilised ravimid.

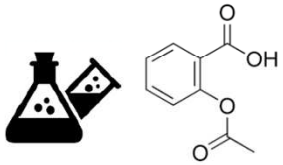
Toivo MAIMETS

Tartu Ülikooli rakubioloogia professor
Euroopa Ravimiameti Uudsete Ravimite komitee (CAT) liige

13.09.2019

BIOLOOGILISED RAVIMID

>120 years



Defineeritud, keemiliselt sünteesitud

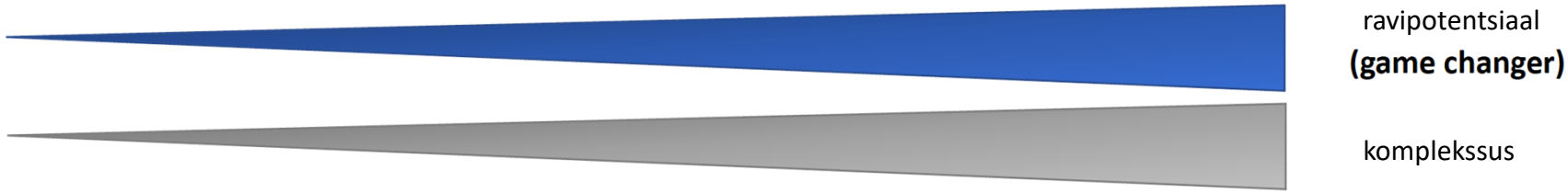
väikesed molekulid

>30 years

Valkudel põhinevad ravimid, toodetud elusrakkudes

>10 years

“elus” ravimid
(geeniteraapia, modifitseeritud geenidega rakud, somaatilised rakud, koetehnoloogiline toode)
Uudsed Ravimid (ATMP)



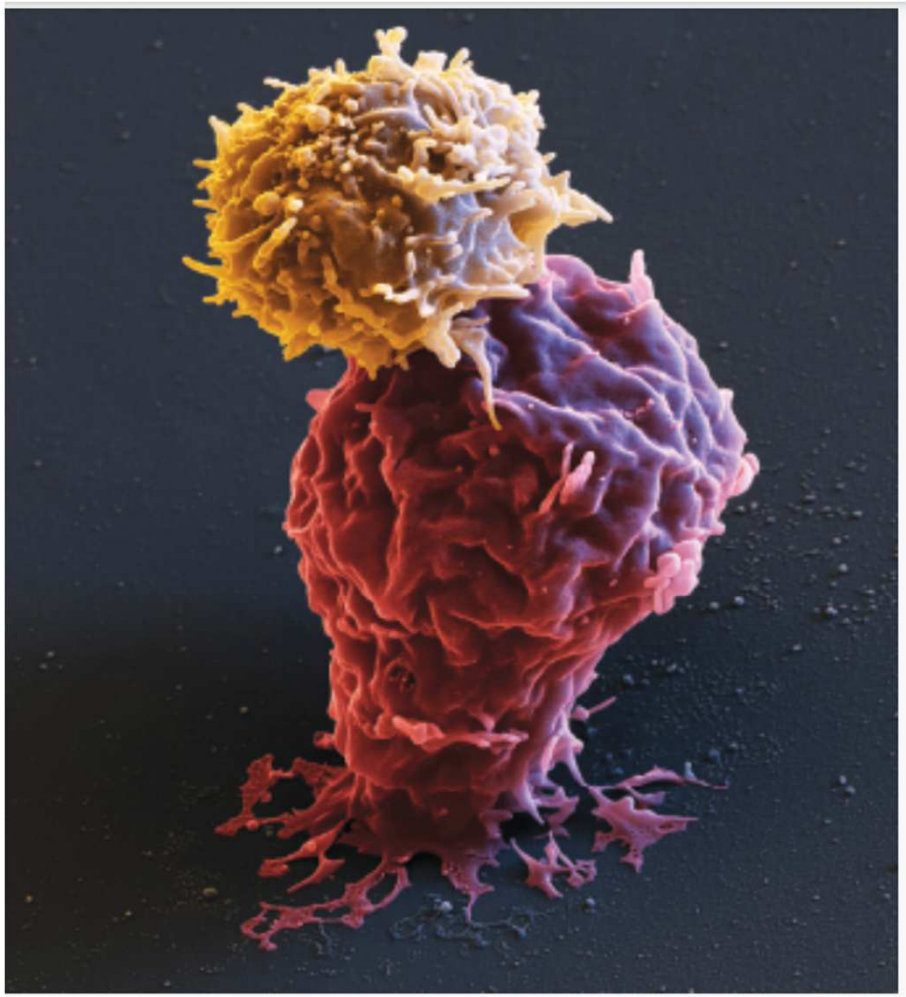
Kompleksed kroonilised haigused vajavad komplekssseid terapeutilisi lähenemisi

BIOLOOGILISED RAVIMID

1. IMMUUNVASTUSE KONTROLLPUNKTIDE INHIBIITORID

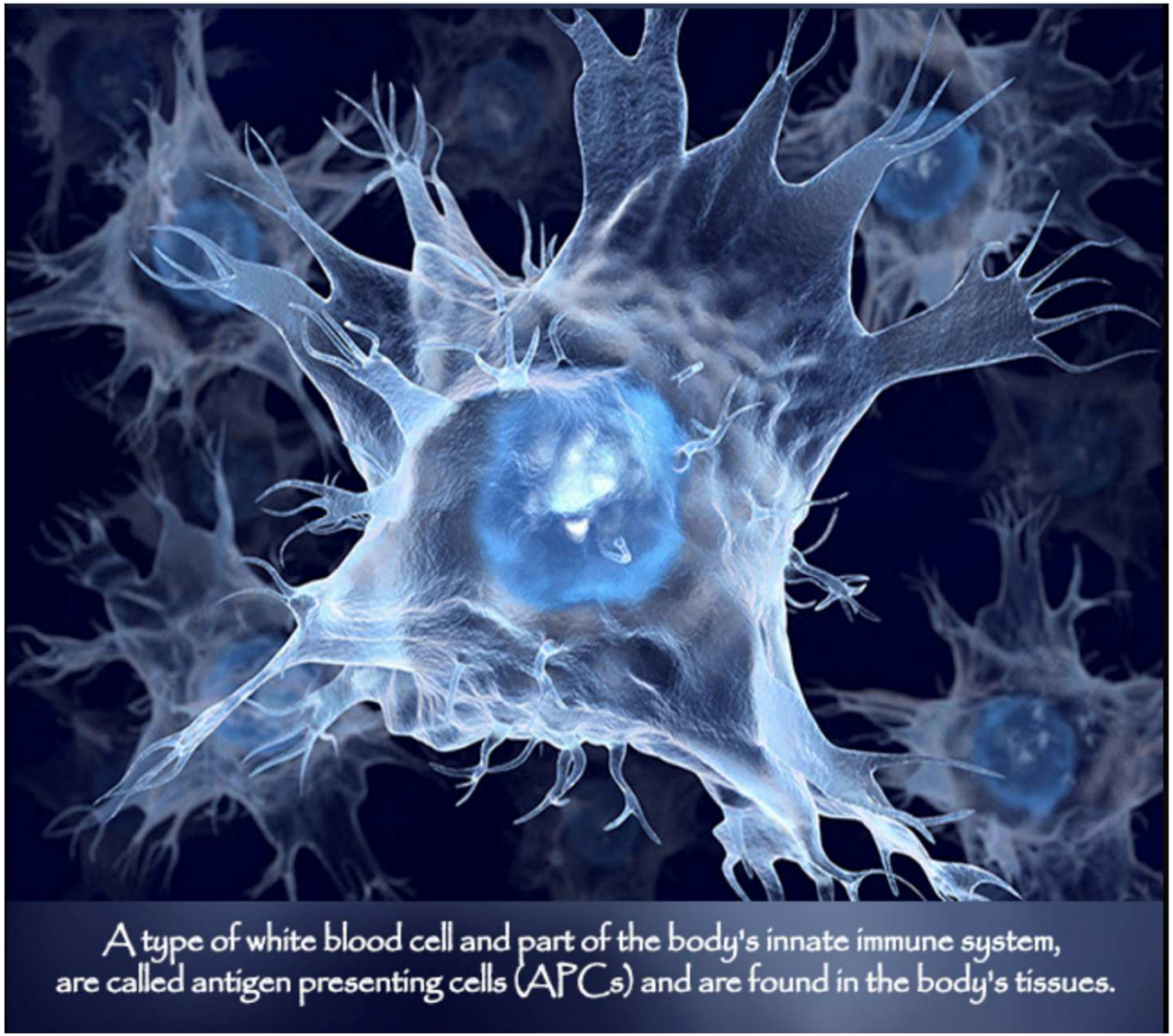
Tasuku Honjo ja James P. Allison - 2018. aasta Nobeli füsioloogia- või meditsiinipreemia “negatiivse immuunregulatsiooni inhibeerimisel põhineva uudse vähiravi meetodi eest.”

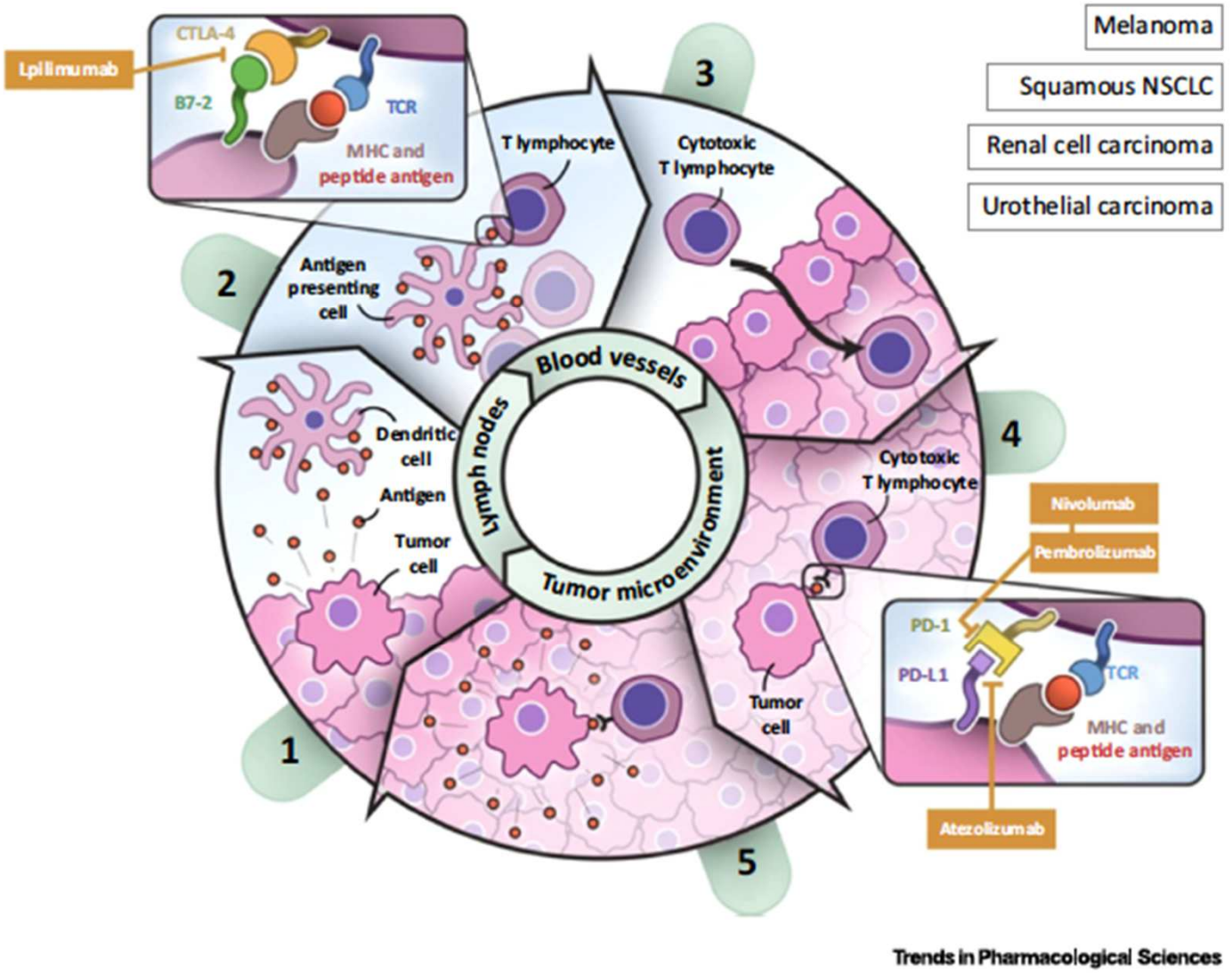
T-lümfotsüüt



This illustration shows a color-enhanced scanning electron microscope image of a CAR T lymphocyte (beige color) attacking a leukemia cell (red color). The binding of the CAR T cell to the tumor cell initiates an immune defense against the cancer cell. Credit: Eye of Science/Science Source.

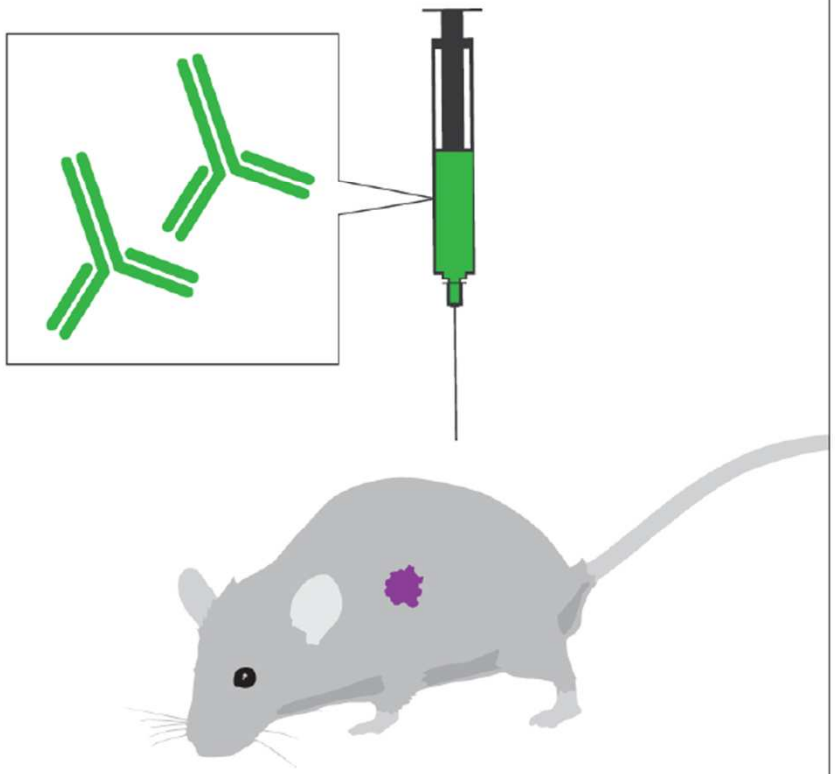
Dendriitrakk

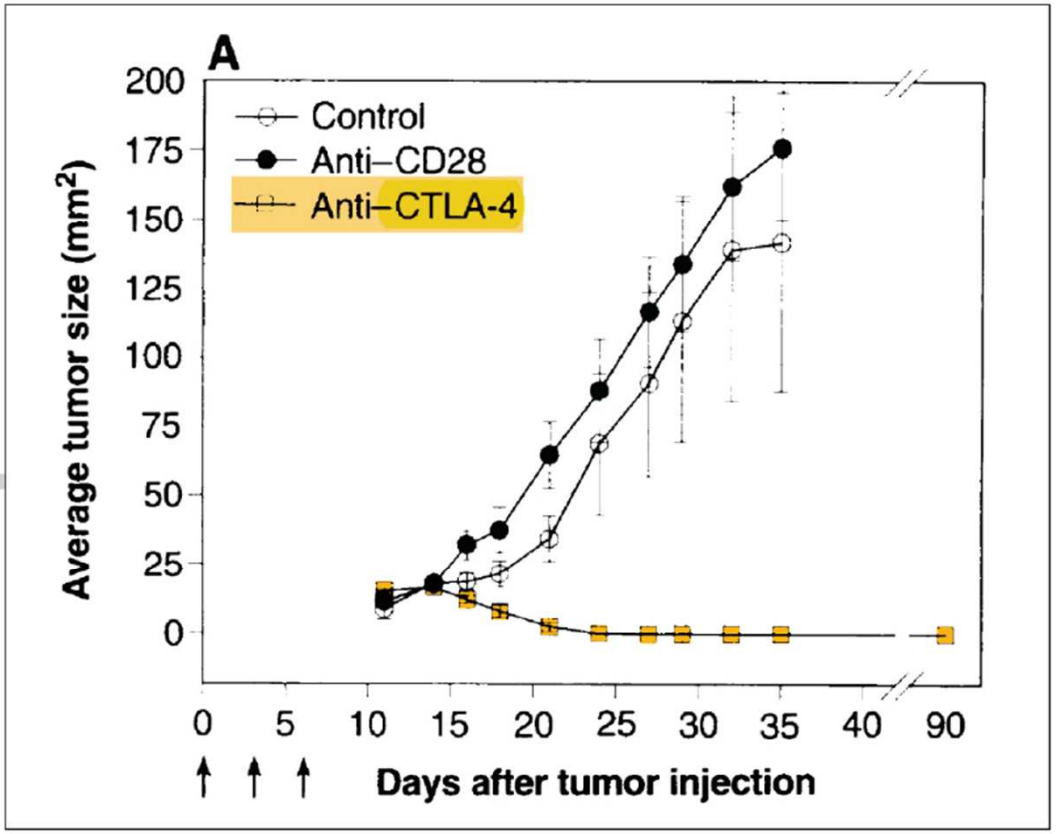




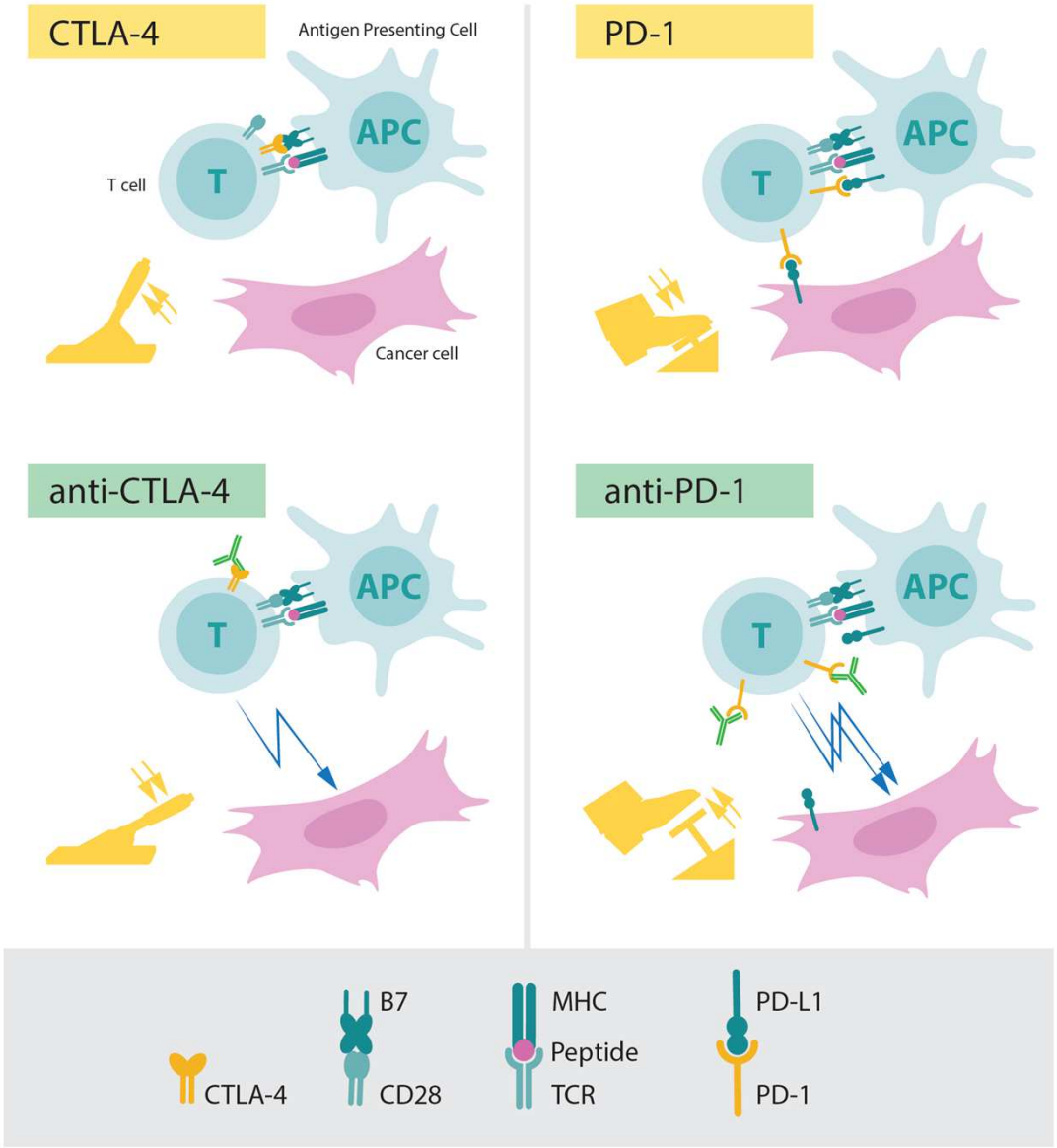
Nicholas C. Dracopoli and Mark S. Boguski (2017)

Anti-CTLA-4 against cancer



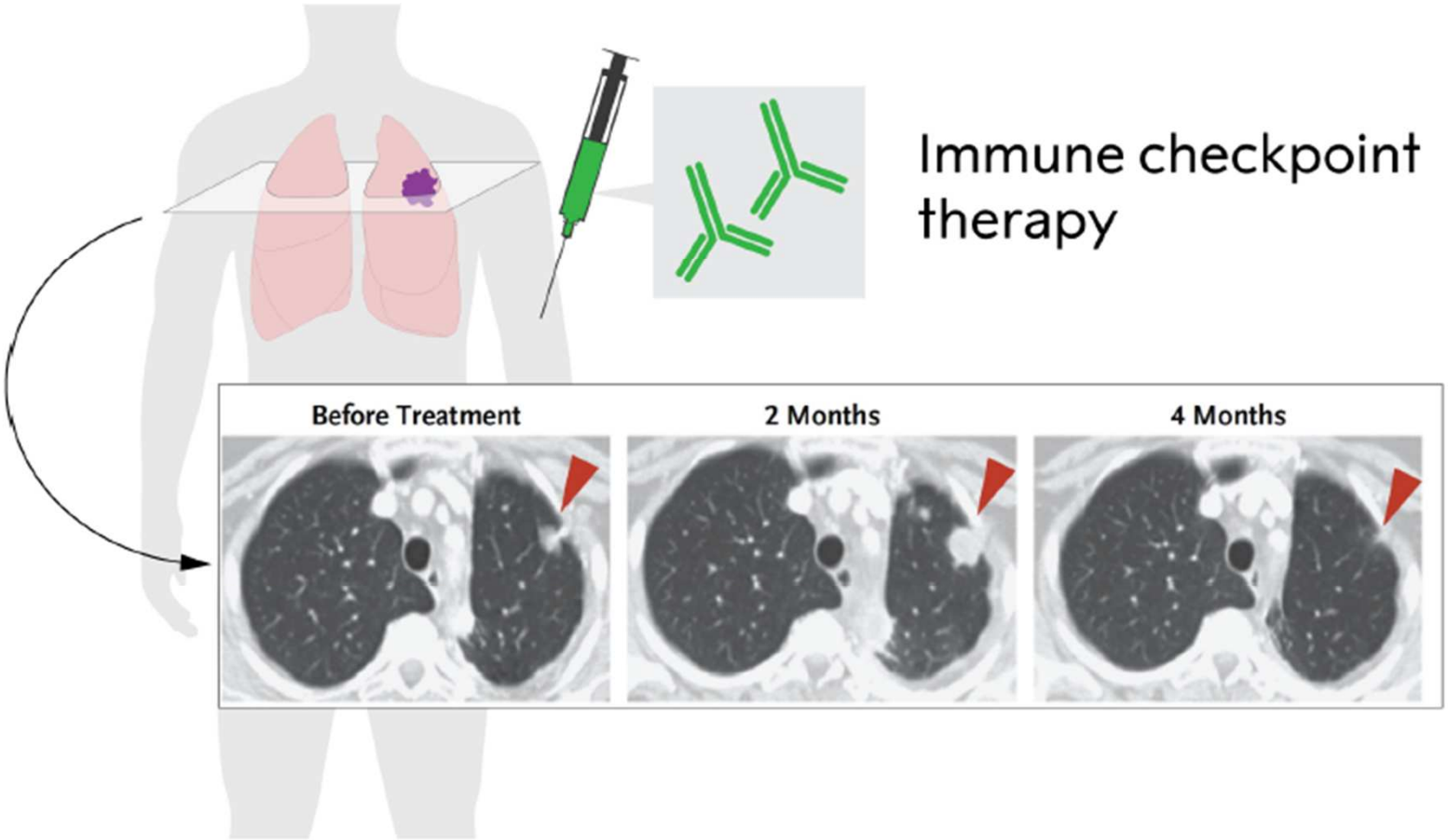


Leach et al., Science, 1996



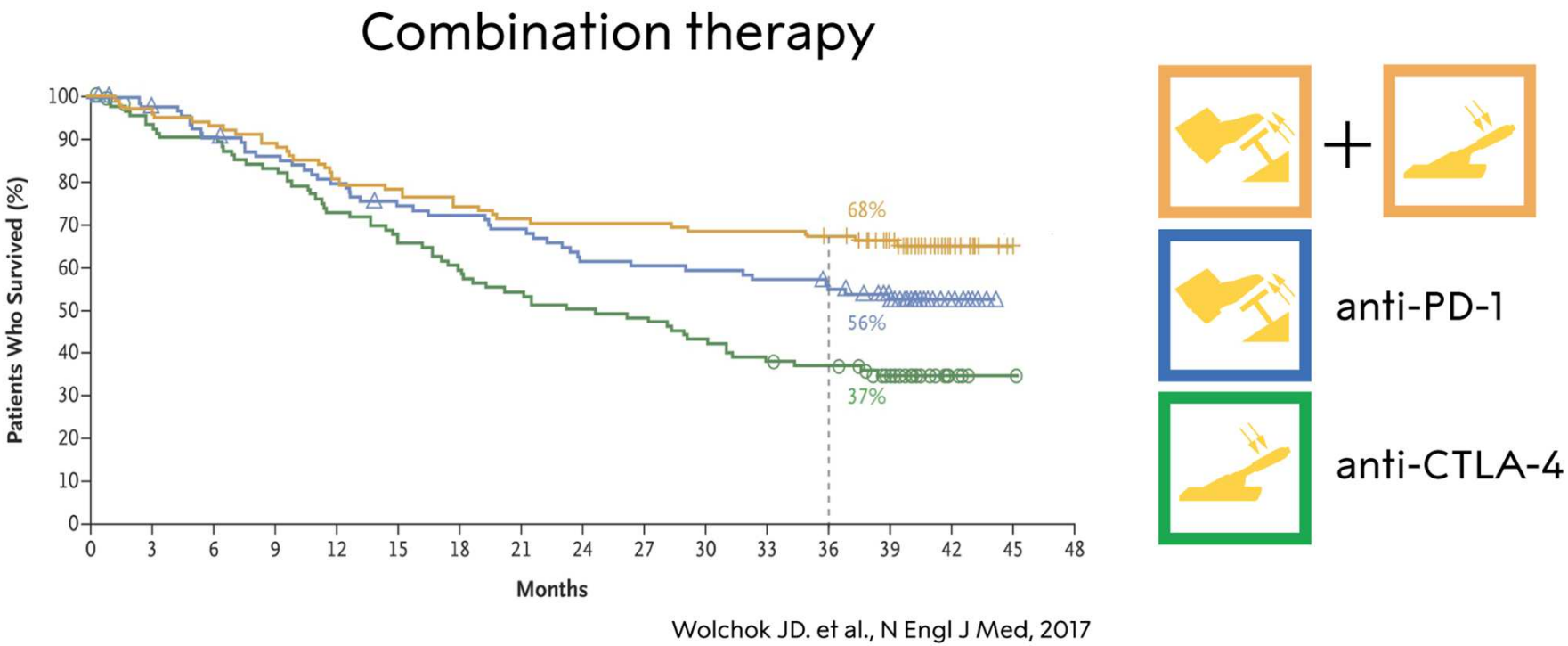
Nicholas C. Dracopoli and Mark S. Boguski (2017)

The role of CTLA-4 and PD-1 as inhibitors of activation (upper panels) and the effect of releasing the corresponding brakes using antibodies (lower panels).



Immune checkpoint therapy in non-small cell lung cancer treated with anti-PD-1 (adopted from Topalian et al., 2012). Please note the pseudoprogression at 2 months due to infiltrating immune cells and the reduced tumor size after 4 months.

Nicholas C. Dracopoli and Mark S. Boguski (2017)



The effect of anti-CTLA-4, anti-PD-1 and combination therapy in a subgroup of patients with melanoma (adopted from Wolchock et al., 2017).

Probleemid:

1. Autoimmuunsusega seonduvad kõrvalnähud (eriti anti-CTLA4 puhul).
   Raskekujuline koliit, endokriinsüsteemi häired, türeoidiit, neerupealiste düsfunktsioon

2. Ravi tulemused kõiguvad ja selle põhjus ei ole selge.
   Parimad tulemused anti-PD-1/PD-L1 raviga (50-90%) Hodgkini lümfoom, Merkeli nahakartsinoom, kasvajad DNA reparatsioonihäirete tõttu ebastabiilsete mikrosatelliitidega, desmoplastsed kasvajad UV-tingitud hulgimutatsioonidega.

   Tulemused sõltuvad kasvaja tüübist, haiguse staadiumist, geneetilisest taustast, varasematest immuunkogemustest, mutatsioonidest kasvajas, eelnevatest infektsioonidest ja vaktsineerimistest, mikrokeskkonnast ja mikrobioomist jne. jne.

3. Resistentsus inhibiitoritele, nii primaarne kui ka ravi tulemusena tekkiv sekundaarne.
   Näiteks β2-mikroglobuliini defitsiidi puhul, vigase IFN-γ retseptori raja puhul.

4. Toimemehhanismid ei ole kaugeltki selged.
   Näiteks reageerivad anti-PD-L1 ravile ka mõned patsiendid, kelle kasvajal ei ekspresseeru PD-L1

2. KIMÄÄRSET ANTIGEENI RETSEPTORIT TOOTVAD T-RAKUD

(*Chimeric Antigen Receptor T Cells*)

AREZZO KIMÄÄR

Etruskid, 399 a EMA

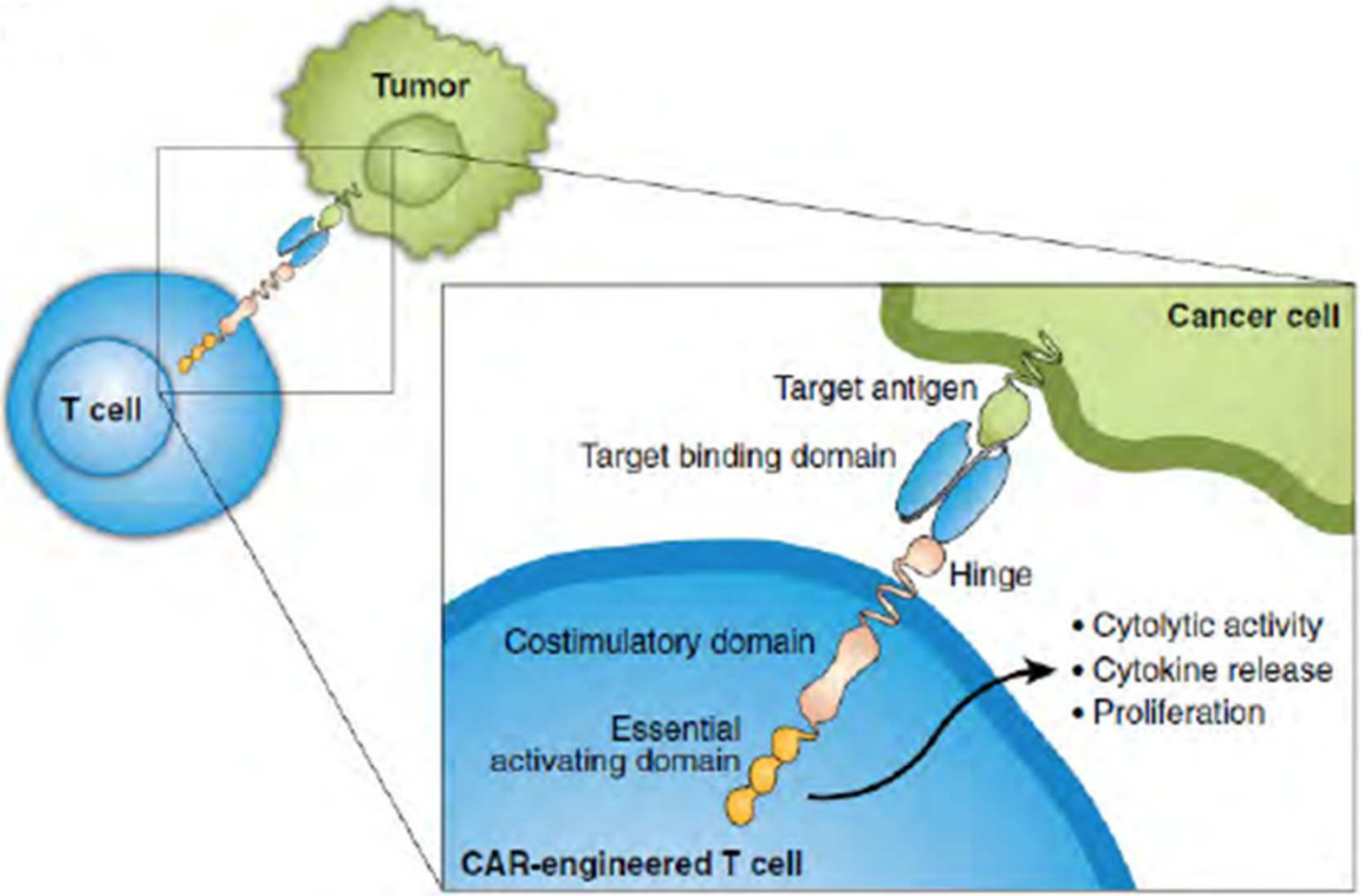
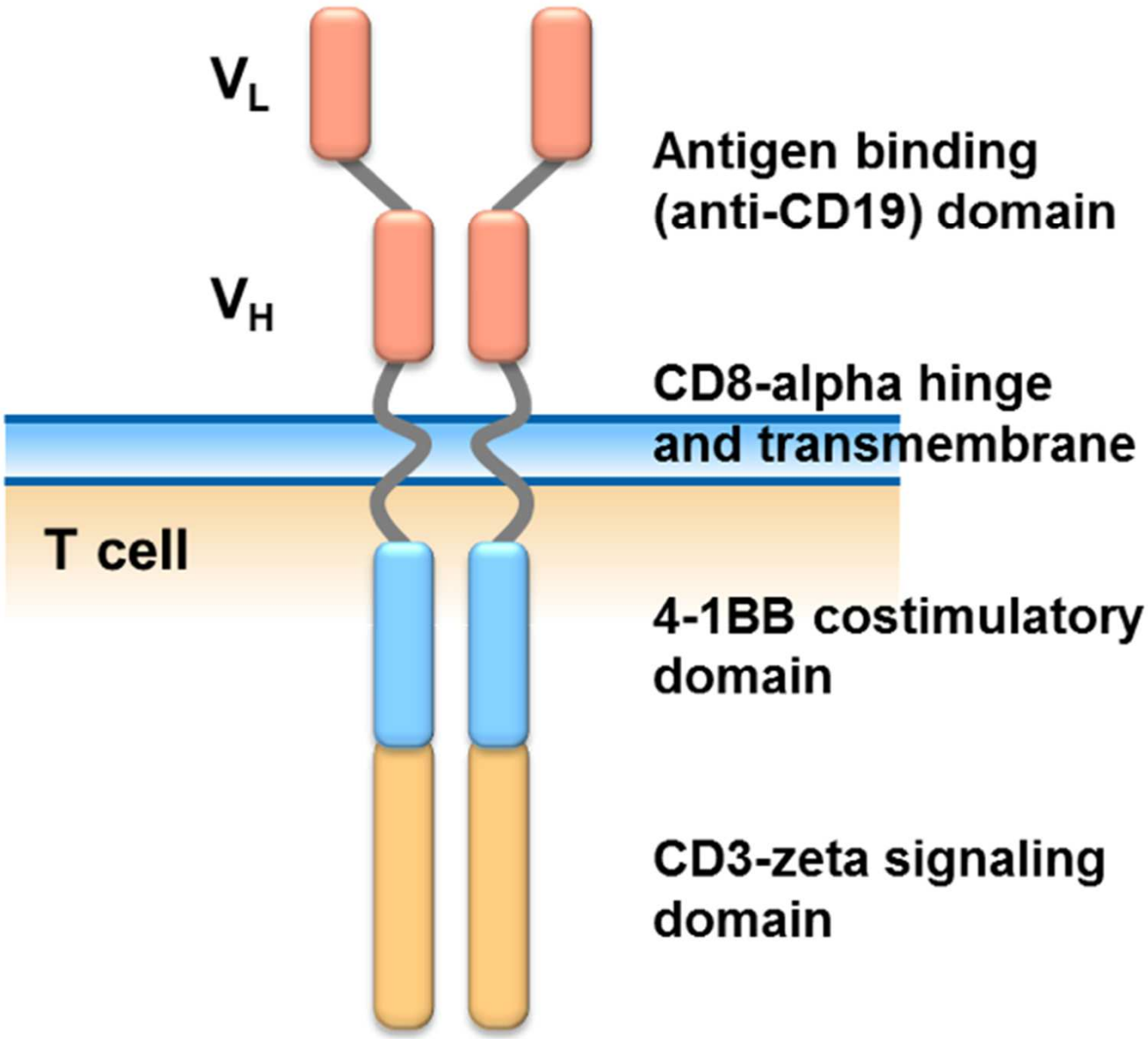
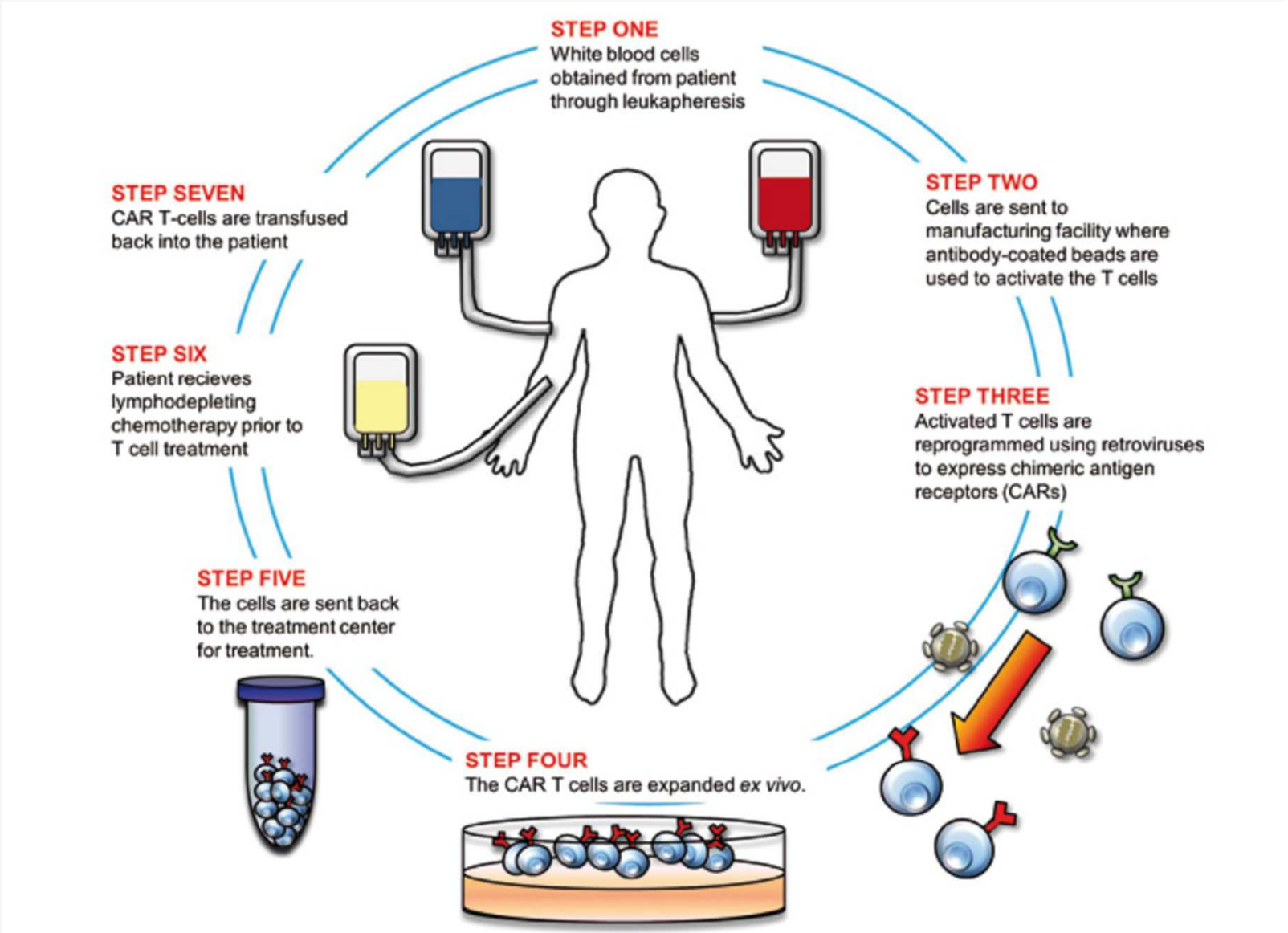


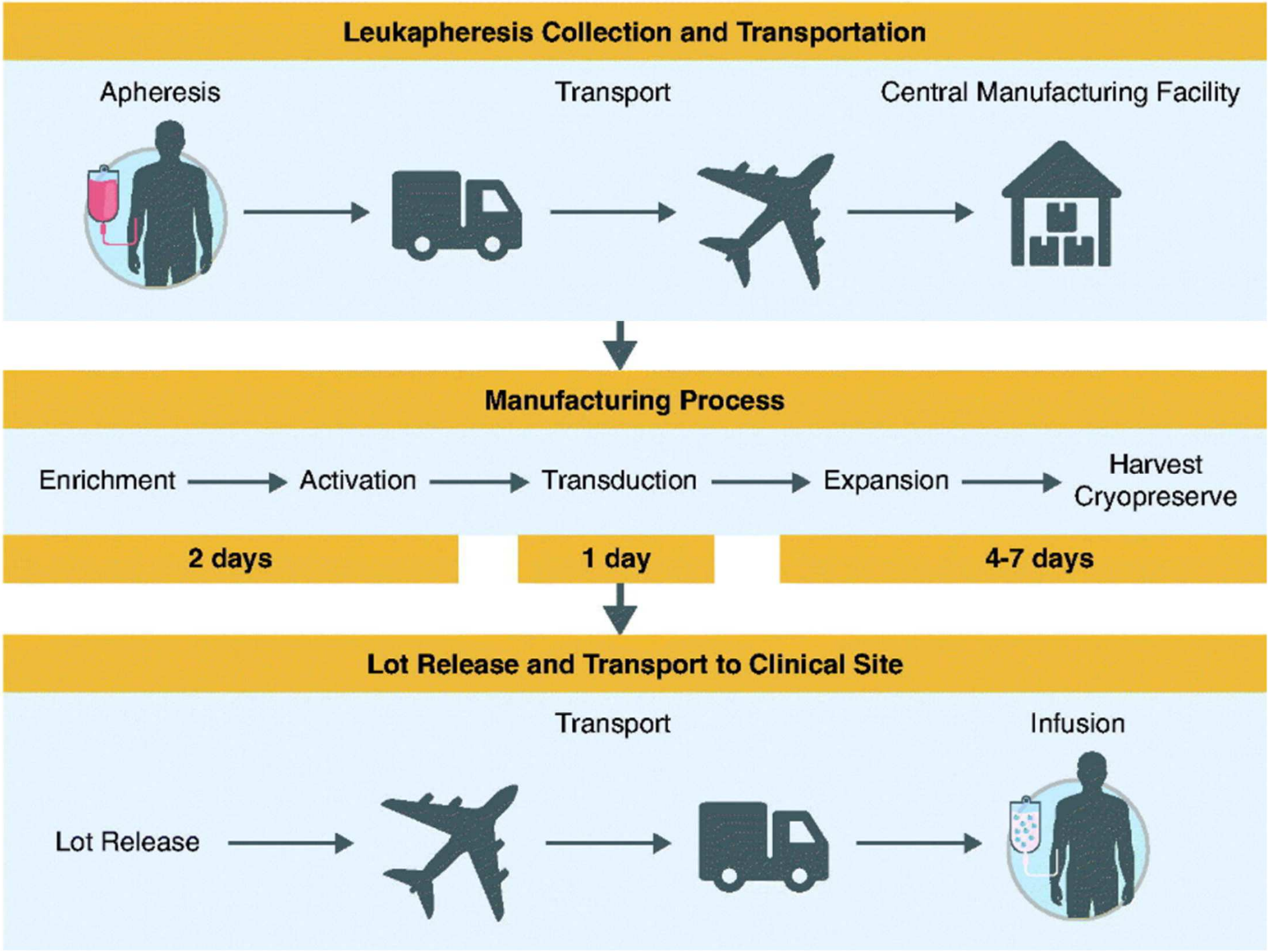
FIGURE CAR T-cell mechanism of action. CAR T-cells are derived from a patient's own T cells that have been genetically modified to express a synthetic immune receptor on their surface. This receptor couples the specificity of an antibody for a specific tumor-associated antigen with the T cell activating machinery and allows T cells to kill tumor cells in an MHC-independent fashion. Reproduced under a Creative Commons license: Roberts ZJ, et al. Axicabtagene ciloleucel, a first-in-class CAR T-cell therapy for aggressive NHL. Leuk Lymphoma 2017. doi: 10.1080/10428194.2017.1387905.

Tisagenlecleucel construct (Kymriah)





Source: Debbie King (2017). FDA Approves First CAR T-Cell Therapy – The evolution of CAR T-Cell Therapy. (CAR T Cell Therapy Workflow) Retrieved from Cell Culture Dish


Leukapheresis Collection and Transportation
Apheresis
Transport
Central Manufacturing Facility
Manufacturing Process
Enrichment
Activation
Transduction
Expansion
Harvest
Cryopreserve
2 days
1 day
4-7 days
Lot Release and Transport to Clinical Site
Transport
Infusion
Lot Release

KYMRIAH (tisagenlecleucel-T), Novartis

RAVIM: Autoloogsed T-rakud, mis on *ex vivo* transdutseeritud vektoriga, mis kodeerib CD19-spetsiifilist kimäärset antigeeni retseptorit.

KLASSIFIKATSIOON: ATMP (GTMP)

NÄIDUSTUSED: patsiendid vanuses 3 kuni 25 aastat relapseerunud või refraktoorse **B-rakulise akuutse lümfoblastse leukeemiaga** (ALL);

täiskavanud patsiendid relapseerunud või refraktoorse **difuusse suure B-raku lümfoomiga** (DLBCL), kellel ei saa rakendada autoloogset tüviraku transplantatsiooni.

Table 3 Efficacy of available treatments for paediatric and young adult r/r ALL patients compared to tisagenlecleucel

	Clofarabine mono[1]	Clofarabine+ etoposide+ cyclo[2]	Clofarabine+ etoposide+ cyclo[3]	Blinatumomab[4]	Tisagenlecleucel in Study B2202
Patients, N	61	25	17	70	75
≥3 prior regimens	62%	28%	NA	7%	60%
ORR (CR+CRi)	20%	44%	76%	39%	81.3%
Median OS	3.0 months	2.5 months	9.0 months	7.5 months	19.1 months
12 months OS	20%	30%	33%	40%	76.4%
Early mortality (within 30 days)	25%	20%	NA	7%	3%

[1] Jeha 2006, [2] Hijiya et al 2011, [3] Locatelli et al 2009, [4] von Stackelberg et al 2016
[5] Full analysis set (FAS)

ORR – overall response rate
OS – overall survival
CR – complete remission
CRi – complete remission with incomplete blood count recovery

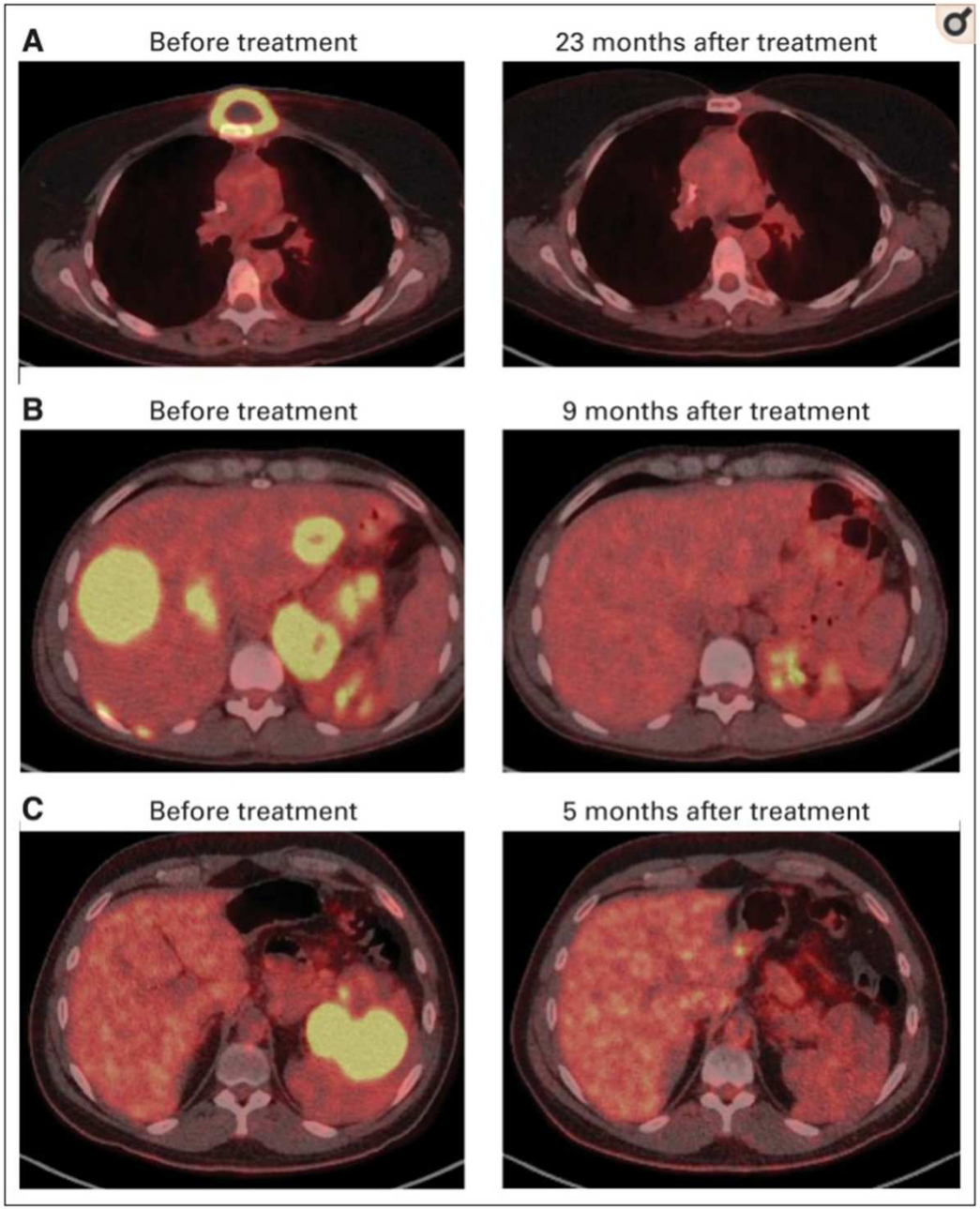


Fig 2.
Complete remissions (CRs) of chemotherapy-refractory **large-cell lymphomas** in patients receiving anti-CD19 chimeric antigen receptor T cells. (A) Positron emission tomography (PET)/computed tomography (CT) scans show CR of chemotherapy-refractory primary mediastinal B-cell lymphoma (PMBCL) in patient No. 2. (B) PET/CT scans demonstrate CR of lymphoma in patient No. 8 who had chemotherapy-refractory PMBCL with extensive liver involvement. (C) PET/CT images show CR of diffuse large B-cell lymphoma, not otherwise specified, in patient No. 14, who had extensive splenic lymphoma.

Kochenderfer et al 2015 J.Clin.Oncol. 2015 33(6):540-9.

CAR-T rakuravimi probleemid:

Safety: Cytokine release syndrome

- Symptoms and signs of CRS (≥ grade 3): Pyrexia, hypotension, hypoxia
- Onset at median day 2, resolution median day 8
- 2 deaths following CRS (anoxic brain injury, haemophagocytic lymphohistiocytosis)

	n	%
Overall (n=119)	110	92
Worst grade 1 or 2	94	79
Worst grade 3	11	9
Worst grade 4	4	3
Worst grade 5	1	1

Hind:

Reimbursement is negotiated by individual European countries, and the picture so far looks mixed.

In Germany, Novartis has set a list price of **€320,000** ($371,000), which will be subject to the usual negotiations and cost-benefit assessments with insurers.

In the UK, NICE made a speedy agreement with Novartis to green-light Kymriah at **£282,000** ($361,000), for children and adults with refractory or relapsed B-cell ALL – less than the $475,000 price listed in the US.

Novartis have said they spent more than $1 billion since 2012 on bringing Kymriah to market.

https://cancerworld.net/cutting-edge/the-car-t-cell-revolution-what-does-it-offer-and-can-we-afford-it/

RAKUBIOLOOGIA ÕPPETOOL, TARTU ÜLIKOOL

UUED MÄRKLAUAD VÄHIRAKKUDEL, MILLELE SIHTIDA CAR-T RAVIMEID

Mutated neo-antigens in hepatocellular carcinoma (HEPAMUT)

ERA-NET: Aligning national/regional translational cancer research programmes and activities
TRANSCAN-2

The Project

Background and rationale: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide. Identification and immunological validation of specific mutated neo-antigens may help at improving therapeutic outcome in HCC patients.

Hypothesis: The hypothesis is that identification and immunological validation of HCC-specific mutated neo-antigens will be critical for developing immunotherapy strategies for better clinical outcome in HCC patients.

Aims: The primary aim is the identification and immunological validation of mutated neo-antigens specific to HCC. Specific aims will be: 1) evaluate the mutational rate in HCC and predict the presentation of neo-epitopes by HLA-A2*01 allele; 2) assess the frequency of specific T cells to such mutant epitopes in HCC patients, before and after treatment with checkpoint inhibitors (CI); 3) validate the immunogenicity of neo-epitopes in an HLA-transgenic mice and their therapeutic effect in a PDX animal model; 4) identify mutated full-length proteins on the surface of HCC cancer cells; 5) develop MAbs to such mutated proteins and validate their specificity in a PDX animal model.

Potential impact: HCC specific mutated neo-antigens will provide a source of immunogens for immunotherapies to be used alone or in combination with HCC specific shared wild-type antigens identified within the ongoing FP7-funded HEPAVAC project (Coordinator L. Buonaguro).

HEPAMUT CONSORTIUM:

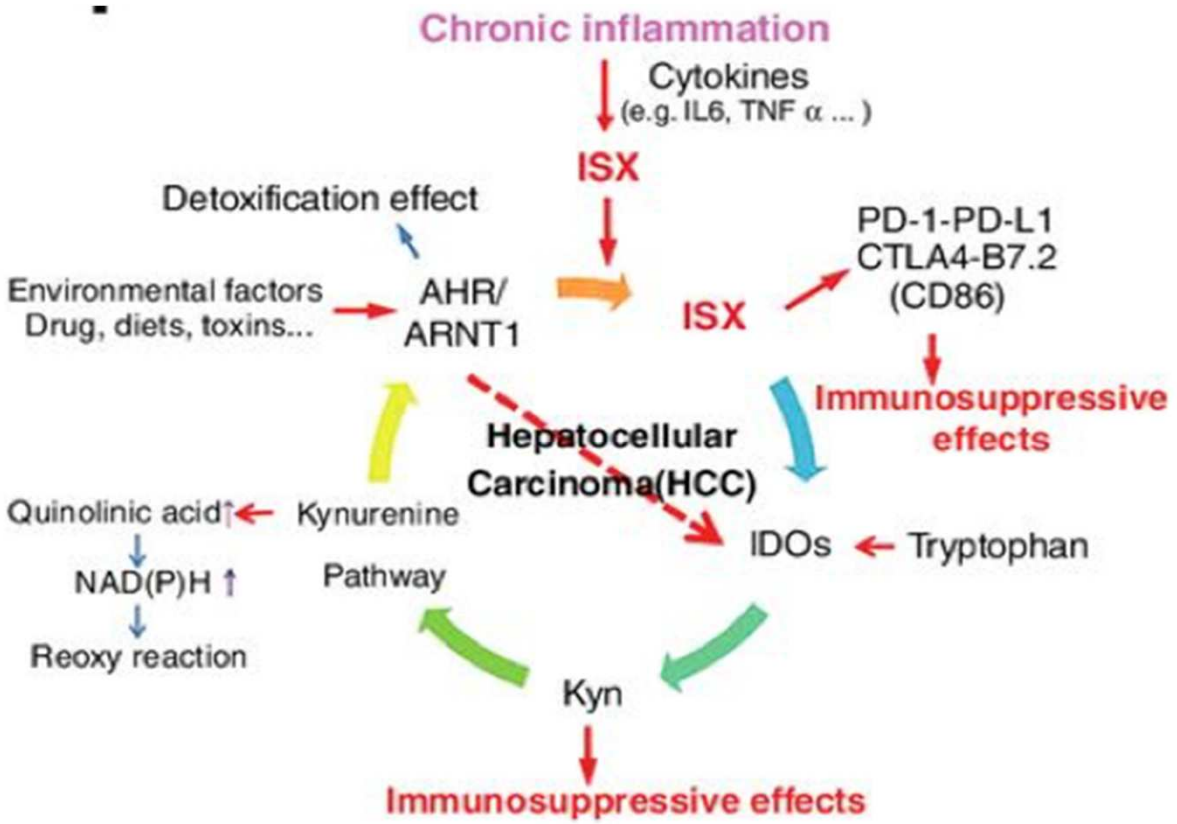
1. National Cancer Institute "PASCALE”, IT, Prof. Luigi Buonaguro
2. INSERM, FR, Prof. Danila Valmori
3. Universidad de Navarra, ES, Prof. Bruno Sangro
4. University Hospitals Leuven - KU Leuven, BE, Prof. Frederic Amant
5. San Raffaele Hospital, IT, Prof. Lucia Lopalco
6. University of Tartu, EE, Prof. Toivo Maimets
7. Riga Stradins University, LV, Prof. Edvins Miklasevics

- In the analysis with all samples but ESTP1, a total of 2,116 genes resulted to be differentially expressed in the 5 tumor samples respect to their controls.

EnsemblGeneID	OfficialGeneSymbol	GeneName	MeanExpression	log2FoldChange	P-value	Adjusted p-value
ENSG00000140835	CHST4	carbohydrate sulfotransferase 4(CHST4)	589,8767888	-6,08711757	1,54342E-41	3,63536E-37
ENSG00000182566	CLEC4G	C-type lectin domain family 4 member G(CLEC4G)	2578,689582	-7,064209238	2,96564E-40	3,49264E-36
ENSG00000164619	BMPER	BMP binding endothelial regulator(BMPER)	526,3742823	-5,973920822	2,78115E-39	2,18358E-35
ENSG00000019169	MARCO	macrophage receptor with collagenous structure(MAR	9371,113789	-6,462820256	5,20924E-38	3,06746E-34
ENSG00000145708	CRHBP	corticotropin releasing hormone binding protein(CRHE	6106,242926	-6,720828207	9,00235E-36	4,24082E-32
ENSG00000091622	PITPNM3	PITPNM family member 3(PITPNM3)	429,1724971	-4,056899048	1,66196E-34	6,5243E-31
ENSG00000160339	FCN2	ficolin 2(FCN2)	3834,032702	-7,972870929	5,99557E-34	2,01742E-30
ENSG00000138315	OIT3	oncoprotein induced transcript 3(OIT3)	4577,077258	-6,780906304	1,59305E-31	4,69033E-28
ENSG00000147003	TMEM27	transmembrane protein 27(TMEM27)	884,9113307	-5,380506613	1,57871E-29	4,13166E-26
ENSG00000160323	ADAMTS13	ADAM metallopeptidase with thrombospondin type 1	2982,739933	-3,47778241	3,86402E-27	9,1013E-24
ENSG00000126759	CFP	complement factor properdin(CFP)	1871,500927	-3,70969596	1,17552E-26	2,51711E-23
ENSG00000136011	STAB2	stabilin 2(STAB2)	2763,101227	-6,562571759	8,7225E-26	1,57929E-22
ENSG00000114812	VIPR1	vasoactive intestinal peptide receptor 1(VIPR1)	1247,38118	-4,860207295	8,92261E-26	1,57929E-22
ENSG00000165682	CLEC1B	C-type lectin domain family 1 member B(CLEC1B)	928,1019658	-6,805495168	9,38698E-26	1,57929E-22
ENSG00000125780	TGM3	transglutaminase 3(TGM3)	1224,132555	5,478865722	1,93368E-25	3,03639E-22
ENSG00000133477	FAM83F	family with sequence similarity 83 member F(FAM83F)	738,424675	-7,795625413	5,01436E-25	7,38177E-22
ENSG00000123454	DBH	dopamine beta-hydroxylase(DBH)	1229,843725	-7,214927215	1,03877E-24	1,43924E-21
ENSG00000263761	GDF2	growth differentiation factor 2(GDF2)	1328,800549	-8,310335786	1,11312E-24	1,45658E-21
ENSG00000145287	PLAC8	placenta specific 8(PLAC8)	461,7512344	-3,373458885	3,82895E-24	4,74669E-21
ENSG00000150893	FREM2	FRAS1 related extracellular matrix protein 2(FREM2)	1063,672712	-7,87980112	5,12063E-24	6,03056E-21
ENSG00000104938	CLEC4M	C-type lectin domain family 4 member M(CLEC4M)	1417,654809	-8,309375368	6,54024E-24	7,33565E-21
ENSG00000087237	CETP	cholesteryl ester transfer protein(CETP)	1633,87582	-4,068749119	1,01341E-22	1,08499E-19
ENSG00000001626	CFTR	cystic fibrosis transmembrane conductance regulator(	670,2075234	-6,383483685	1,06018E-22	1,08571E-19
ENSG00000157551	KCNJ15	potassium voltage-gated channel subfamily J member	141,4516447	-3,83780091	1,20261E-21	1,18026E-18
ENSG00000120057	SFRP5	secreted frizzled related protein 5(SFRP5)	1081,214743	-7,108648315	1,7815E-21	1,67846E-18

RAKUBIOLOOGIA ÕPPETOOL, TARTU ÜLIKOOL

PD1-PDL1 INHIBIITORITE RAVI EFEKTIIVSUSE PARANDAMINE



A schematic diagram illustrating a possible self-perpetuating loop in hepatocellular carcinoma. The secretion of proinflammatory cytokines by hepatic tumour cells activates ISX expression and tryptophan metabolism. This promotes inflammation-mediated cell proliferation and malignancy. Overexpressed KYN interacts with AHR receptor and tumorigenesis both in vitro and in vivo. KYN-AHR, CD86-CTAL-4 and PD-1-PD-L1 axis overexpression promotes tumour immunosuppression and enhances tumour growth. AHR expression aggravates the effect of ISX-KYN loop by promoting IDO1 expression (from Wang et al 2017, Cancer Res 2017;77:4065-4077).

Modulation of AHR signalling to boost the activity of PD-1/PD-L1 inhibitors for the treatment of liver tumours.

Background:

Recently, targeting immune checkpoint proteins (PD-1/PD-L1) has shown promise in treatment of several types of cancers, however response rates vary considerably. Accumulating data indicate that aryl hydrocarbon receptor (AHR) is important in liver cancer initiation and progression. Moreover, AHR modulates the interplay between cancer and immune cells and regulates immune cell function including the expression of PD-1/PD-L1.

Hypothesis: modulation of AHR signalling alone or in combination with PD-1/PD-L1 inhibition boosts the anti-tumour effects of adaptive immunity.