CAR-T терапия следующего поколения: стратегии, технологии, мишени

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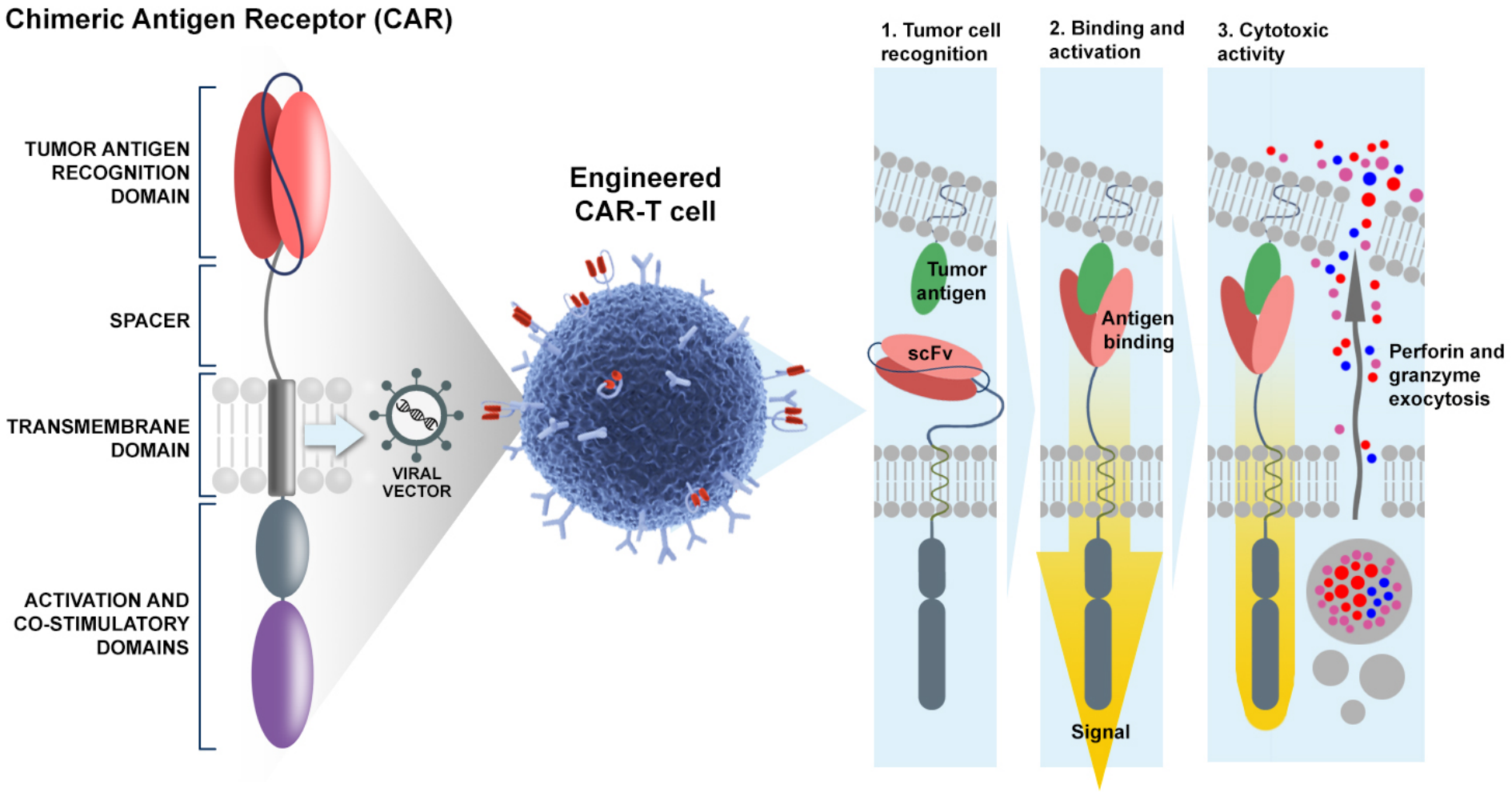
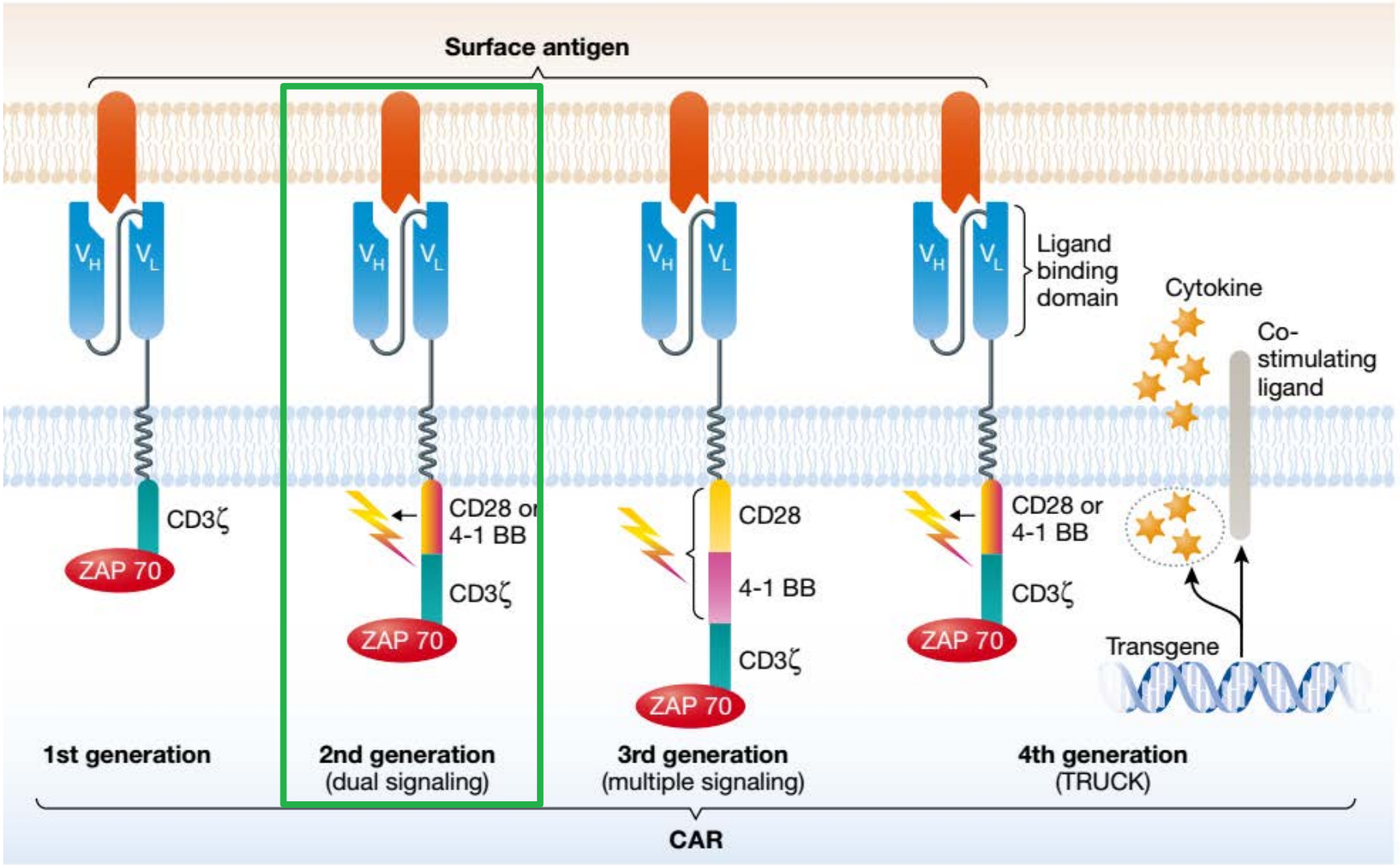
VI Всероссийская конференция
«Актуальные вопросы доклинических и клинических исследований лекарственных средств, биомедицинских клеточных продуктов и клинических испытаний медицинских изделий»

BIOCAD
Biotechnology Company

A “Living Drug”

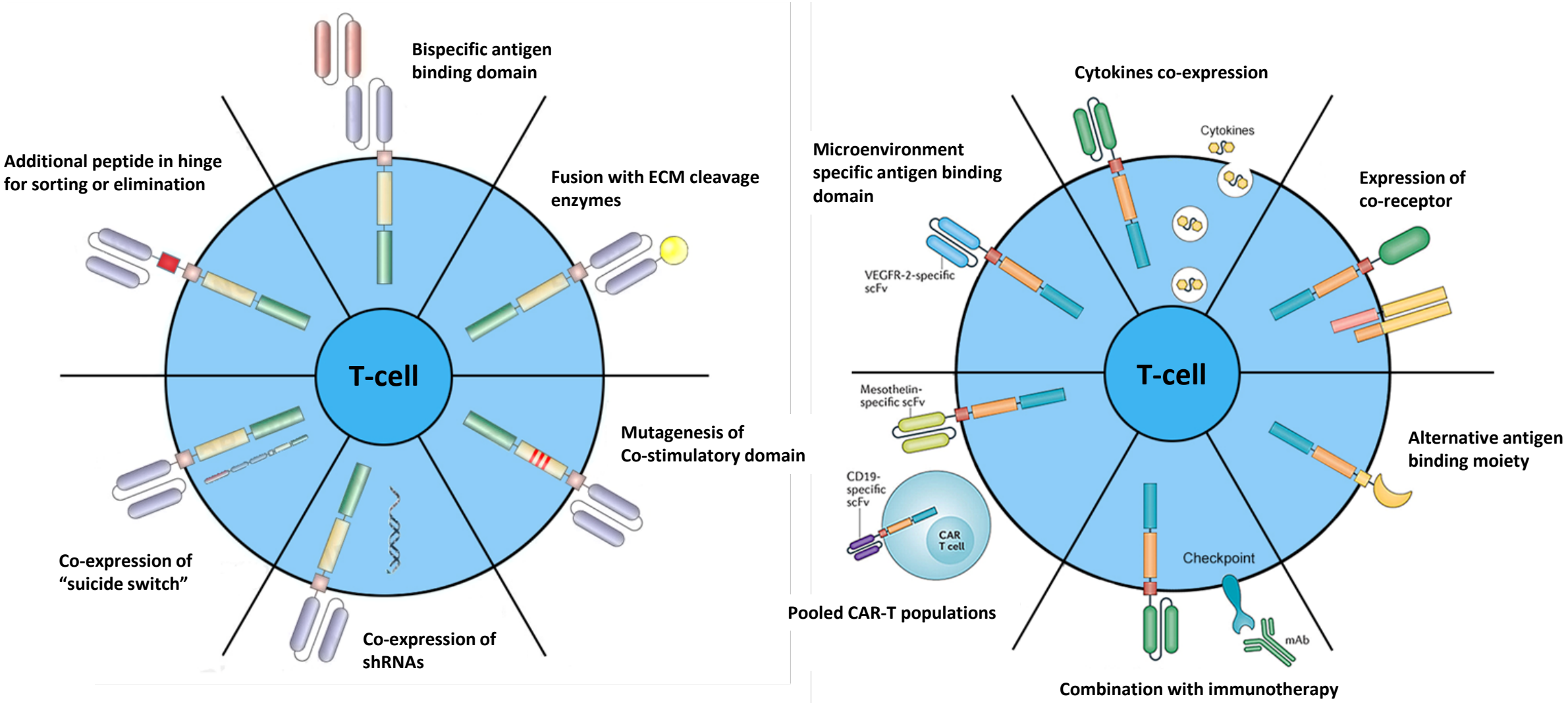


Evolution of CAR concept



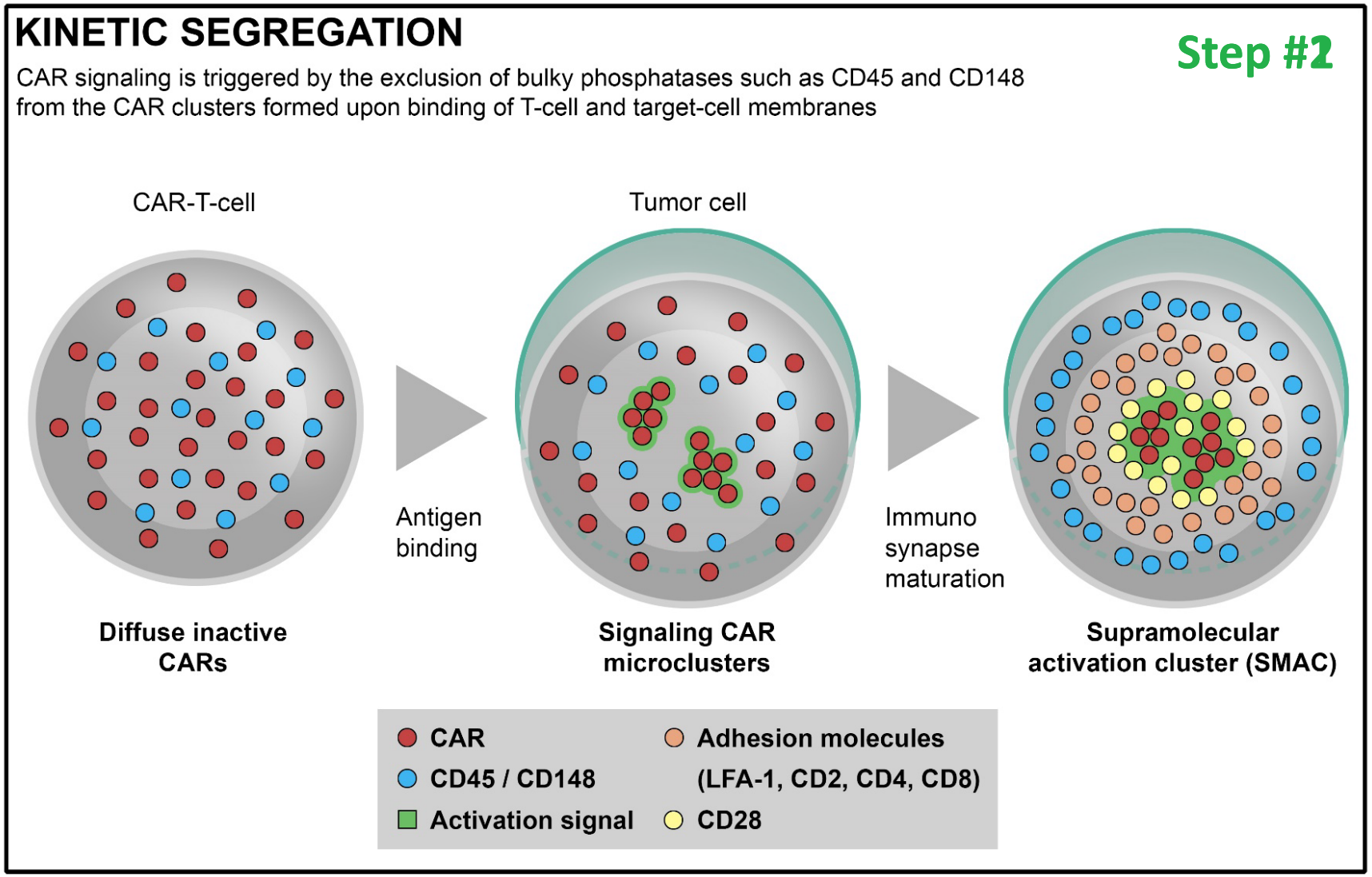
Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells—challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med [Internet]. 2017; : e201607485.

Perspective modifications and cobinations of CAR-T

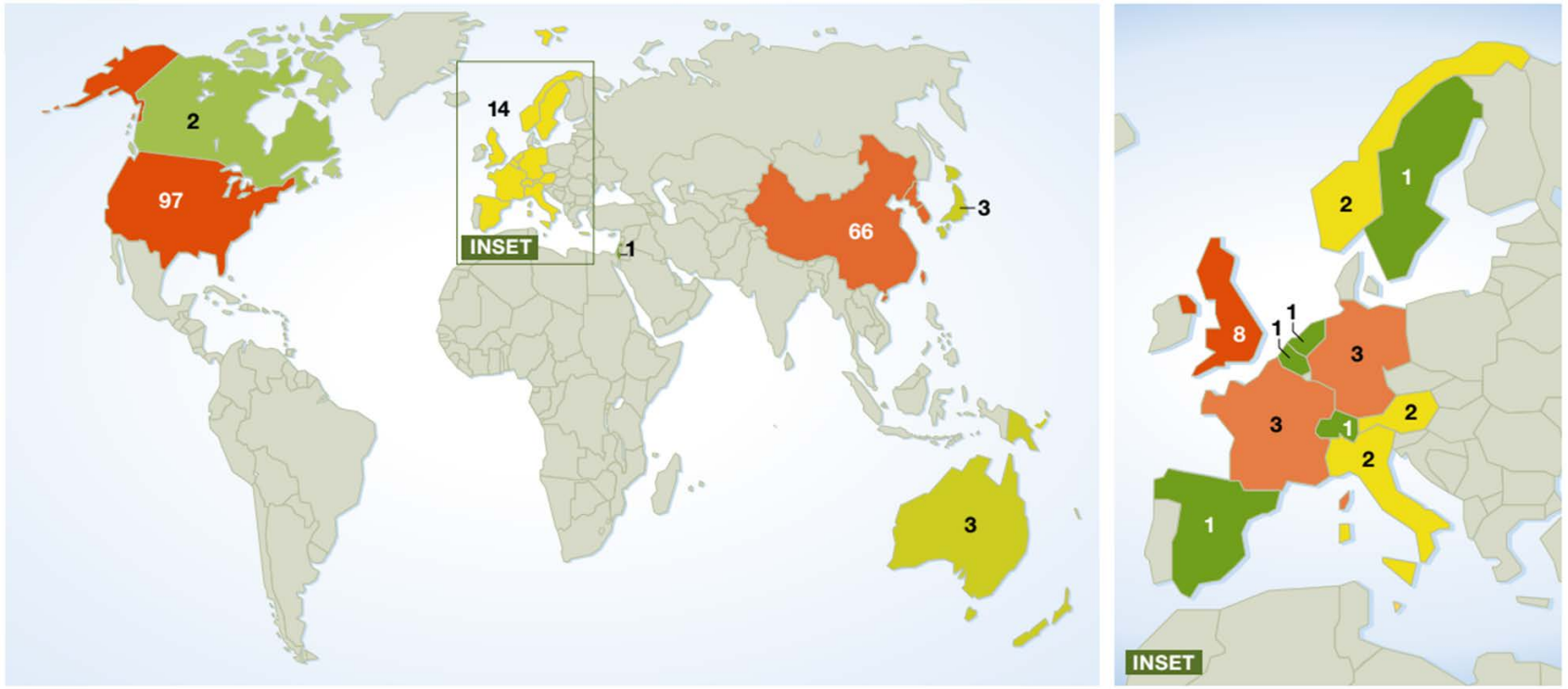


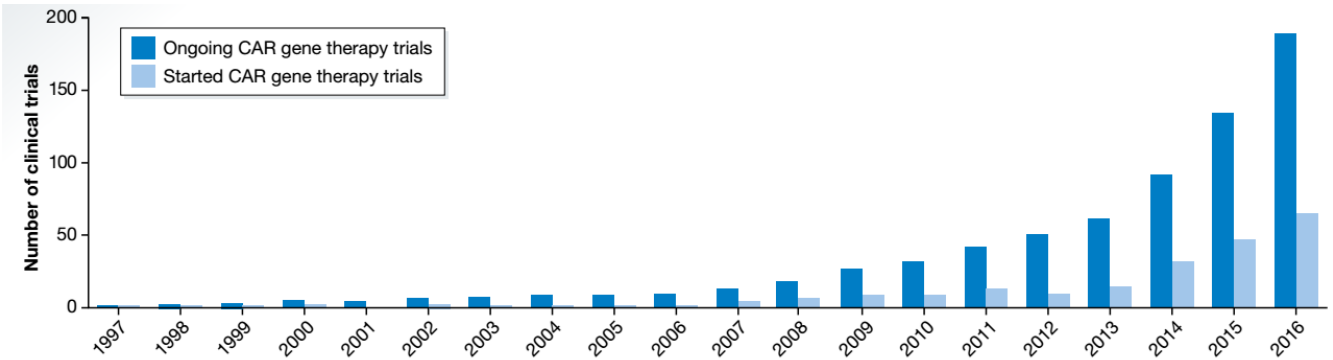
Based on Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells—challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med [Internet]. 2017; : e201607485.

Molecular mechanisms of action

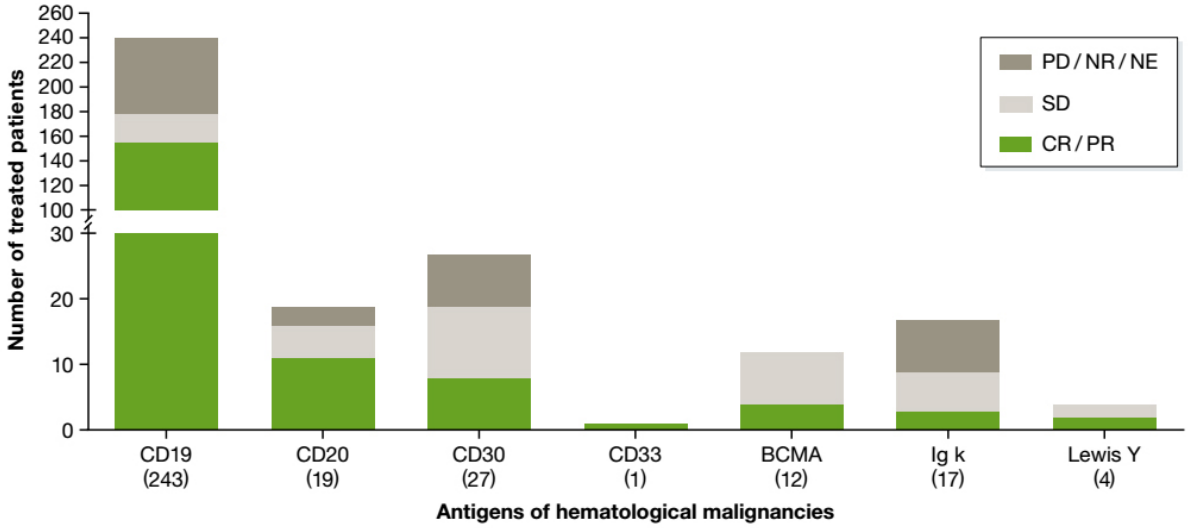


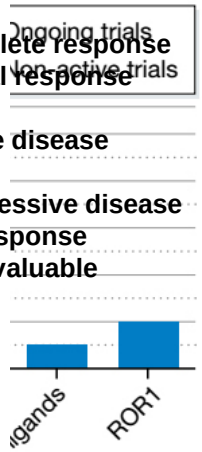
CAR-T clinical trials





CAR-T clinical trials

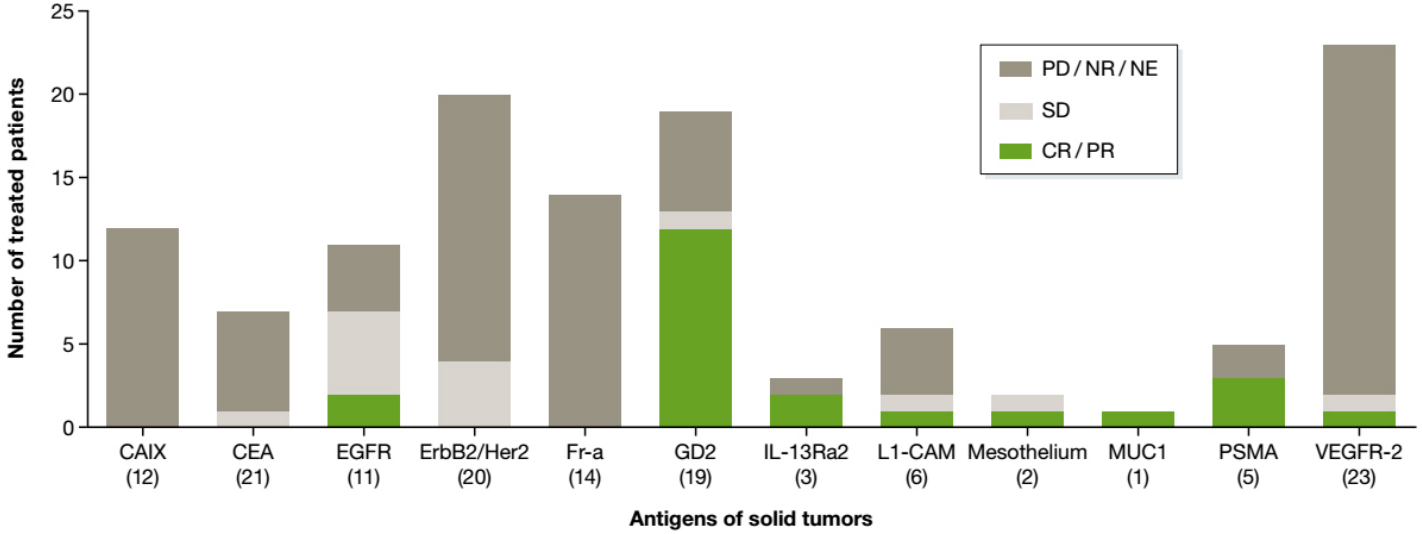


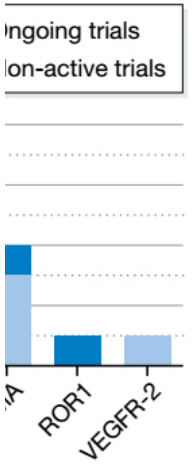


CR - complete response
PR - partial response

SD - stable disease

PD - progressive disease
NR - no response
NE - not evaluable





Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells—challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med [Internet]. 2017; : e201607485.

CAR-T therapy approved

NOVARTIS

Kymriah™ (tisagenlecleucel) suspension for intravenous infusion, formerly CTL019

30.08.2017 FDA approved for refractory or relapsed **acute lymphoblastic leukemia (ALL)**

83% overall remission rate in patient population with limited treatment options and historically poor outcomes

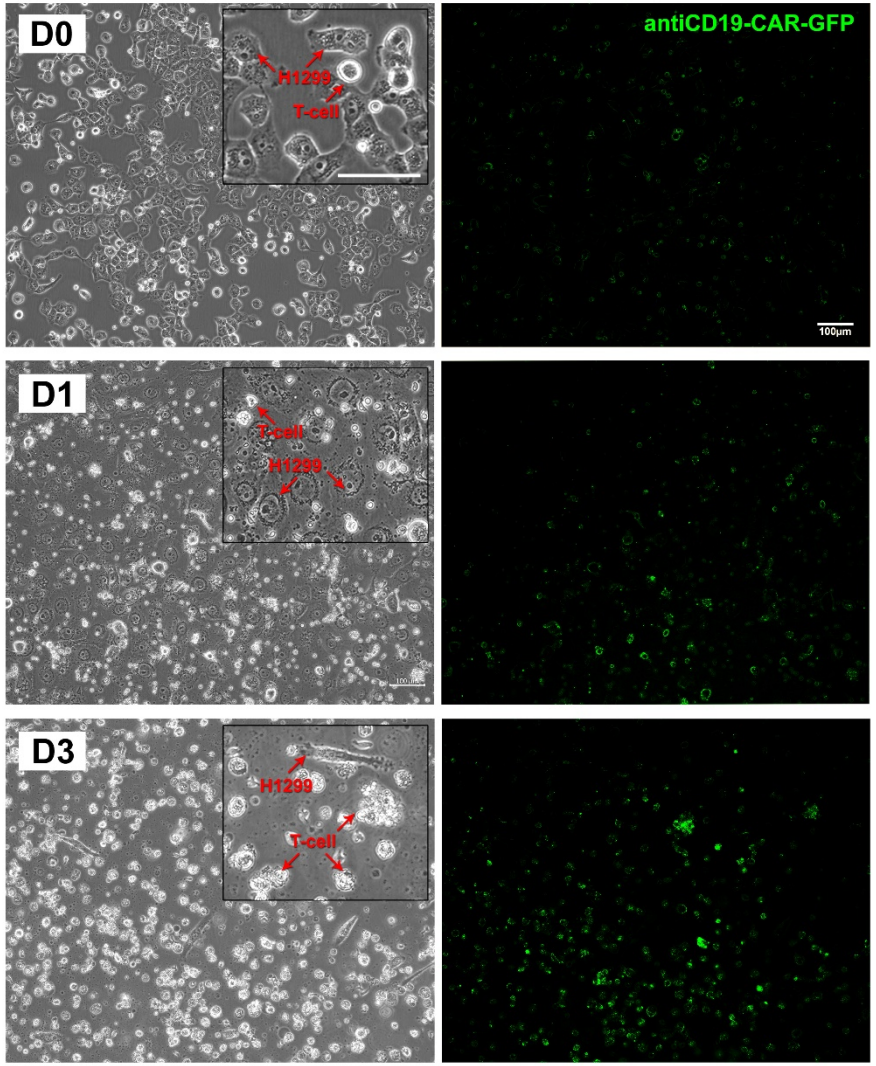
Kite
A GILEAD Company

YESCARTA™ (axicabtagene ciloleucel) suspension for intravenous infusion

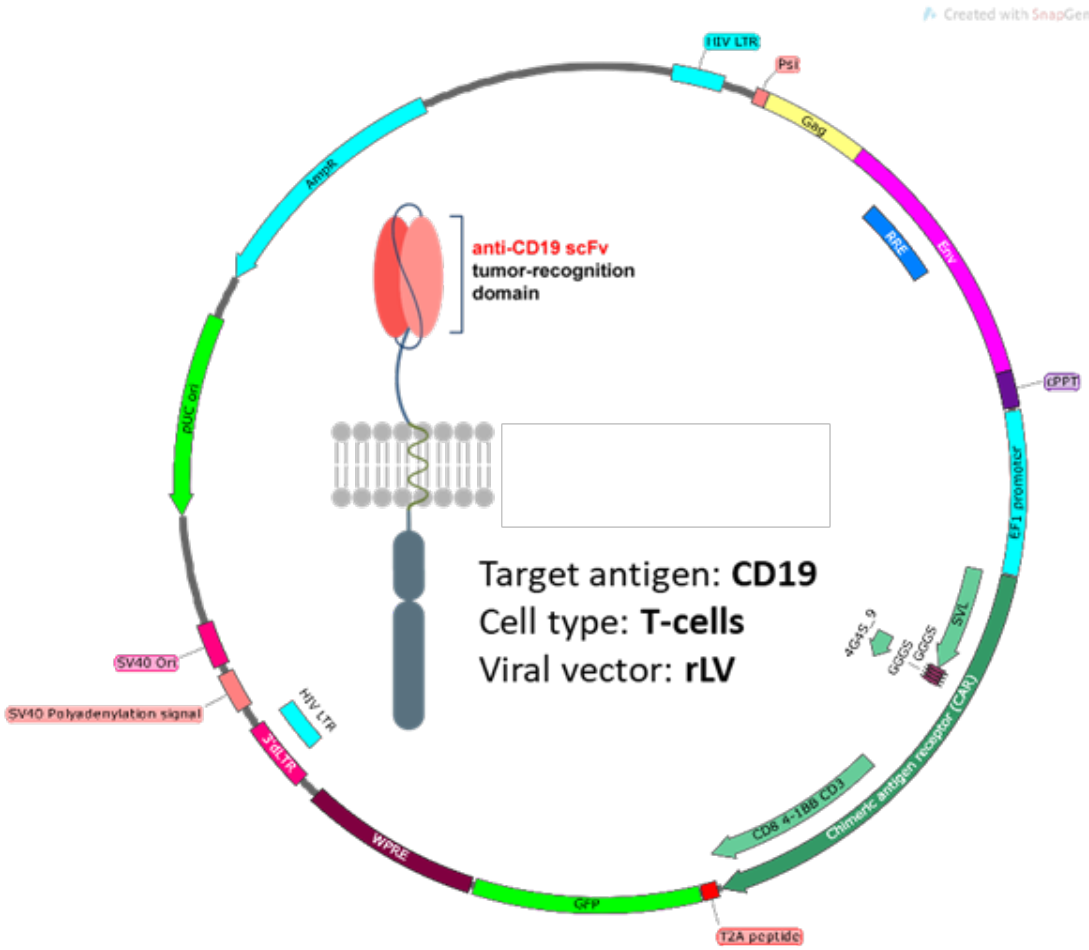
18.10.2017 FDA approved for refractory or relapsed **diffuse large B-cell lymphoma (DLBCL)**

51% complete remission rate in more than 100 adults with refractory or relapsed large B-cell lymphoma

CD19-targeted CAR-T

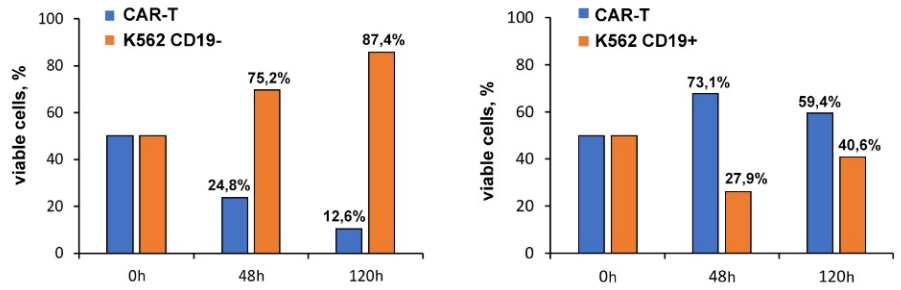


Rapid elimination of CD19+ tumor targets (H1299 CD19+ cell line) *in vitro*

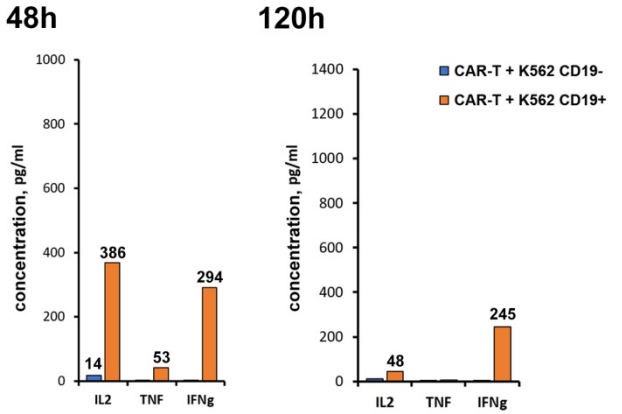


CD19-targeted CAR-T

CD19-targeted CAR-T (41-BB)



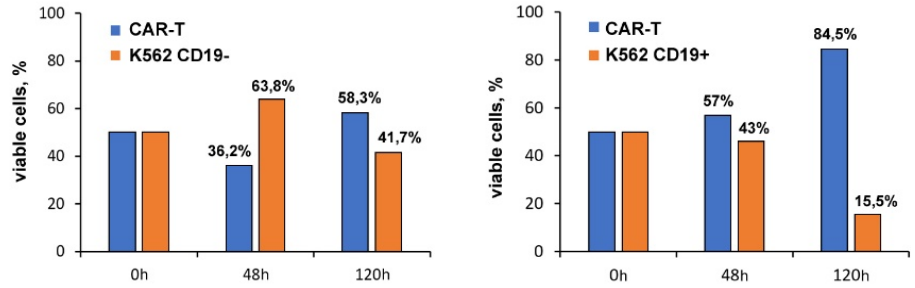
In vitro **cytotoxic activity and proliferation response**



In vitro **cytokine release, co-cultivation with CD19+ targets**

CD19-targeted CAR-T (CD28)

augmented (RIAD + shRNA scramble)



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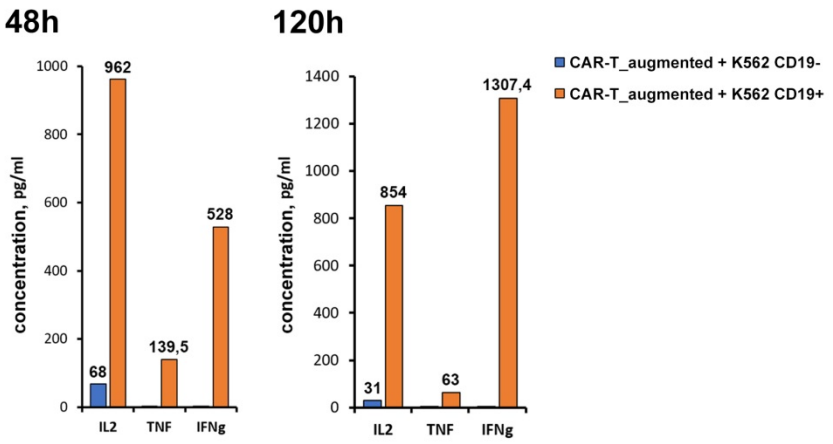
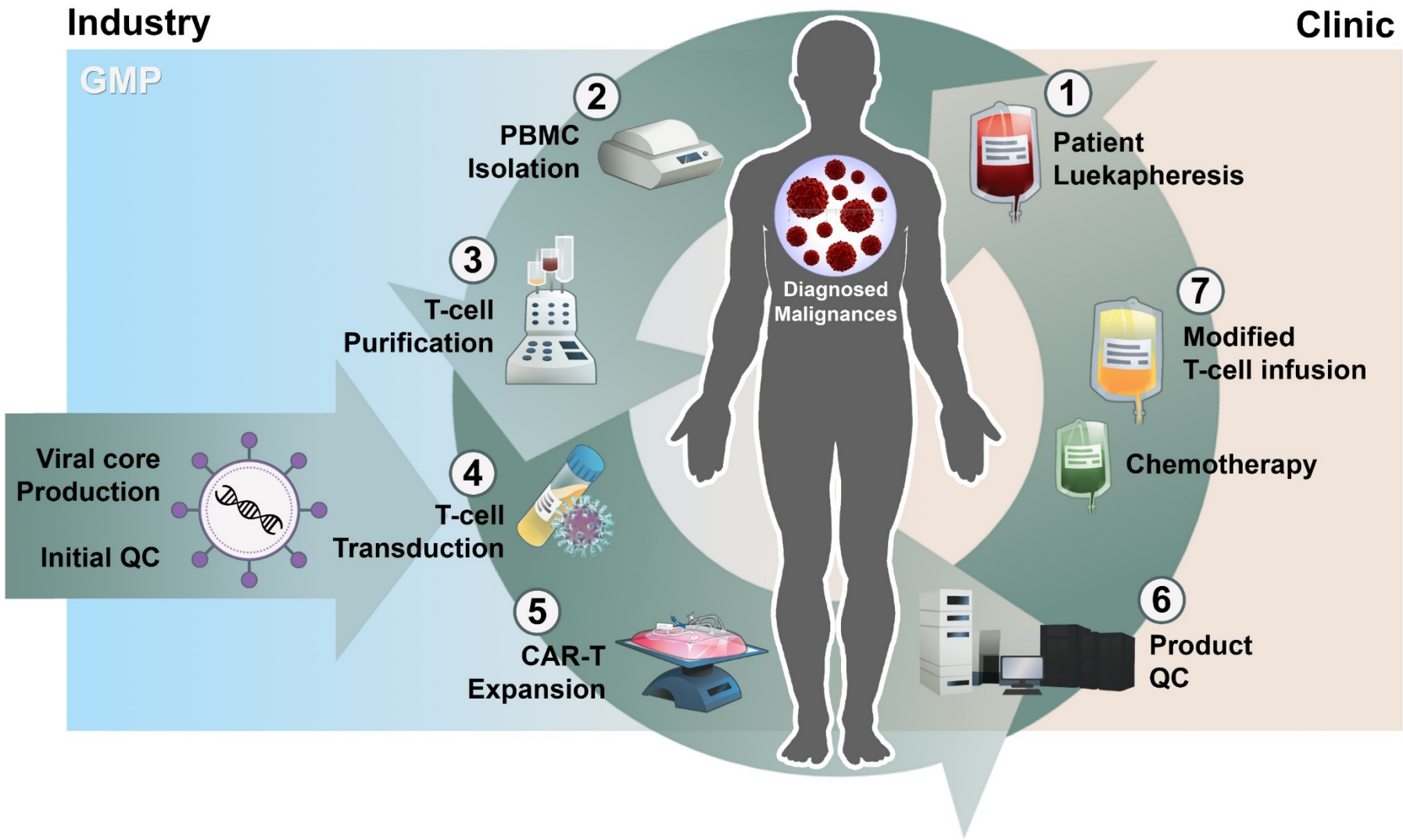


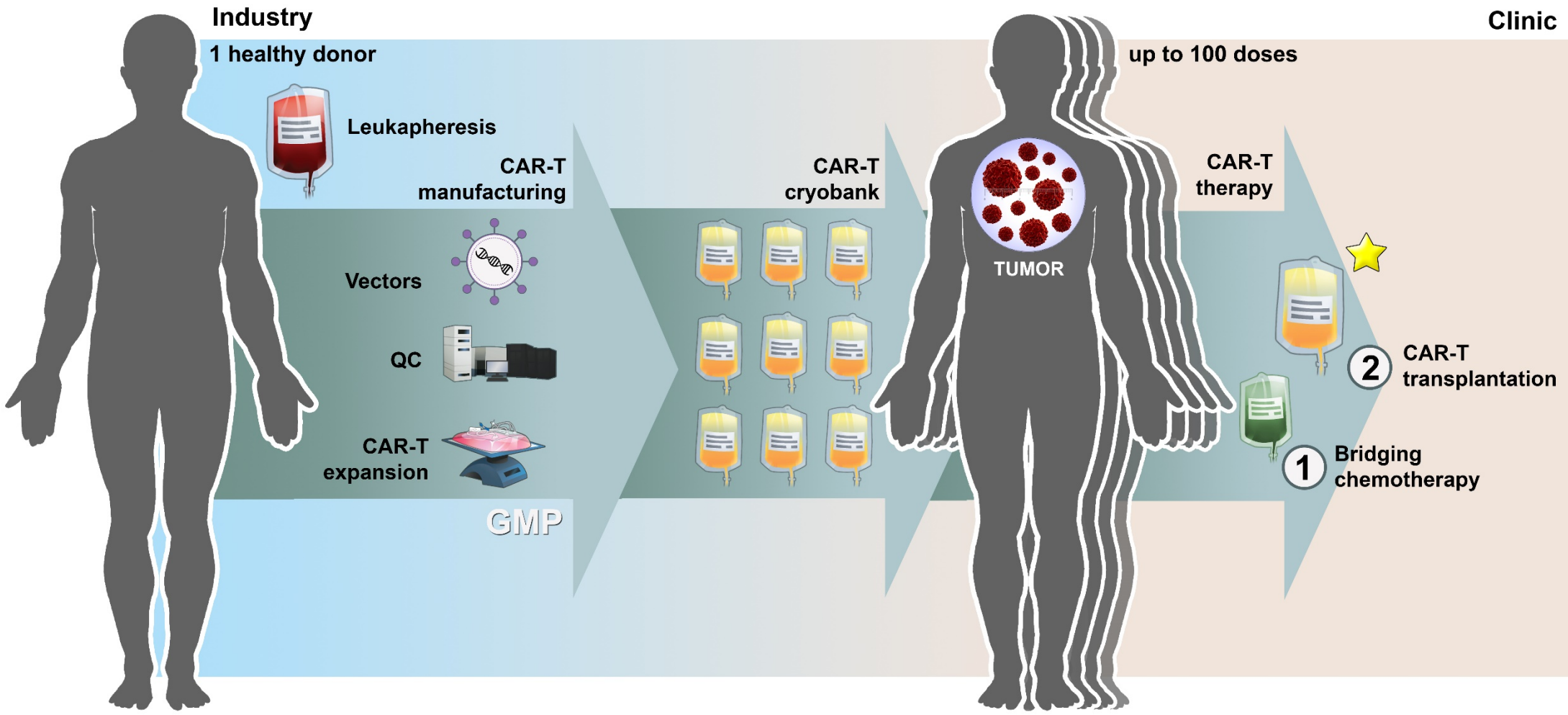
In vitro **cytokine release, co-cultivation with CD19+ targets**

Petukhov, A., Markova, V., Gershovich, P., Titov, A., Smirnov, E., Zaikova, E., … Zaritskey, A. (2017). CAR-T Enhancement by Combined TCR Knockdown and the RIAD Peptide Addition. Clinical Lymphoma Myeloma and Leukemia, 17, S394.

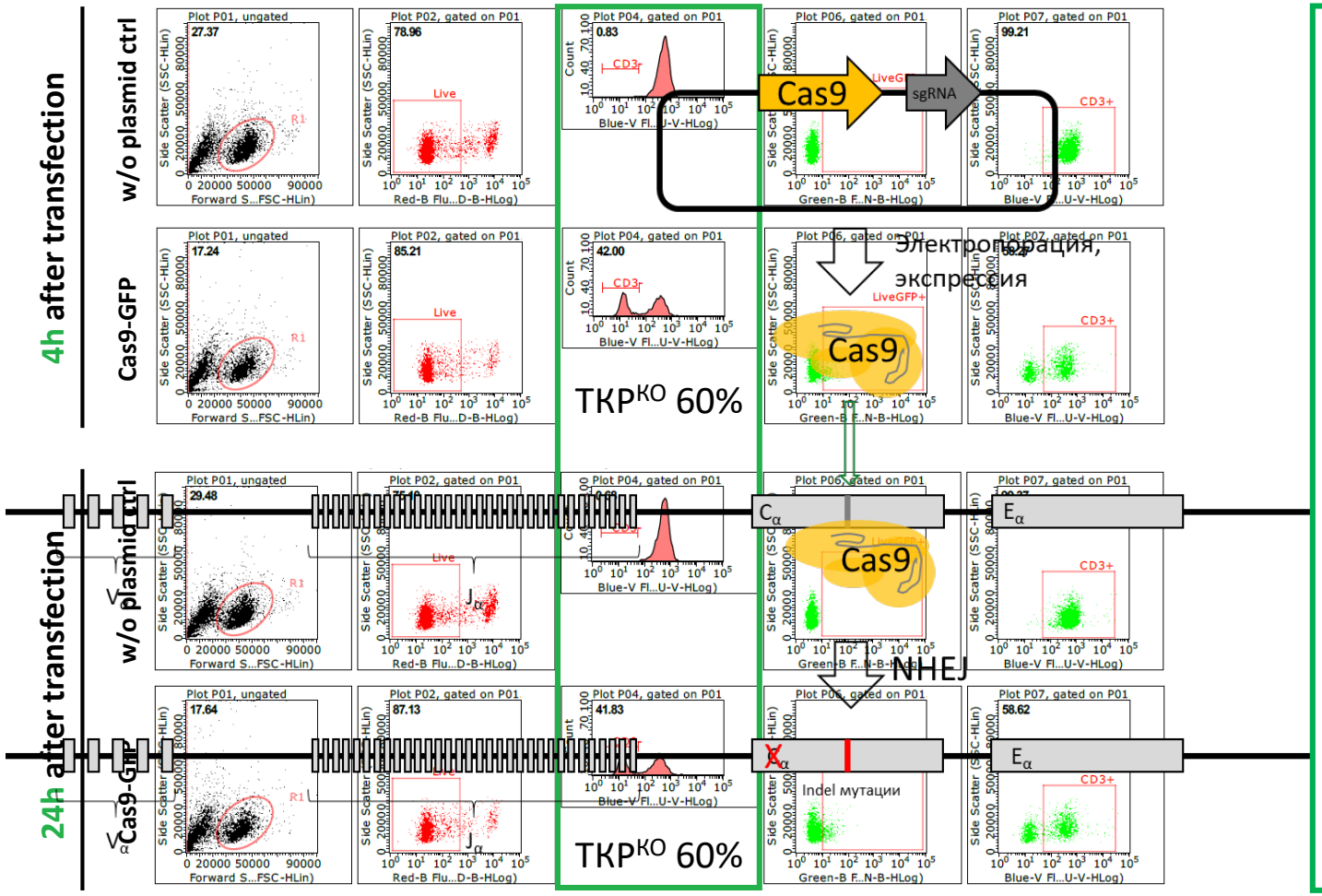
Autologous CAR-T platform



Towards to allogeneic CAR-T



Genome editing tools for TCR knockout

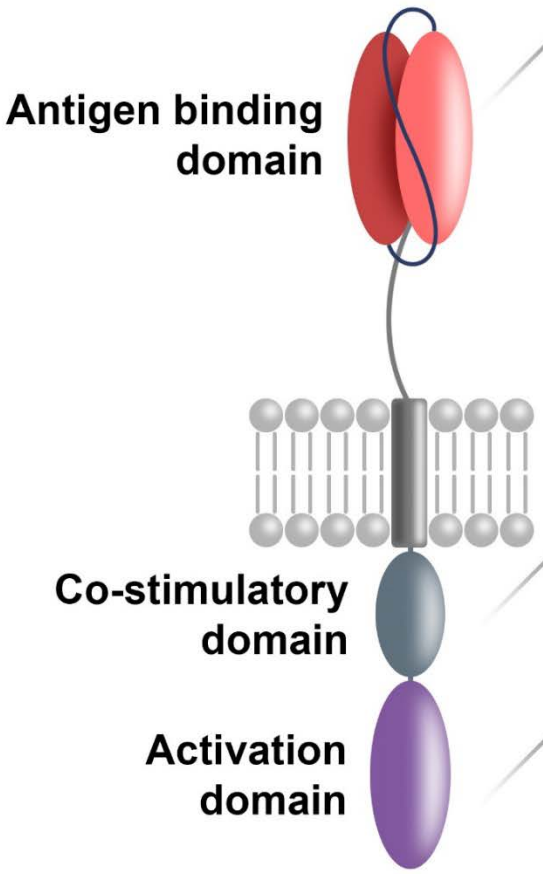


What is real allogenicity?

and… is it safe enough?

- **KO of TCR is mitigating GvHD but not affecting transplant rejection (persistence of CAR-T *in vivo*)**
- **Elimination of HLA / MHC complex could leads to uncontrolled persistence *in vivo***
- **“Suicide switch” may not provide 100% clearance of CAR-T in the case of manifestation of related side effects**

Ongoing directions of CAR engineering in BIOCAD



«in-house» fully human scFv
- + targeting mutated isoforms of TAAs
- + reducing immunoginicity
- + tuning of affinity

towards to next generation of CAR:
- + shRNAs
- + cytokines / superagonistic Abs
- + gene editing tools
- + «suicide switch»

computational modeling of co-stimulatory domains
- + optimized natural domains
- + synthetic siquences

site-directed mutagenesis of CD3z domain
- + enhanced signal transduction

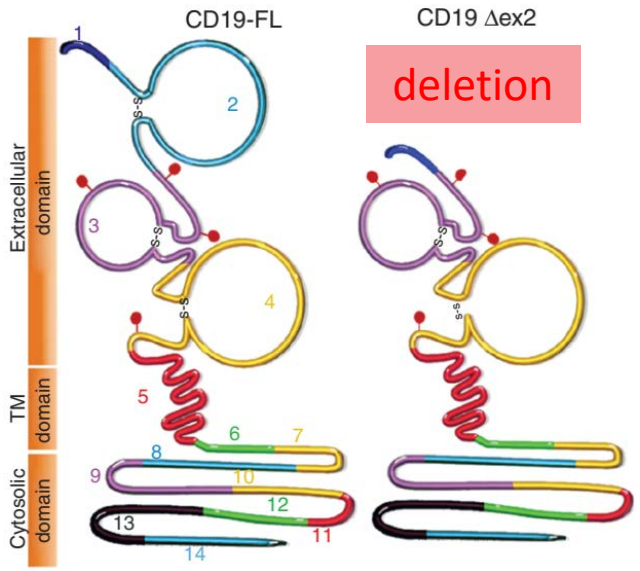
Why it's so important?

CD19 is good example

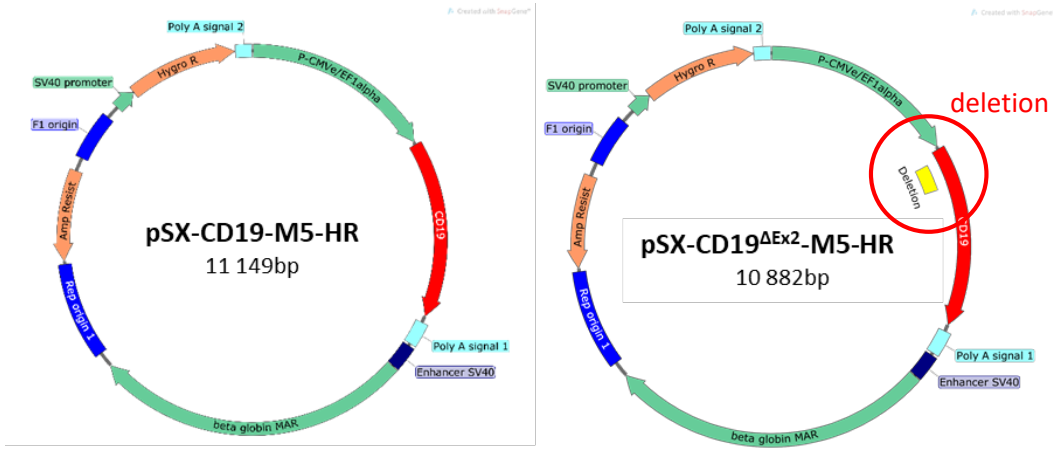
- **Reported cases of CD19 negative relapse after BiTE and CAR-T19 treatment are of concern**
- **Majority of CARs used in ongoing CTs engineered based on mouse mAbs (clone FMC63)**
- **There is only predicted structure of CD19 antigen**
- **There is no complete understanding of CD19 mutated alleles prevalence in population**

Improving efficacy of aCD19 scFv for CAR-T therapy

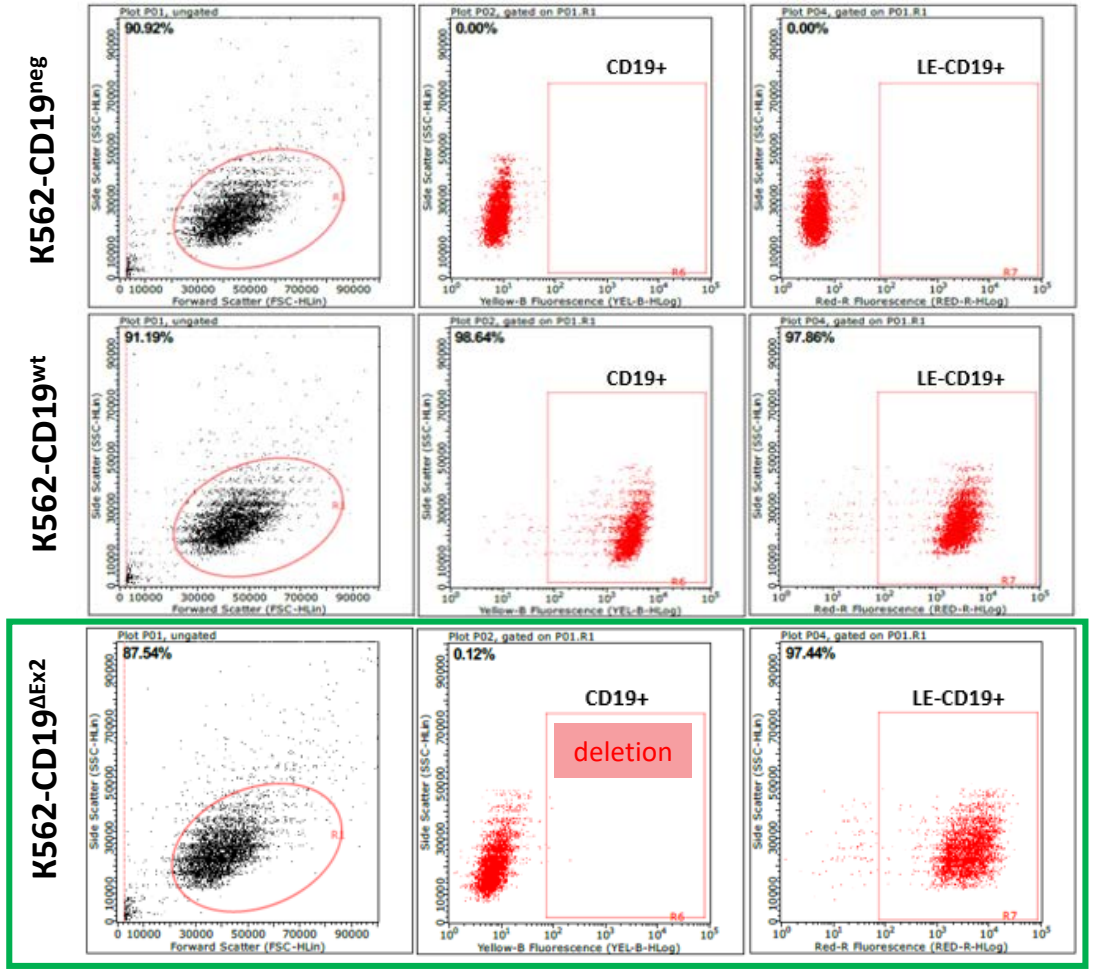
Predicted structure of CD19 Δex2



Sotillo et.al., 2015



$CD19^{+/-/\Delta}$ cell line panel for antibody screening

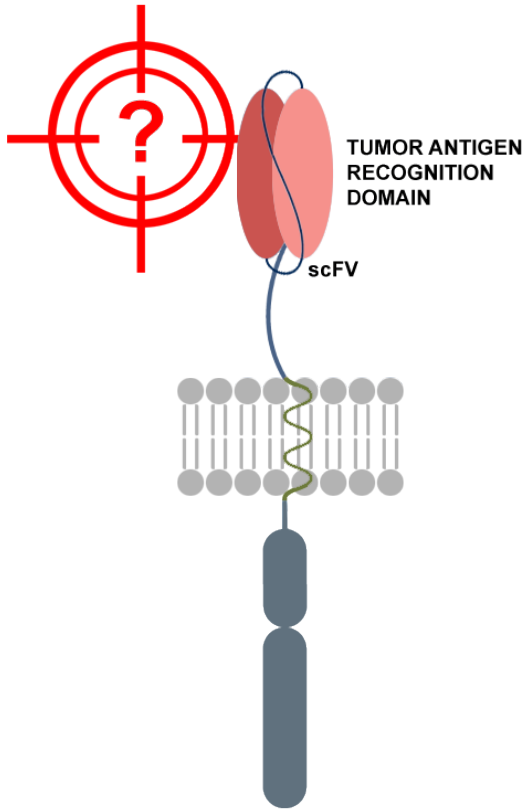


Choosing the solid tumor specific TAAs for CAR-T therapy

Antigen	Type of cancer
HER2	Breast Cancer Ovarian Cancer Lung Cancer Gastric Cancer Colorectal Cancer Glioma Glioblastoma Pancreatic Cancer
Mesothelin	Malignant Pleural Mesothelioma Malignant Epithelial Pleural Mesothelioma Pancreatic Cancer Metastatic Pancreatic (Ductal) Adenocarcinoma Epithelial Ovarian Cancer
IL13Ra	Brain and Central Nervous System Tumors Malignant Glioma Refractory Brain Neoplasm Recurrent Brain Neoplasm Glioblastoma
Mucin 1 (MUC1) protein	Hepatocellular Carcinoma Non-small Cell Lung Cancer Pancreatic Carcinoma Triple-Negative Invasive Breast Carcinoma
GD2	Neuroblastoma Sarcoma
EGFR / EGFRvIII	Malignant Glioma Glioblastoma

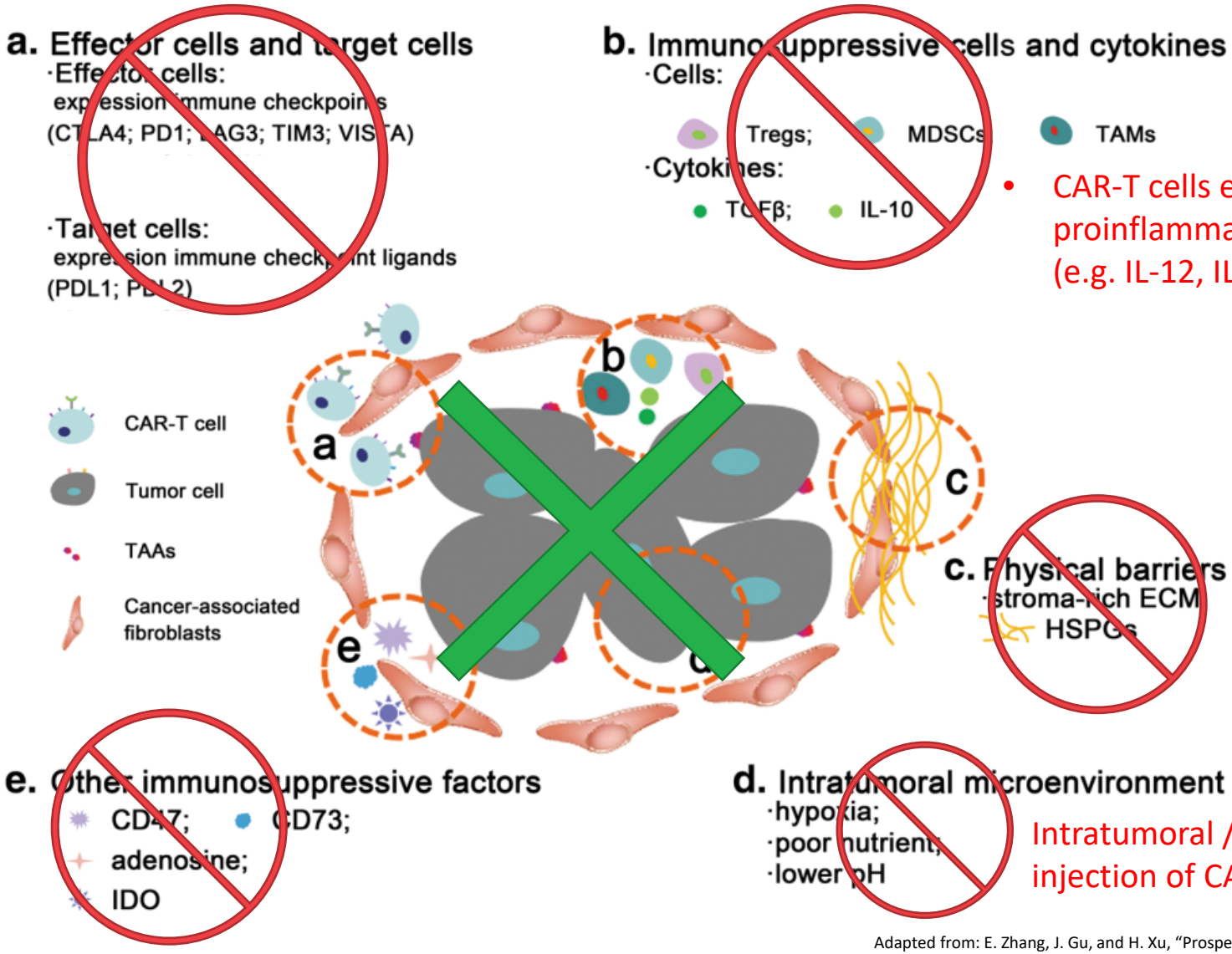
and other…

Solid tumor markers are of great interest



Barriers for efficient CAR-T therapy of solid tumors

Combination with immunotherapeutic MABs (e.g. aPD1)



- CAR-T cells expressing proinflammatory cytokines (e.g. IL-12, IL-15)

CAR-modified T cells express receptors or enzymes increasing their ability to move to the tumor site (e.g., CCR2b, CCR4, heparanase)

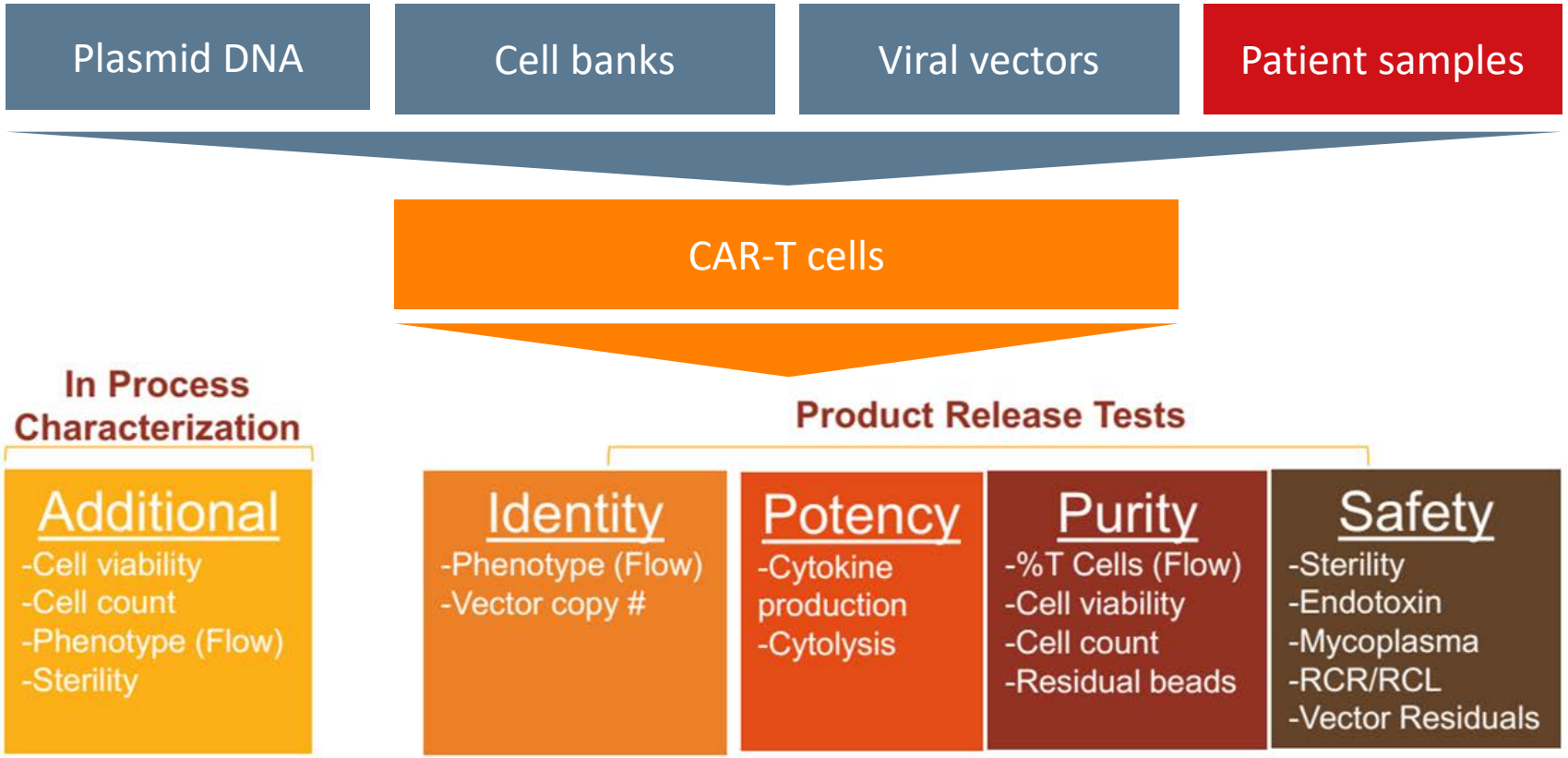
Blockage of soluble tumor suppressive mediators

Intratumoral / post surgical injection of CAR-T

Adapted from: E. Zhang, J. Gu, and H. Xu, "Prospects for chimeric antigen receptor-modified T cell therapy for solid tumors," Mol. Cancer, vol. 17, no. 1, pp. 1–12, 2018.

Elaboration of comprehensive QC for autologous CAR-T products

CAR-T biomedical cell-based product QC main blocks:



Limitations:

- Flow cytometry based assays are rather variable
- Duration of release tests are limited due to short period between manufacturing and patient treatment
- Regulatory requirements are not well defined yet

Conclusion

- CAR-T therapy is exceptionally efficient against hematologic malignances
- Treatment of solid tumors with CAR-T is now feasible
- CAR-T biomedical cell-based products have every chance of being approved for marketing in Russia
- Biomedical cell-based products could face the regulatory gaps since there is no such type of drugs on market yet

Acknowledgments

Ivanov R.A., PhD
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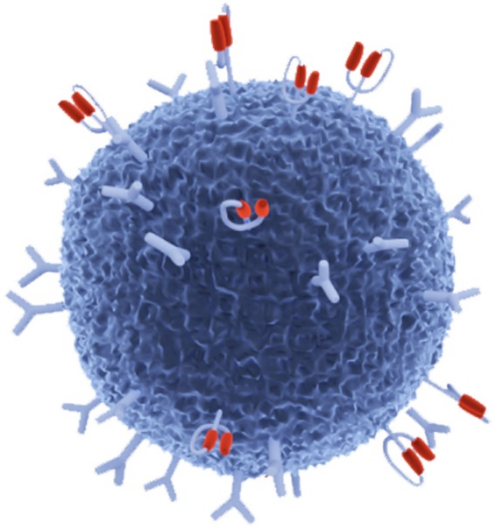
Zaikova E.K.



Thank you for your attention!