SMART DIASPORA 2023

Personalized vaccines in immuno-oncology

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1. CAR-T/NK cells immunotherapies

Personalized Approaches in Immuno-oncology

CAR-T Cells Revolution

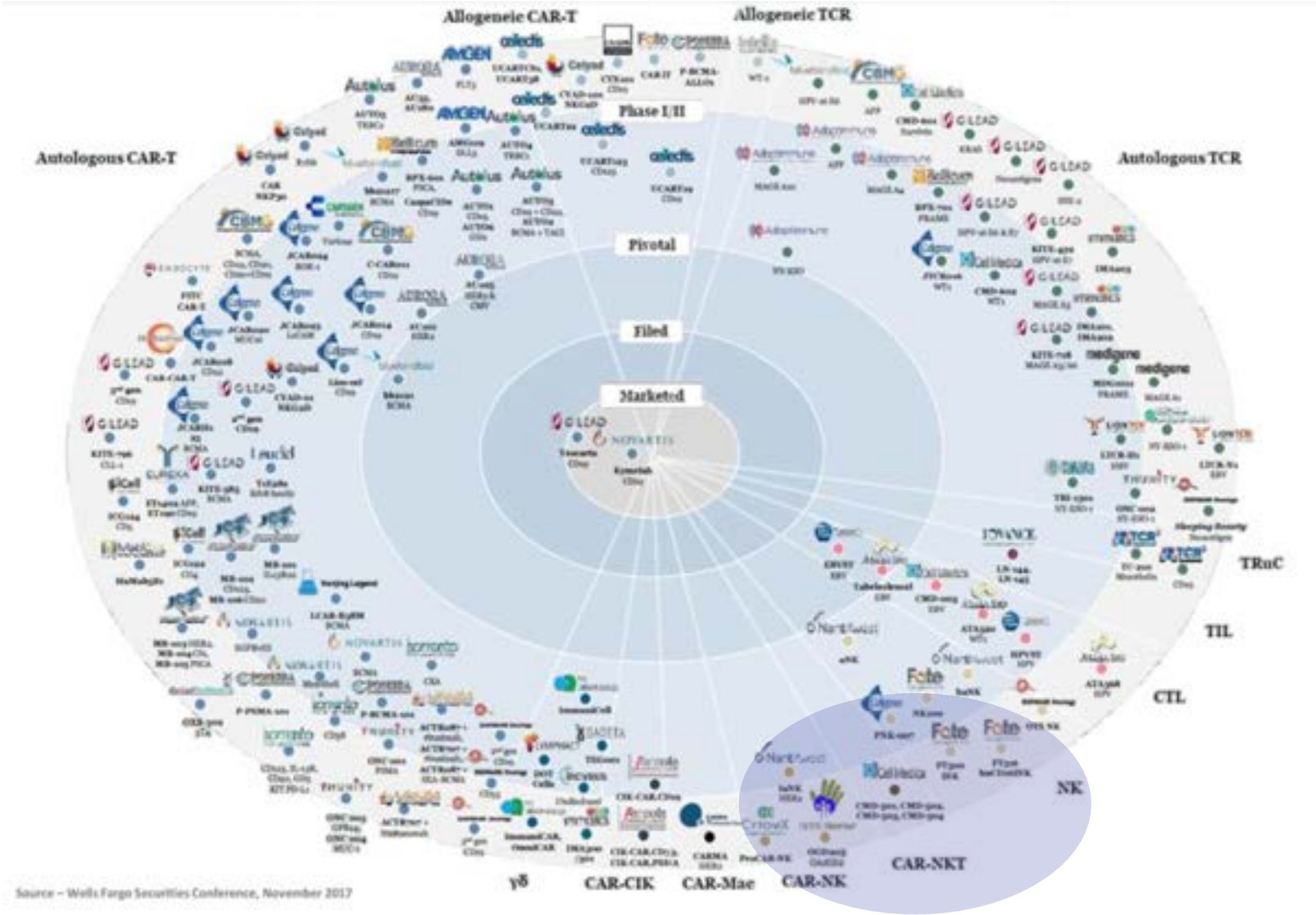
30 August 2017 - FDA approves Novartis' Kymriah as first CAR-T cancer immunotherapy for refractory B cell ALL

18 October 2017 - Kite's Yescarta is approved for the treatment of adult patients with relapsed or refractory large B cell lymphoma

"This remarkable approval is the beginning of what we see as a chance to transform the way in which we treat cancer."

Helen H. Heslop, MD, President of the American Society of Gene & Cell Therapy

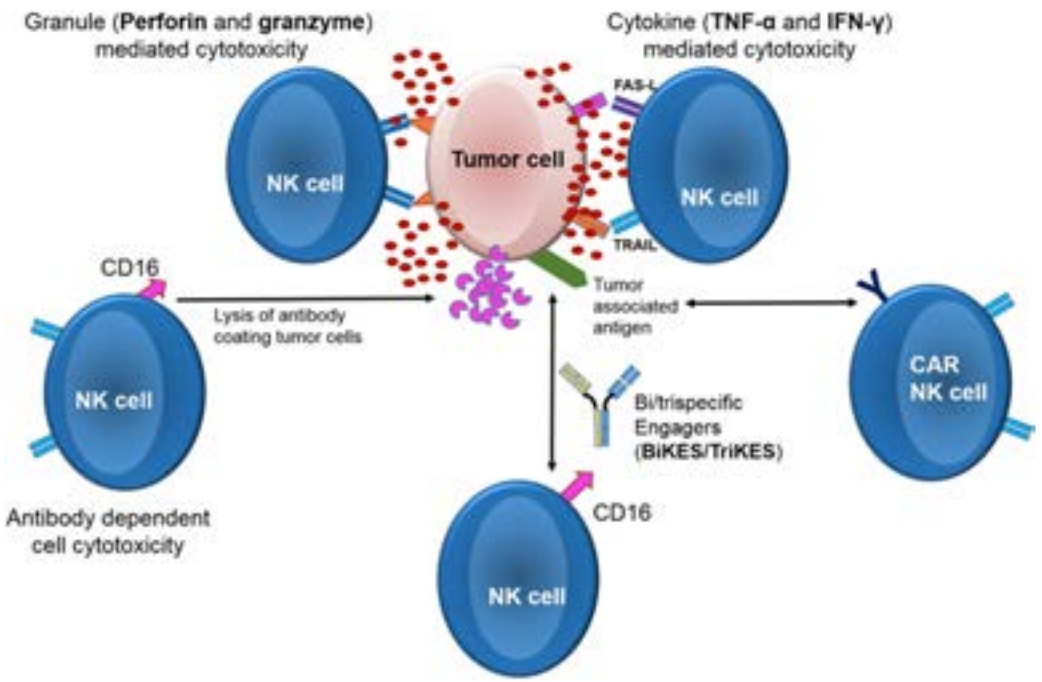
Global Landscape of Adoptive Cell Therapies



A Better Platform for CAR-based Therapies?

Advantages of equipping a mixed population of NK-polarized activated peripheral blood derived killer cells with a chimeric antigen receptor directed against a tumor antigen:

- a broad non-MHC restricted recognition of diverse tumor targets through either the CAR, NK-cell activating receptors or endogenous TCR receptors of NKT cells triggered by glycolipids presented via CD1d
- a combination of NK, NKT and CTLs can target both MHC class I expressing cancer cells as well as those that have downregulated their MHC expression
- potent cytolytic activity sustained by multiple modes of killing
- even low numbers of NKT cells administered with T cells have a significant role in reducing GVHD, thus supporting allogeneic immunotherapy
- less manipulation and better *ex vivo* expansion



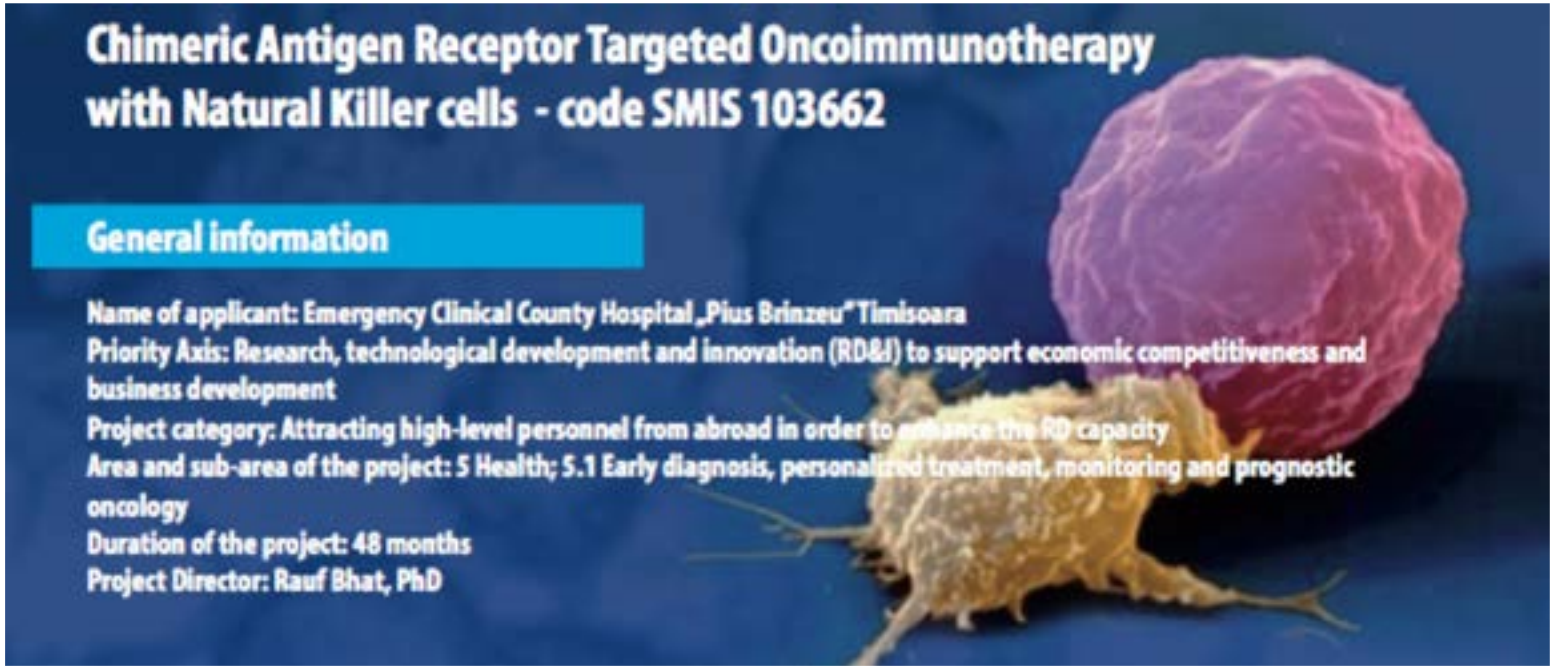
CAR-NK Clinical Trials

Row	Clinical trial identifier	CAR target	Disease	Status	Phase	NK cell source	Study location
1	NCT03692767	CD22	Refractory B-cell lymphoma	N	I	U	Allife Medical Science and Technology Co., Ltd., Beijing, China
2	NCT03690310	CD19	Refractory B-cell lymphoma	N	I	U	Allife Medical Science and Technology Co., Ltd., Beijing, China
3	NCT03692663	PSMA	Prostate cancer	N	I	U	Allife Medical Science and Technology Co., Ltd., Beijing, China
4	NCT03692637	Mesothelin	Epithelial ovarian cancer	N	I	U	Allife Medical Science and Technology Co., Ltd., Beijing, China
5	NCT03415100	NKG2D ligands	Solid tumours	R	I	Auto/allo PBMCs	The Third Affiliated Hospital of Guangzhou Medical University, China
6	NCT02944162	CD33	Leukemias	U	I/II	NK92	PersonGen Bio Therapeutics Co., China
7	NCT02892695	CD19	Leukemia/lymphoma	R	I/II	NK92	PersonGen Bio Therapeutics Co., China
8	NCT03579929	CD19	Leukemia/lymphoma	N	I/II	CB	M.D. Anderson Cancer Center, USA
9	NCT03056339	CD19	Leukemia/lymphoma	R	I/II	CB	M.D. Anderson Cancer Center, USA
10	NCT03383978	5.28	Glioblastoma	R	II	NK92	Johann W. Goethe University, Germany
11	NCT02742727	CD7	Leukemia/lymphoma	R	I/II	NK92	PersonGen Bio Therapeutics Co., China
12	NCT02839954	MUC1	Solid tumours	R	I/II	U	PersonGen Bio Therapeutics Co., China
13	NCT03049449	CD30	Lymphomas	R	I	U	National Institutes of Health Clinical Center, USA
14	NCT02274584	CD30	Lymphomas	U	I/II	U	University of Florida, US & Peking University Cancer Hospital, China

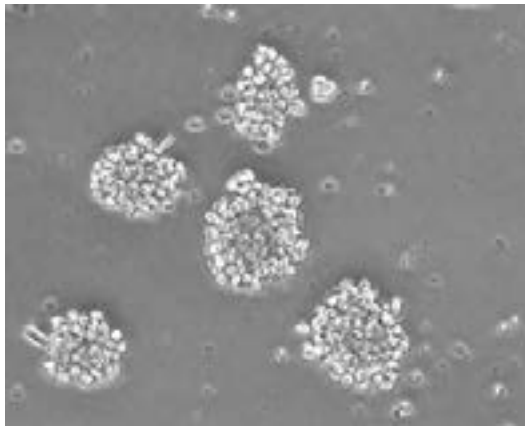
CAR, chimeric antigen receptor; NK, natural killer; N, not yet recruiting; R, recruiting, U, unknown.

CAR-NK Objective

- To develop an experimental prototype of novel CARs (chimeric antigen receptors), specifically suited for NK (natural killer) cell-based therapies in cancer

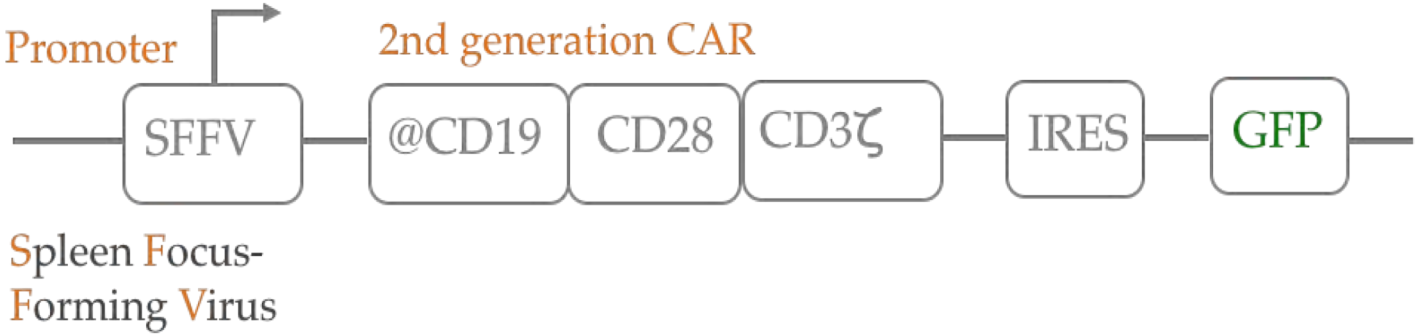


Generation of anti-CD19 CAR-NK92



NK-92 cells cultured in XVIVO10 medium + 5%HuPlasma + 500U/mL IL-2 were transduced with LentiONE vectors (GEGTech), at MOI=20-50, in the presence of BX795 and polybrene.

- vector from Wels group at Georg Speyer Haus, Frankfurt; lentivirus produced at OncoGen

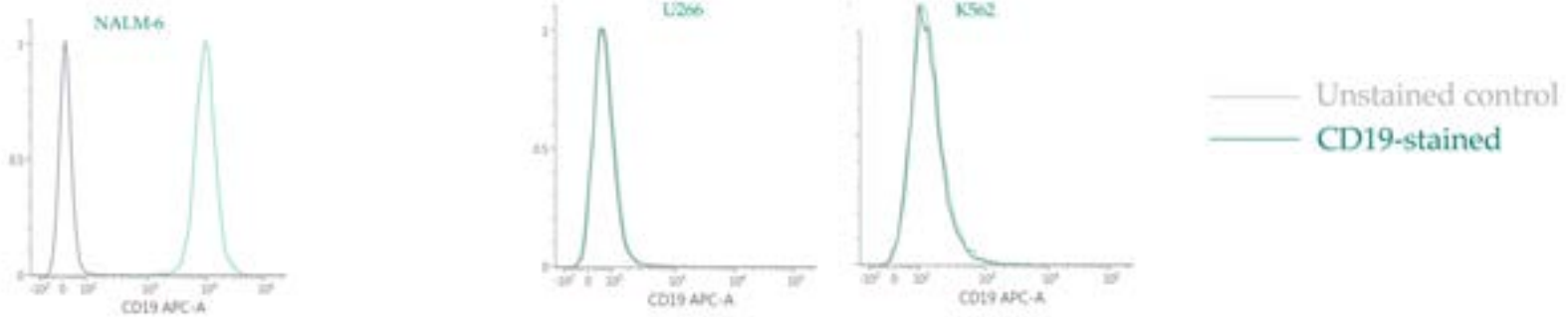


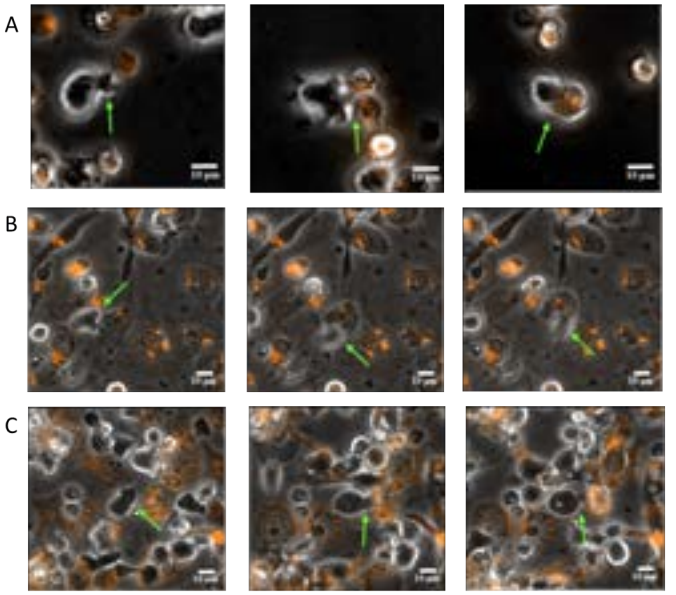
- 2nd generation LV expression plasmid, with 4th generation Lenti-X packaging system (Clontech)

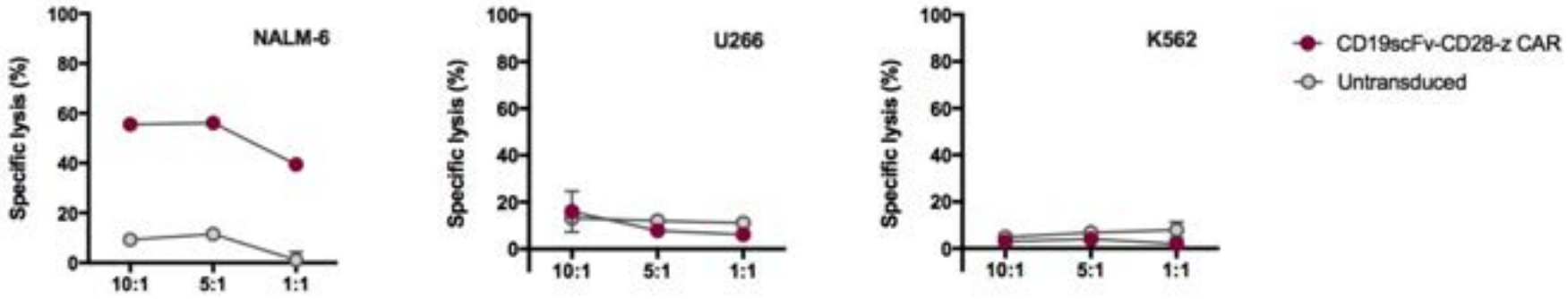
CAR-NK92 Cells Kill Target Cells Specifically

CD19+ target cells

CD19- target cells

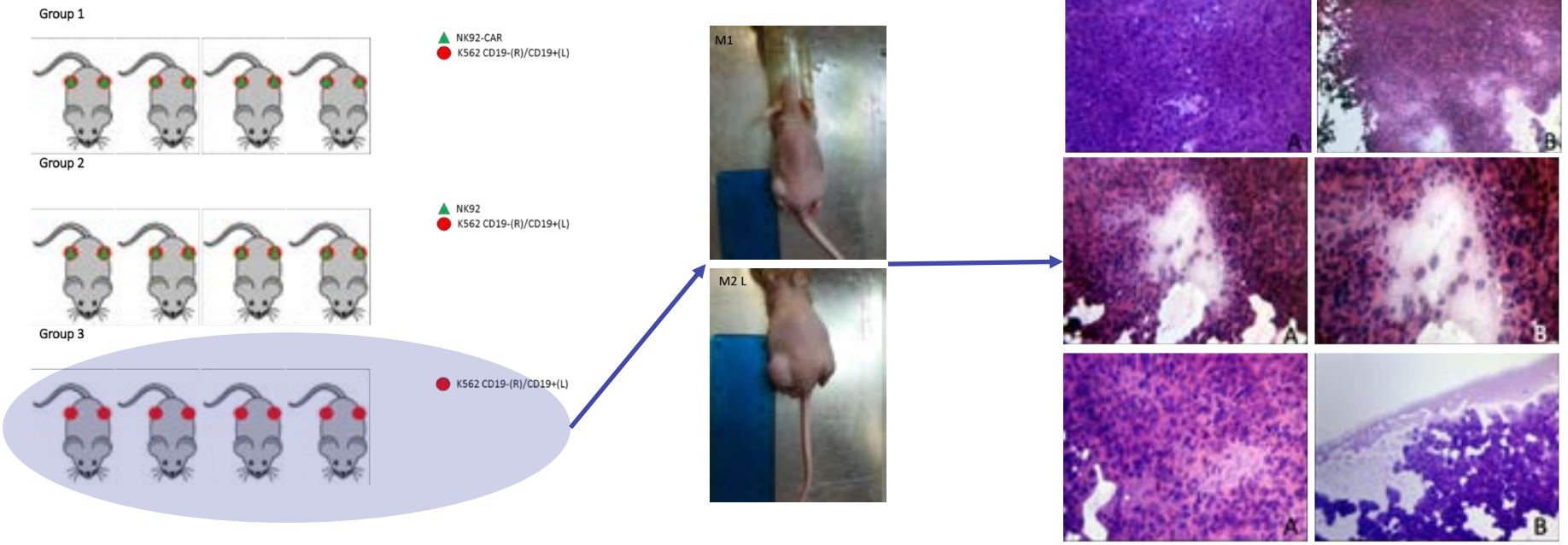






Animal Model

- Effector cells: NK92-CAR (anti-CD19)and NK92 cells
- Target cells: K562 CD19- and K562 CD19+ cells

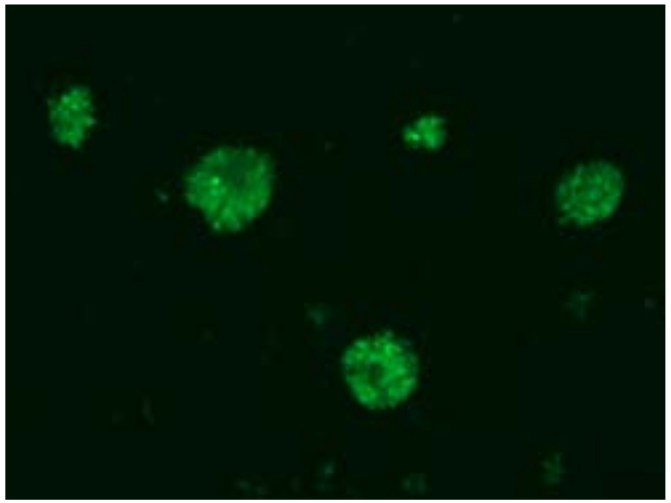


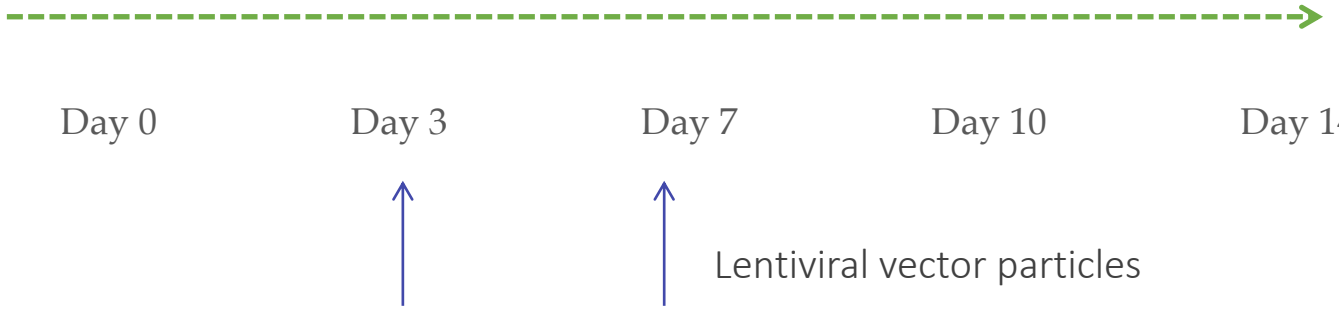
Ex vivo aspect of tumors in HE staining showed hypercellularity, plaque-forming units, mononuclear cells with atypical mitosis

- 6 weeks after daily monitoring the mice, tumors growth and development was detected only in control group

Generation of anti-EGFR CAR Cells

- Transduction by spinoculation, followed by incubation with the virus for 24hrs;
- Cell were transduced after activation with cytokines for 3 or 7d.

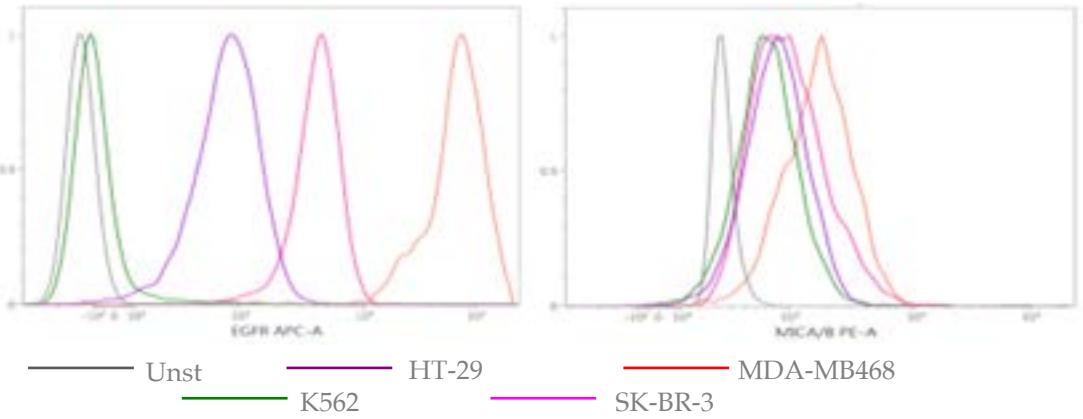




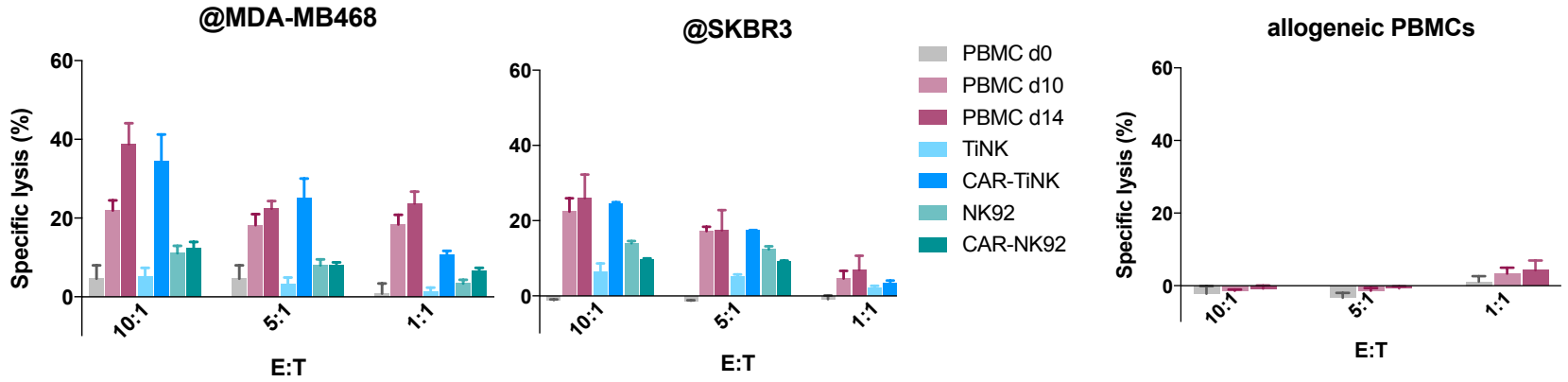
Lentiviral vector particles

- very high titer lentivirus (10^9 TU/mL) from GEGTech (Paris)

Cytotoxicity against EGFR+ Targets



Target cells were adherent tumor cell lines MDA-MB-468, SK-BR-3, HT-29, which were analyzed for the expression of EGFR, as well as NKG2D ligand MICA/B by flow cytometry.



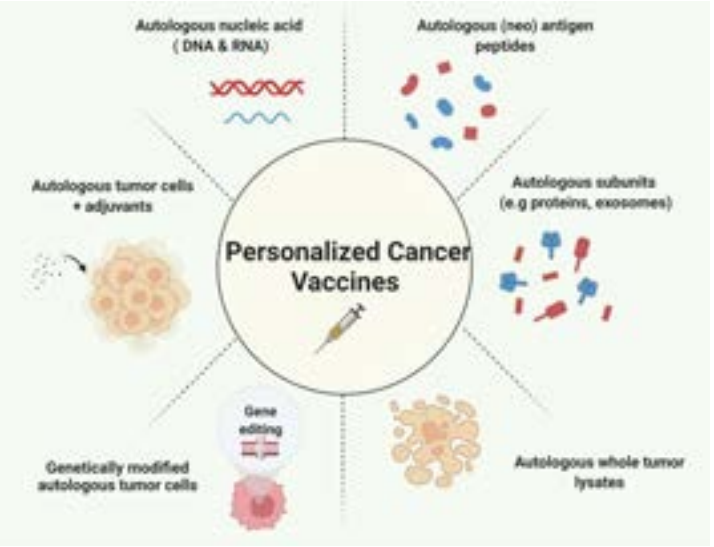
- When transduced with an anti-EGFR-CAR, NK cells will specifically kill EGFR+ tumor cells, but not self or allogeneic healthy cells.

2. Anti-tumor vaccines

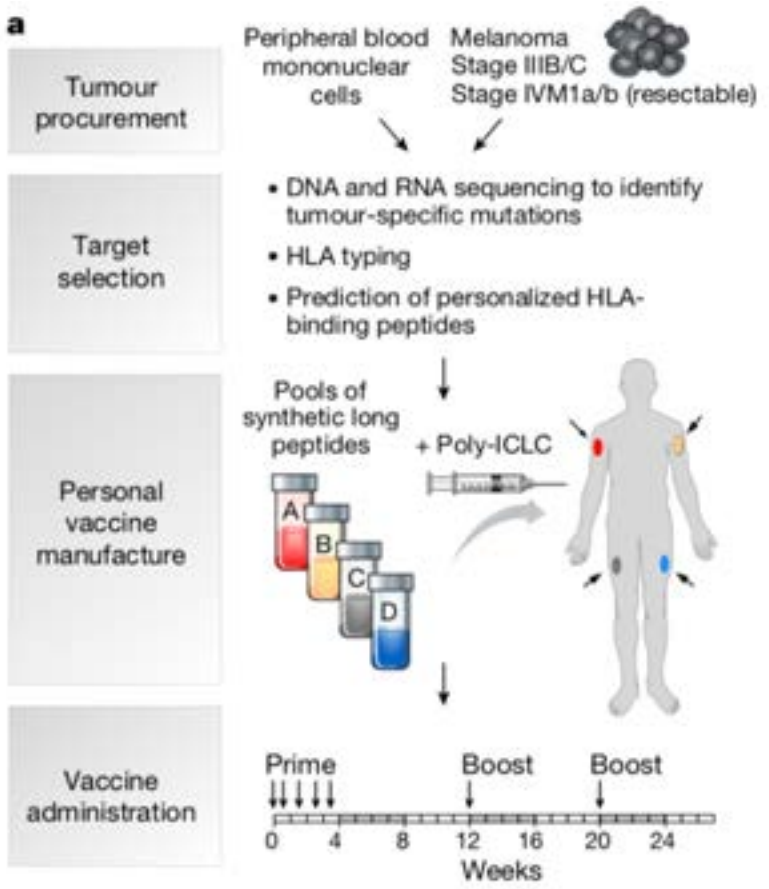
Personalized Approaches in Immuno-oncology

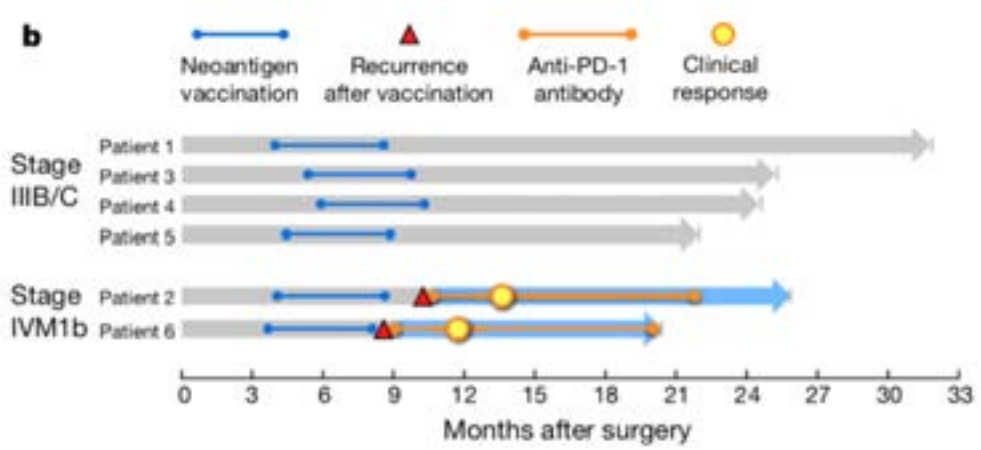
Conventional anti-tumor immunotherapy - Vaccines

- Sipuleucel-T (Provenge™) – prostate metastatic cancer; DCs are pulsed with HLA-A*02:01-restricted peptides derived from prostate specific membrane antigen (PSMA);
- GVAX - autologous vaccin with GM-CSF transduced cells; GVAX recruits DCs for tumor antigen presentation and activation of CTLs;
- Canvaxin™ - 3 melanoma cell lines combined with BCG as adjuvant for melanoma;
- Belagenpumatucel-L – allogenic vaccine containing 4 genetically modified NSCLC tumor cell lineswhich secrete anti-sense oligonucleotides for immunosupressor TGF-β2 cytokine;
- Oncophage/Vitespen – Gp96 in renal cancer;
- Trovax – MVA (modified vaccinia strain Ankara) vector-based vaccine for renal cancer – targets 5T4 antigen;
- CimaVax-EGF – N. Meningitidis + EGF vaccine in NSCLC;
- Adstiladrin - nadofaragene firadenovec (rAd-IFN/Syn3) – adenovirus-based gene therapy in urinary bladder cancer (NMIBC)



Synthetic Long Peptides Vaccine in Cancer





Clinical trials using SLPs in the US in 2022 (> 30)

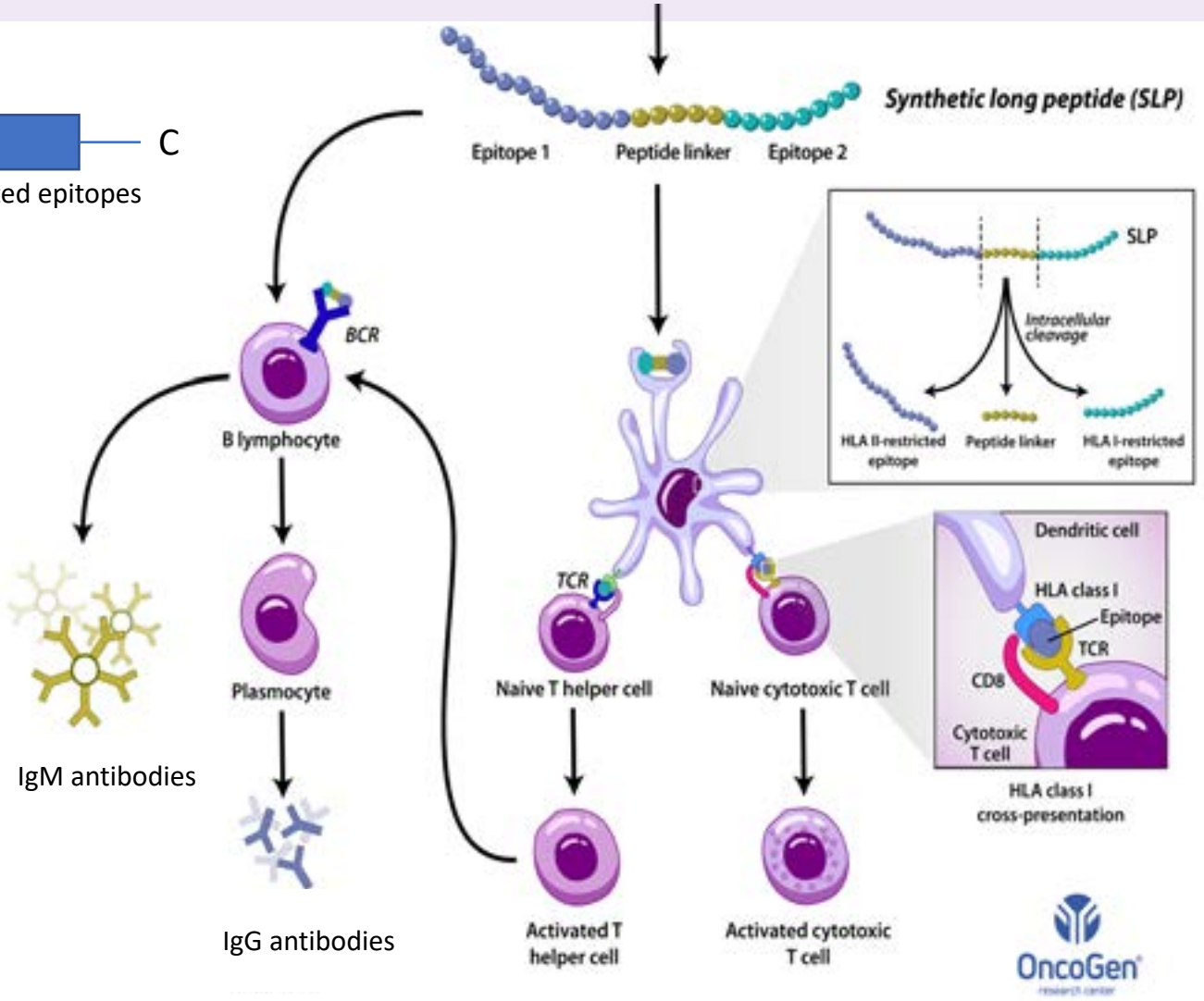
Trial ID	Phase	Cancer type	Institution/ Company Sponsors	Vaccine Platform	Patient Status	Patient Accrual Target
NCT02287428	Phase 1	Glioblastoma	Dana-Farber Cancer Institute, The Ben & Catherine Ivy Foundation, Accelerate Brain Cancer Cure, Merck Sharp & Dohme Corp.	Synthetic long peptide	Active, not currently recruiting	46
NCT02348320	Phase 1	Breast cancer	Washington University School of Medicine	DNA	Recruiting	30
NCT02721043	Phase 1	Multiple solid tumors	Icahn School of Medicine at Mount Sinai	Synthetic long peptide	Recruiting	20
NCT03300843	Phase 2	Multiple solid cancers	National Cancer Institute	Dendritic cell	Recruiting	86
NCT02897765	Phase 1	Melanoma, lung cancer, bladder cancer	Neon Therapeutics, Bristol-Myers Squibb	Synthetic long peptide	Active, not currently recruiting	55
NCT02950766	Phase 1	Kidney cancer	Dana-Farber Cancer Institute, Bristol-Myers Squibb, Oncovir	Synthetic long peptide	Recruiting	15
NCT03068832	Phase 1	Pediatric brain tumor	Washington University School of Medicine	Synthetic long peptide	Not yet recruiting	10
NCT03380871	Phase 1	Lung cancers	Neon Therapeutics, Merck Sharp & Dohme Corp.	Synthetic long peptide	Active, not currently recruiting	15
NCT03422094	Phase 1	Glioblastoma	Washington University School of Medicine, Bristol-Myers Squibb	Synthetic long peptide	Recruiting	30

An immunogenic personal neoantigen vaccine for patients with melanoma. Patrick A. Ott et al., *Nature*, 2017

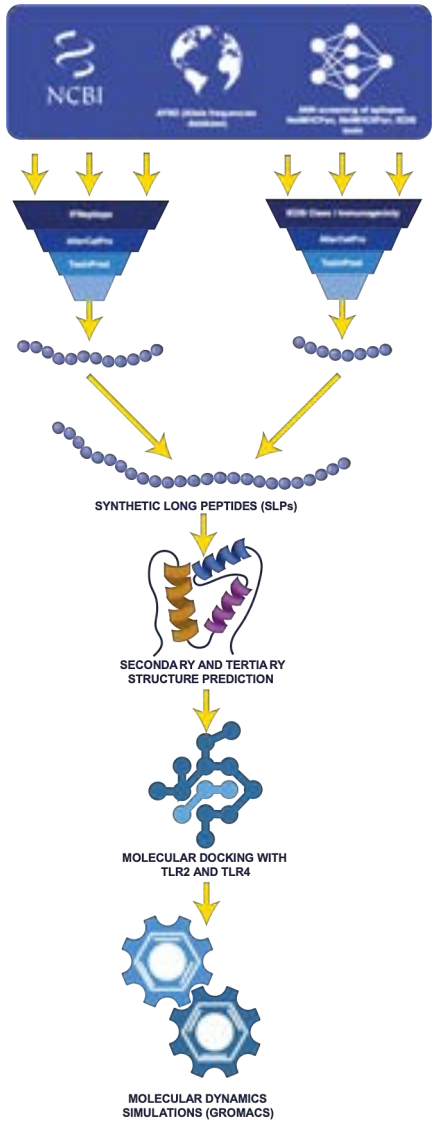
Synthetic Long Peptides Vaccine in Cancer – OncoGen strategy

Advantages of SLPs use:

- easy manufacturing process with high yield and purity;
- increased stability during storage and transport;
- combination of HLA class I and class II restricted epitopes, providing bidirectional stimulation of CD4+ and CD8+ T cells via canonical antigen presentation and cross-presentation;
- robust, specific cytotoxic T-cell response against malignant cells, amplified by the CD4+-mediated pro-inflammatory cytokine secretion.



Synthetic Long Peptides Vaccine in Cancer – anti-HPV 16 and 18



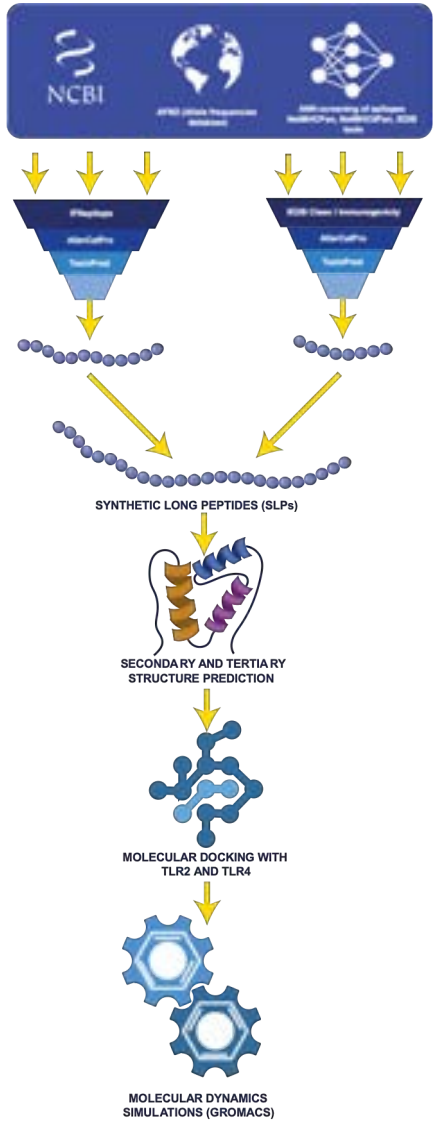
- Epitope identification: **Immune Epitope Database (IEDB)** or by **neo-antigen prediction** using the ANN-based software (NetMHCPan, NetMHCIIPan). MHC Class I and II **strong binders** with **high promiscuity** and **high degree of conservation** were selected so that the population coverage is maximal with a minimum number of peptides;
- SLPs Design: **Two models of SLPs were designed:**

1. N-terminal class II-restricted peptide, a flexible, a 6-mer cathepsin-cleavable linker (LLSVGG) (Rabu *et al.*), and a C-terminal class I-restricted epitope.

2. 2 subunits (class II-linker-class I) joined by the cathepsin and ERAP-cleavable linker LRMK.

Synthetic Long Peptides Vaccine in Cancer – anti-HPV 16 and 18

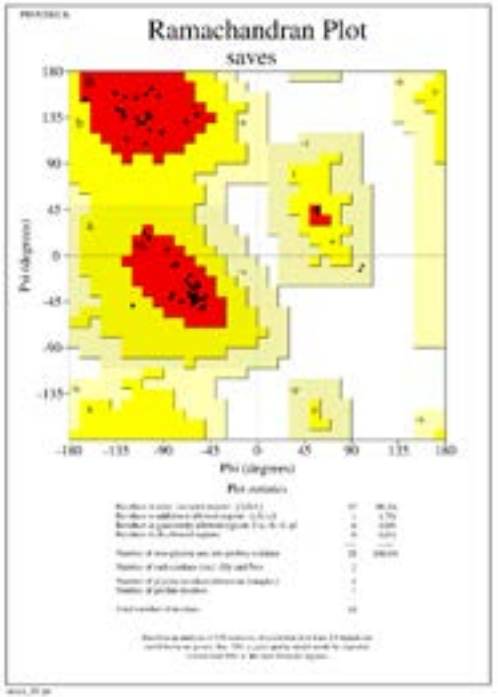
- **Allergenicity and toxicity screening -** AllerCatPro, ToxinPred and ToxIBTL.
- **Physico-chemical property analysis** (ProtParam)
- **Antigenicity analysis** was performed using VaxiJen 2.0. A VaxiJen score >0.4 reflects high antigenicity.
- 3-mer and 9-mer fragments generated from Robetta were used for **tertiary structure prediction** using Rosetta ab initio.
- **3D-structure validation** of the constructs was based on the QMEAN4 score, PROCHECK and Ramachandran plot analysis.
- **Molecular docking with Toll-like receptor 2 and 4** (PDB id: 6NIG and 3FXI) and structure refinement were performed using HADDOCK 2.4.
- **Molecular dynamics (MD) simulations** were performed using GROMACS package.



Synthetic Long Peptides Vaccine in Cancer – anti-HPV 16 and 18

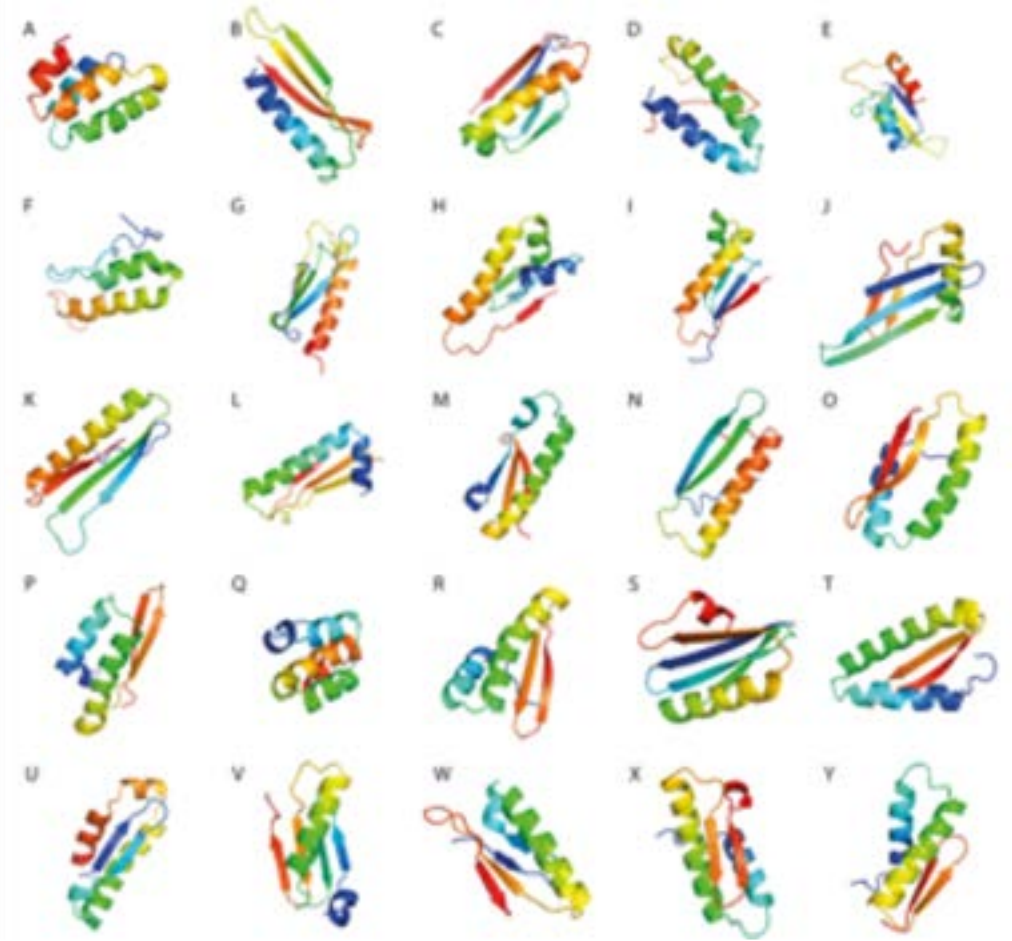
- **25 SLPs constructs were generated**, including both HLA class I and class II restricted epitopes from E6 and E7 proteins, identified from IEDB or predicted using NetMHCPan and NetMHCIIPan;
- None of the SLPs displayed *in silico* allergic or toxic properties;
- Population coverage studies provided 98.18% coverage for class I epitopes and 99.81% coverage for class II peptides in the IEDB World population allele set;

Class I		
Coverage	Average hit	PC90
98.18%	6.48	2.47
Class II		
Coverage	Average hit	PC90
99.81%	15.29	9.03

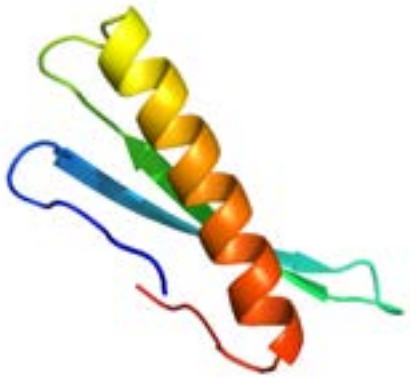


Ramachandran plot for the peptide: MLDLQPETTDLYCYELLS VGGKFYSKISEYLRMKLKF YSKISEYRHYCYLLSVGGL FLNTLSFV.

Synthetic Long Peptides Vaccine in Cancer – anti-HPV 16 and 18



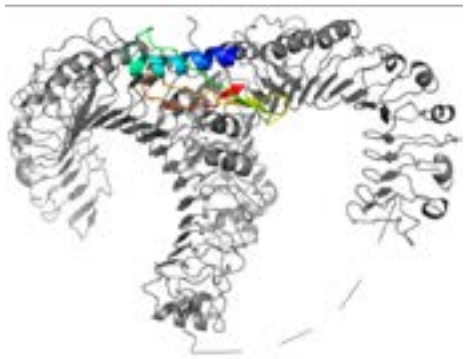
3D structure of the 25 SLPs visualized by PyMol



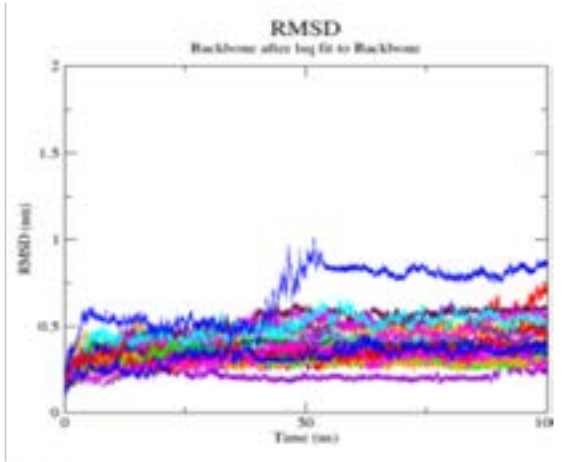
Molecular visualization of the peptide MLDLQPETTDLYCYELLSVGGKFYSKISEYLRMKLKFYSKISEYR HYCYLLSVGGLFLNTLSFV using PyMol software.

- 3D structure ab initio prediction provided good quality models (>90% of residues in most favorable regions, QMEAN4 score = 0.7, Z-score > -2).

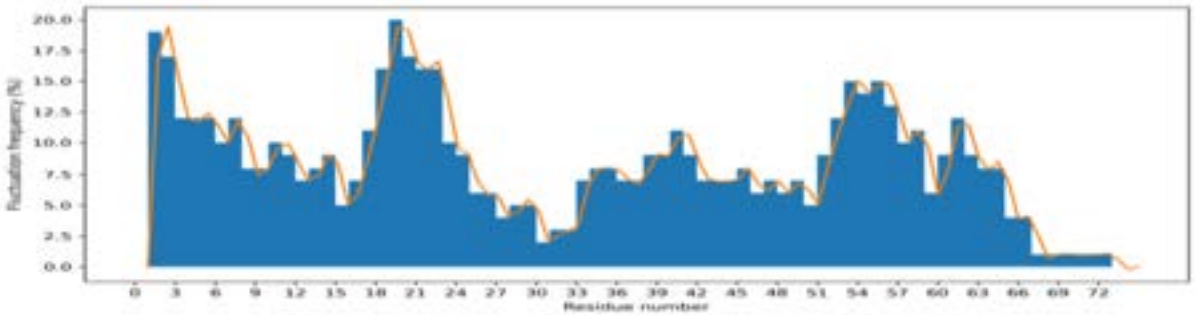
Synthetic Long Peptides Vaccine in Cancer – anti-HPV 16 and 18



One synthetic long peptide in complex with toll-like receptor 2.



RMSD plots for the 25 SLPs. All peptides reach a stable conformation after 40 ns of MD simulation



Plot showing the mean RMSF per each residue. It is worth noting that residues 15-25 and 51-57 express the highest flexibility, while 30-35, the lowest.

- Molecular docking with toll-like receptor 2 identified potential intrinsic TLR2 agonist activity, while molecular dynamics studies of SLPs in water suggested good stability with favorable thermodynamic properties.

Conclusion

- The available anti-HPV vaccines exert a highly potent prophylactic effect by interfering with HPV keratinocyte adhesion and subsequent infection and malignant keratinocyte transformation;
- Due to the lack of L1 antigen expression in neoplastic epithelial cells, VLP-based vaccination has no effect on already constituted infection;
- Therefore, there is an urgent need for a therapeutic vaccination platform, and **SLPs** could be the elected one.

SMART DIASPORA 2023

Thank you!

Research team

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