UniversitätsSpital Zürich

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CAR-T cells for the treatment of solid tumours

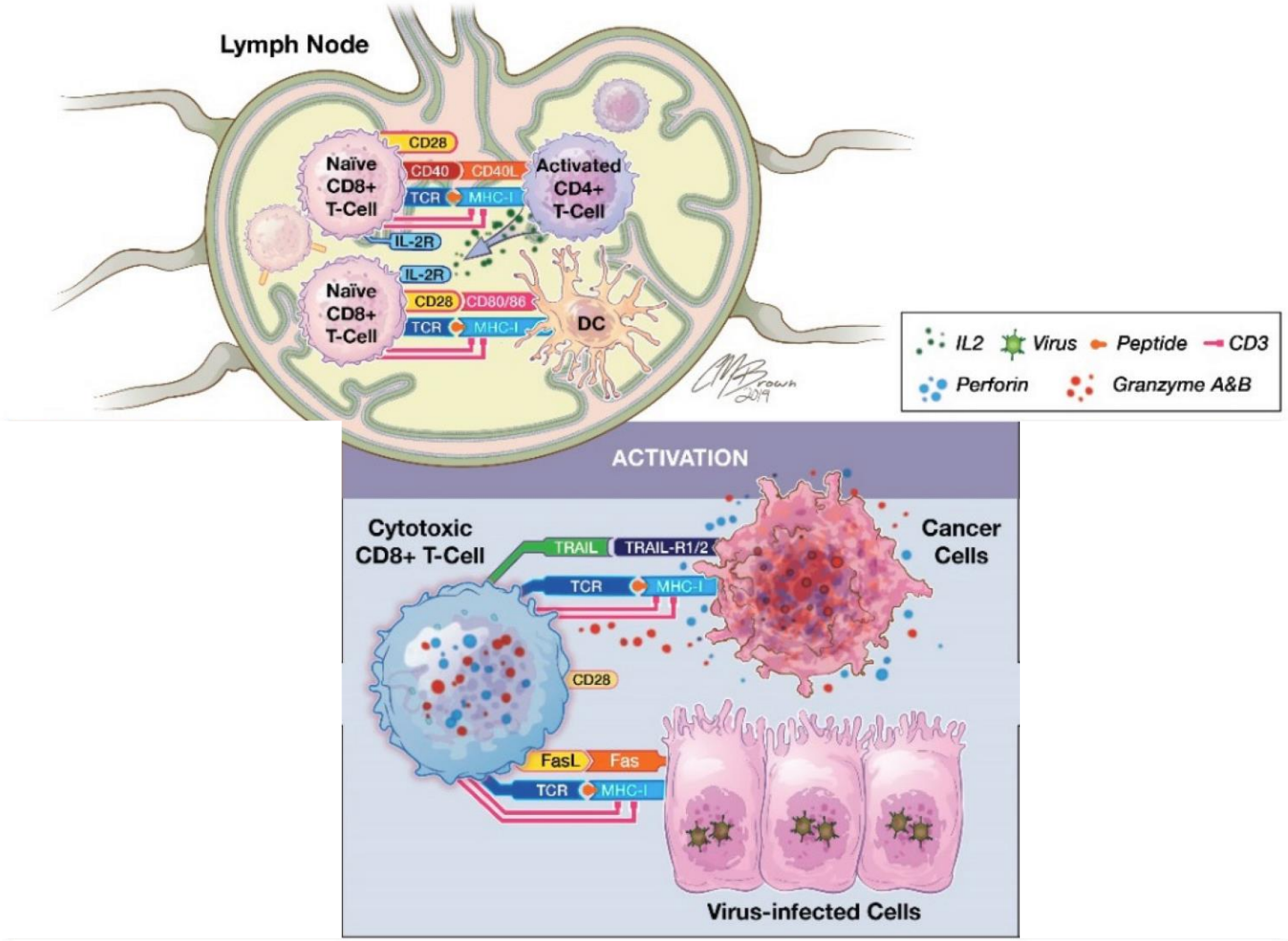
TJC

Marc Emmenegger

Group Prof. Dr. Adriano Aguzzi

15.12.2020

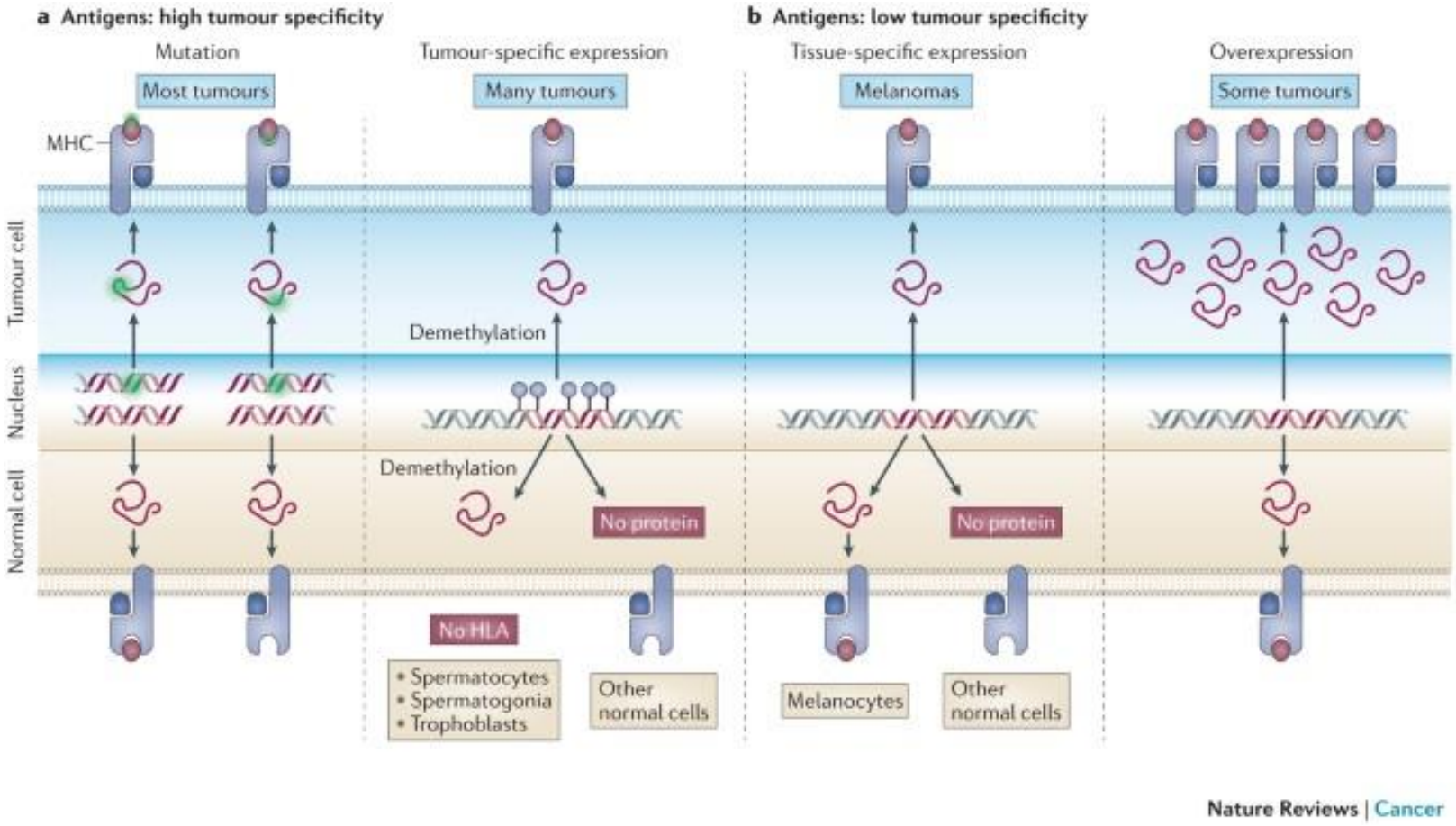
Tumours and the immune system



Huff et al. Int. J. of. Mol. Sci. 2019

Tumours and the immune system

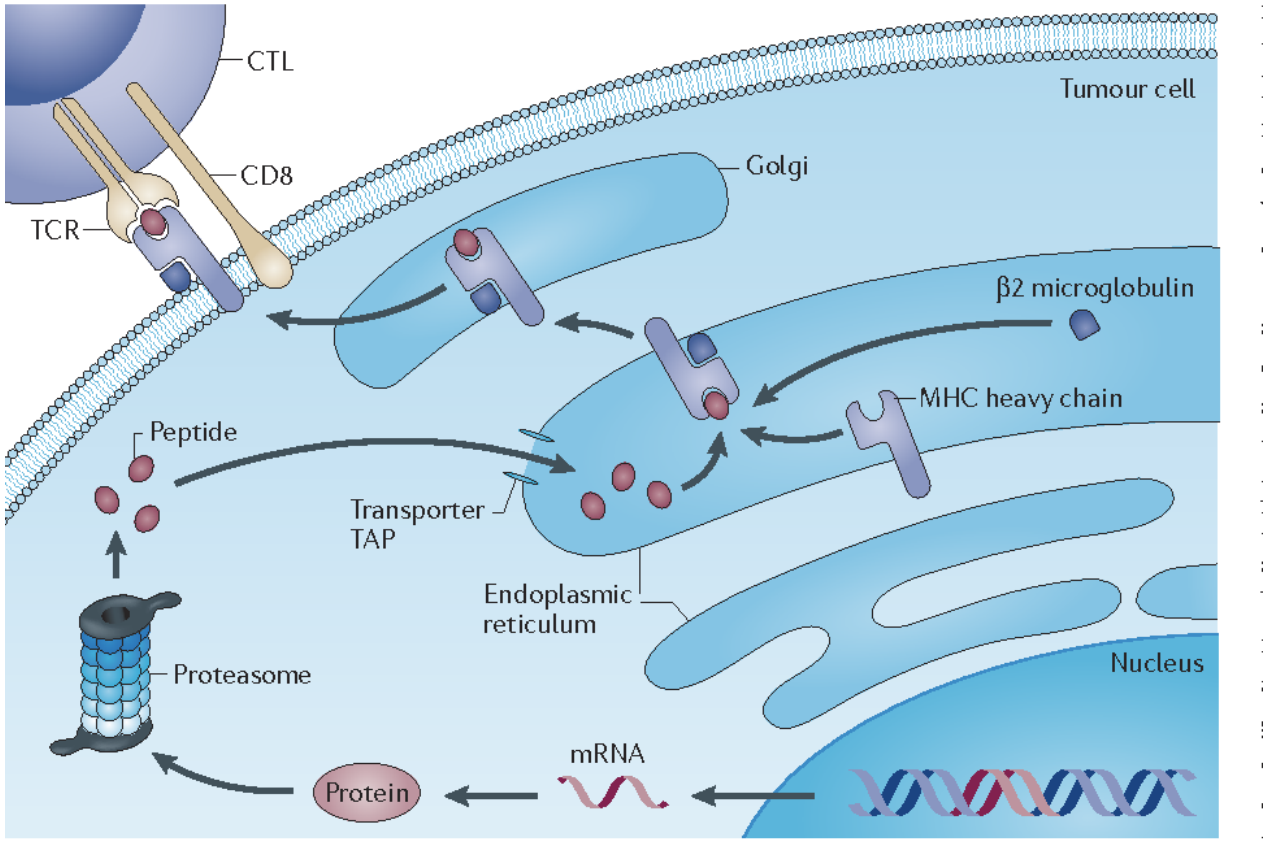
- Cancer cells present self-peptides on self-MHC, with exceptions.
  - Neoantigens through mutations
  - Neoantigens through tumour-specific expression
  - Tissue-specific expression
  - Overexpression (Gejman et al. eLIFE. 2018)



Coulie et al. Nat. Rev. Cancer. 2014.

Tumours and the immune system

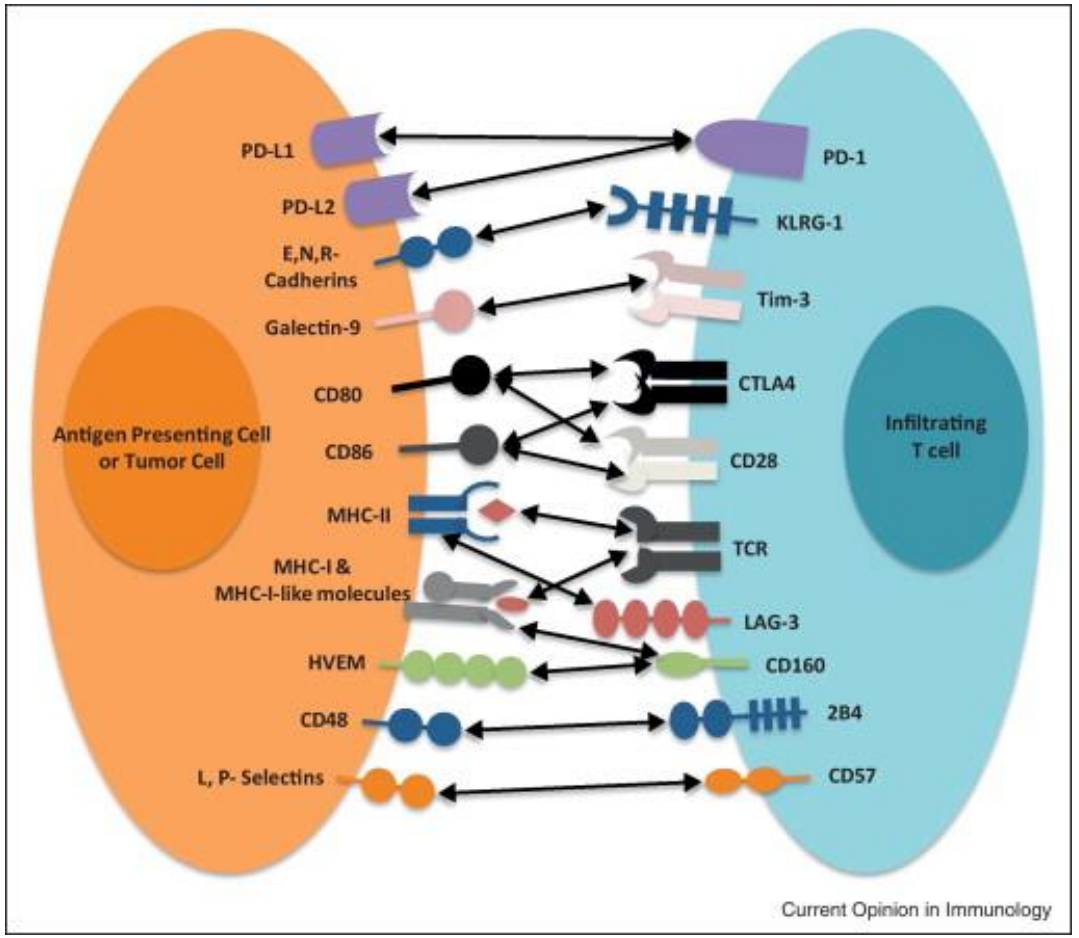
- Degradation of protein by proteasome.
- Peptides transported via TAP into ER.
- Loaded onto MHC and presented on surface.
- Interaction with CTL via
  - MHC – MHC/CD8
- CTLs releases granzymes and perforines to kill tumour cells.



Coulie et al. Nat. Rev. Cancer. 2014.

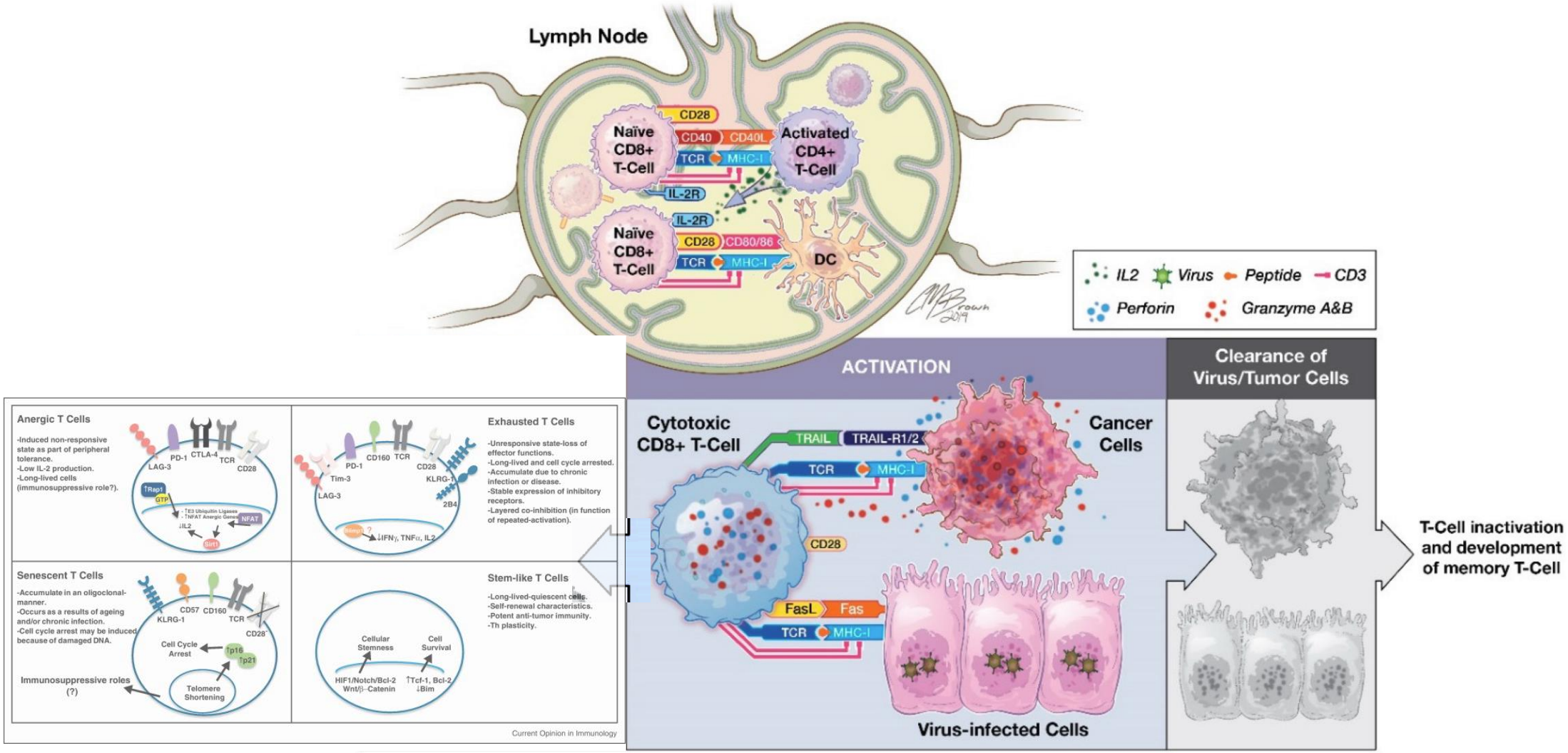
Tumours and the immune system

- Some tumours learn to express a multitude of co-**inhibitory/stimulatory** receptors.
- Normally expressed on APCs to regulate the immune response.



Crespo et al. Current Opinion in Immunology. 2013.

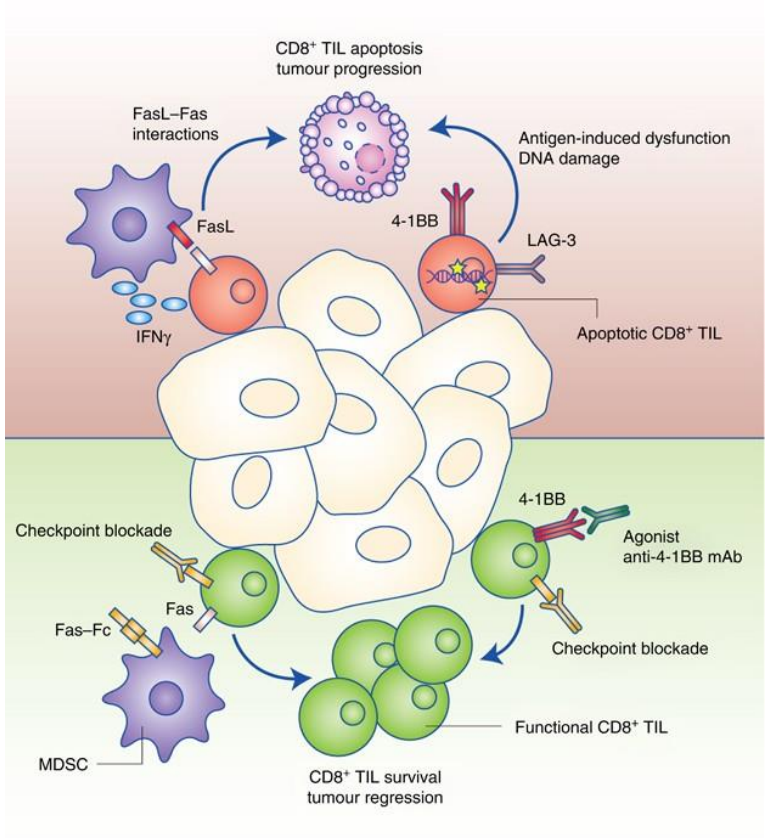
Tumours and the immune system



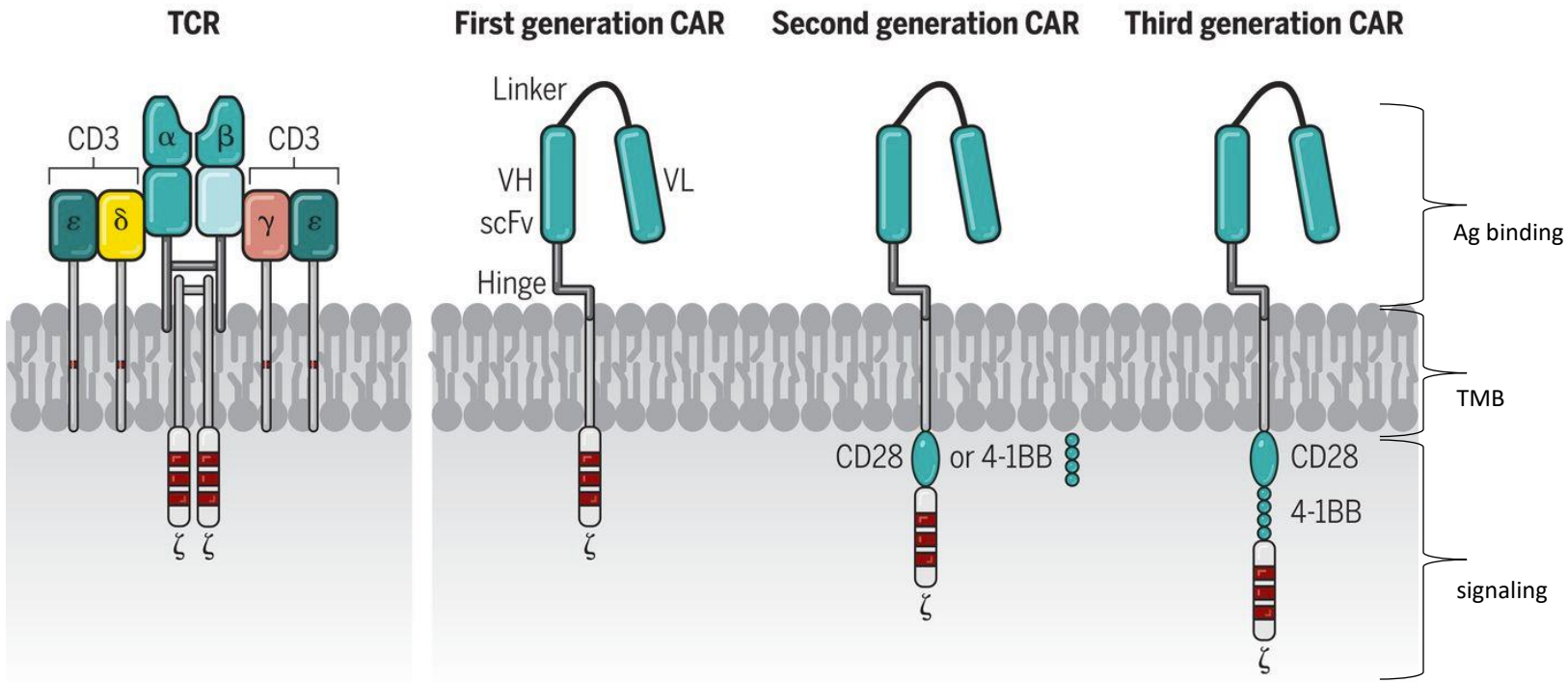
Huff et al. Int. J. of. Mol. Sci. 2019
Horton et al. BJC. 2018.
Crespo et al. Current Opinion in Immunology. 2013.

Re-establishing anti-tumour immunity

Immune checkpoint blockade



CAR-T cell therapy



→ MHC-independent recognition of antigen

Huff et al. Int. J. of. Mol. Sci. 2019
Horton et al. BJC. 2018.
Crespo et al. Current Opinion in Immunology. 2013.
June et al. Science. 2018.

FDA-approved therapy

ORIGINAL ARTICLE

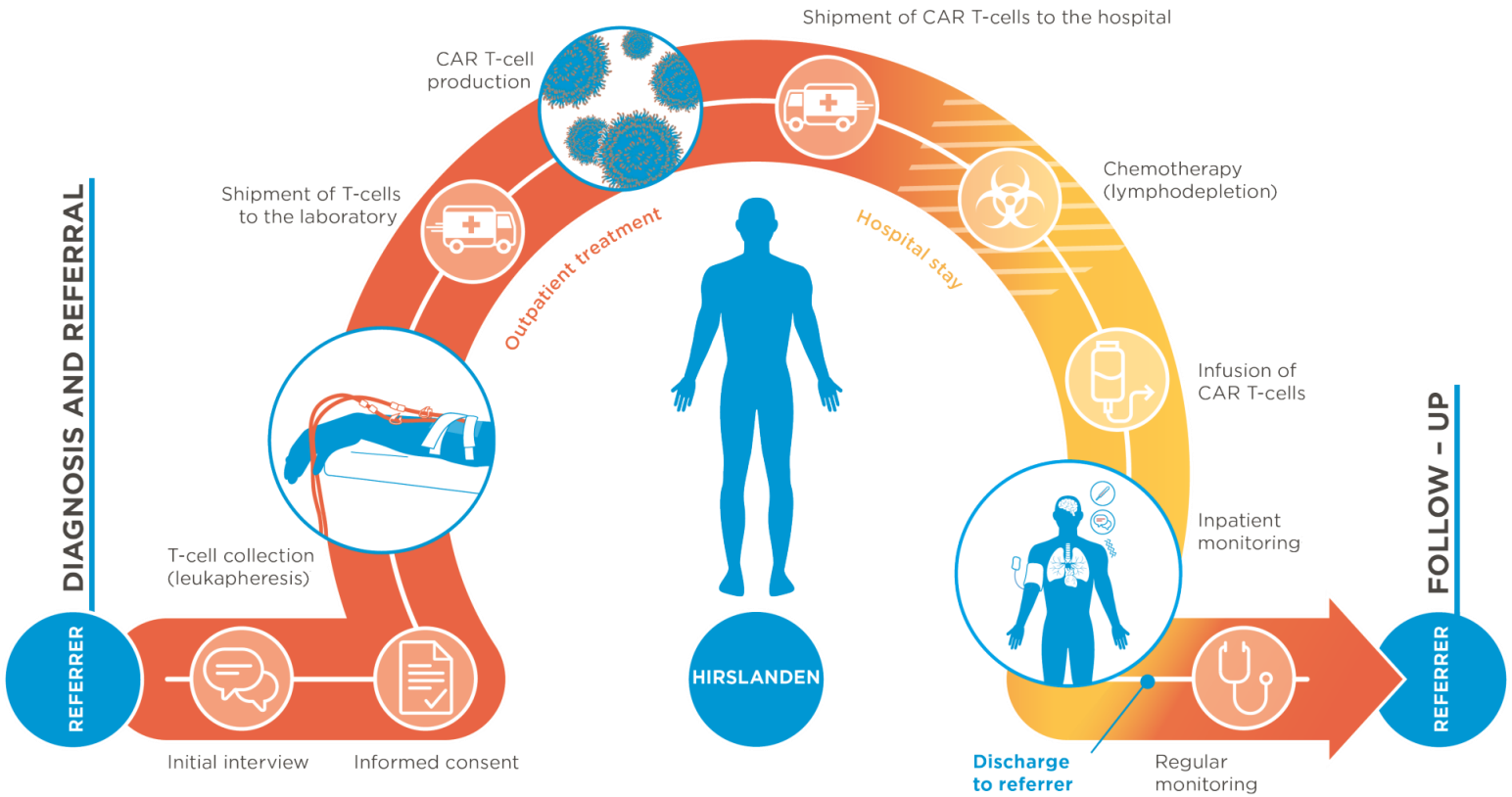
Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma

Sattva S. Neelapu, M.D., Frederick L. Locke, M.D., Nancy L. Bartlett, M.D., Lazaros J. Lekakis, M.D., David B. Miklos, M.D., Ph.D., Caron A. Jacobson, M.D., M.M.Sc., Ira Braunschweig, M.D., Olalekan O. Oluwole, M.B., B.S., M.P.H., Tanya Siddiqi, M.D., Yi Lin, M.D., Ph.D., John M. Timmerman, M.D., Patrick J. Stiff, M.D., et al.

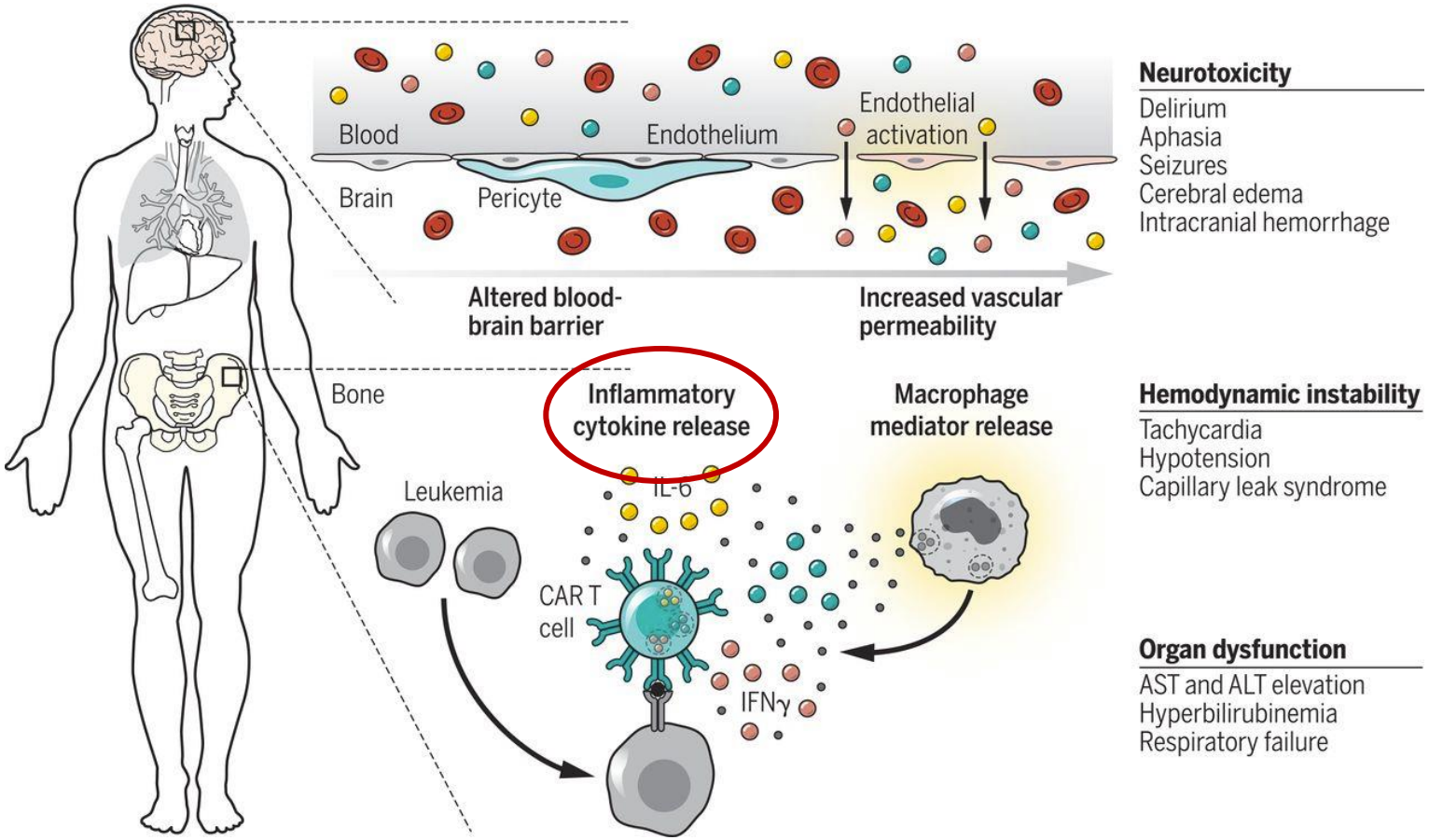
ORIGINAL ARTICLE

KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma

Michael Wang, M.D., Javier Munoz, M.D., Andre Goy, M.D., Frederick L. Locke, M.D., Caron A. Jacobson, M.D., Brian T. Hill, M.D., Ph.D., John M. Timmerman, M.D., Houston Holmes, M.D., Samantha Jaglowski, M.D., Ian W. Flinn, M.D., Ph.D., Peter A. McSweeney, M.D., David B. Miklos, M.D., et al.



Side effects



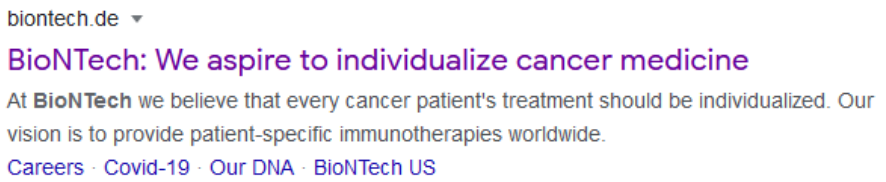
June et al. Science. 2018.

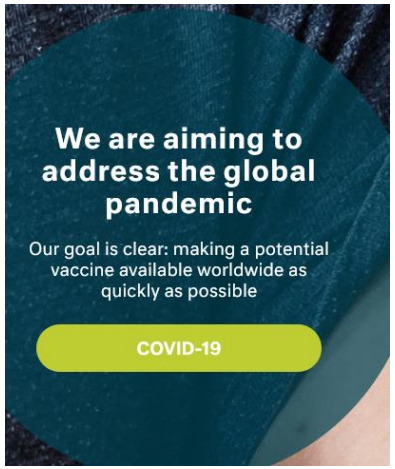
CANCER IMMUNOTHERAPY

An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors

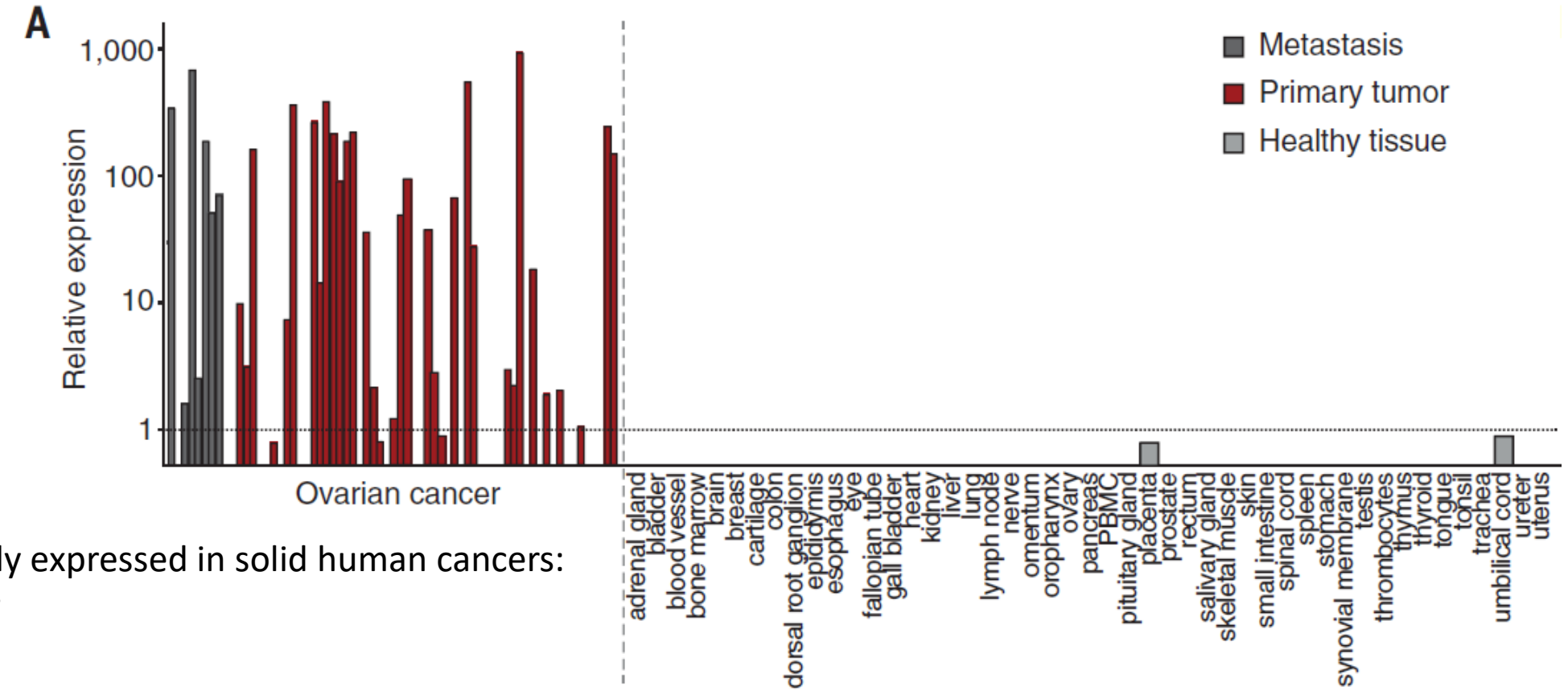
Katharina Reinhard[1]*, Benjamin Rengstl[1]*, Petra Oehm[1]*, Kristina Michel[1], Arne Billmeier[1], Nina Hayduk[1], Oliver Klein[1], Kathrin Kuna[1], Yasmina Ouchan[1], Stefan Wöll[1], Elmar Christ[1], David Weber[2], Martin Suchan[2], Thomas Bukur[2], Matthias Birtel[1], Veronika Jahndel[1], Karolina Mroz[1], Kathleen Hobohm[1], Lena Kranz[1], Mustafa Diken[2], Klaus Kühlcke[1], Özlem Türeci[1]†, Ugur Sahin[1,2,3]†‡

- CAR-T cell therapy for **solid tumours** challenging and much less effective.
- One key hurdle: limited number of cancer-specific cell-surface targets – to limit off-tumour on-target toxicity.



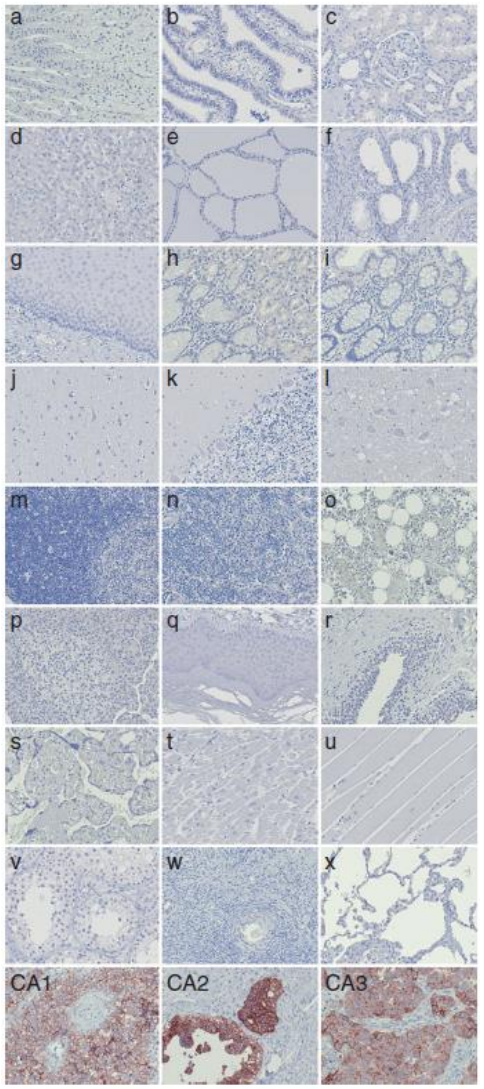


No significant expression of claudin 6 (CLDN6) in more than 160 non-cancerous human samples from more than 50 adult tissues



Frequently expressed in solid human cancers:
Testicular
Ovarian
Uterine
Lung adenocarcinoma

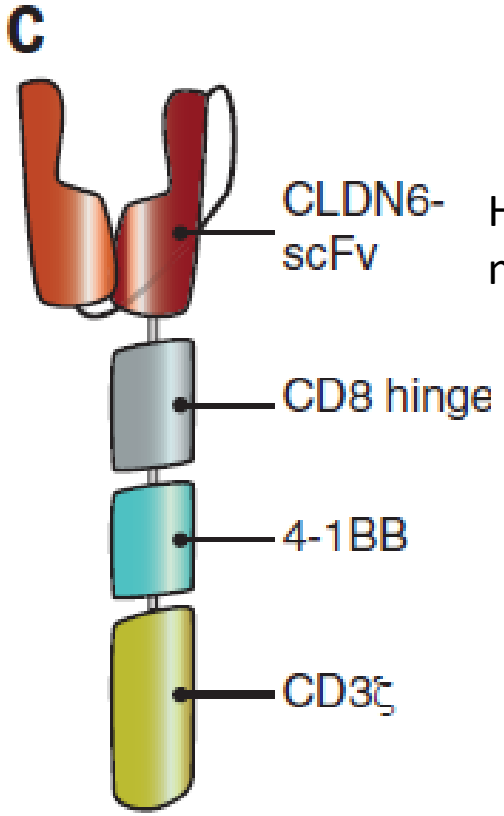
IHC analysis of Claudin 6



Human tissues

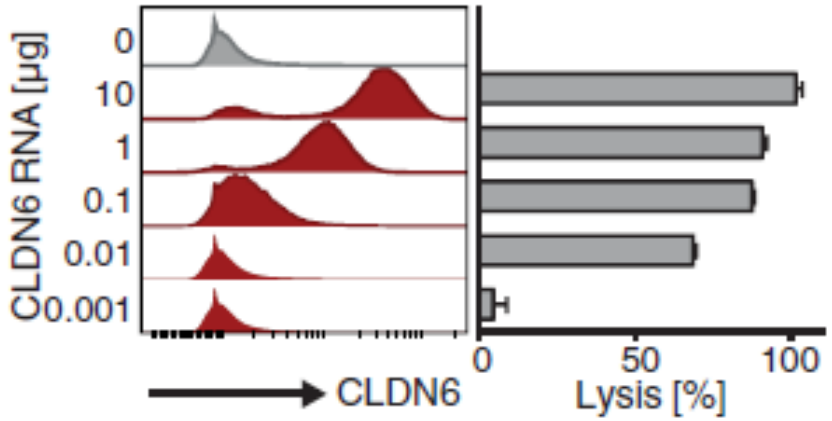
Cancer

Design of second generation CLDN6-CAR with 4-1BB costimulatory domain



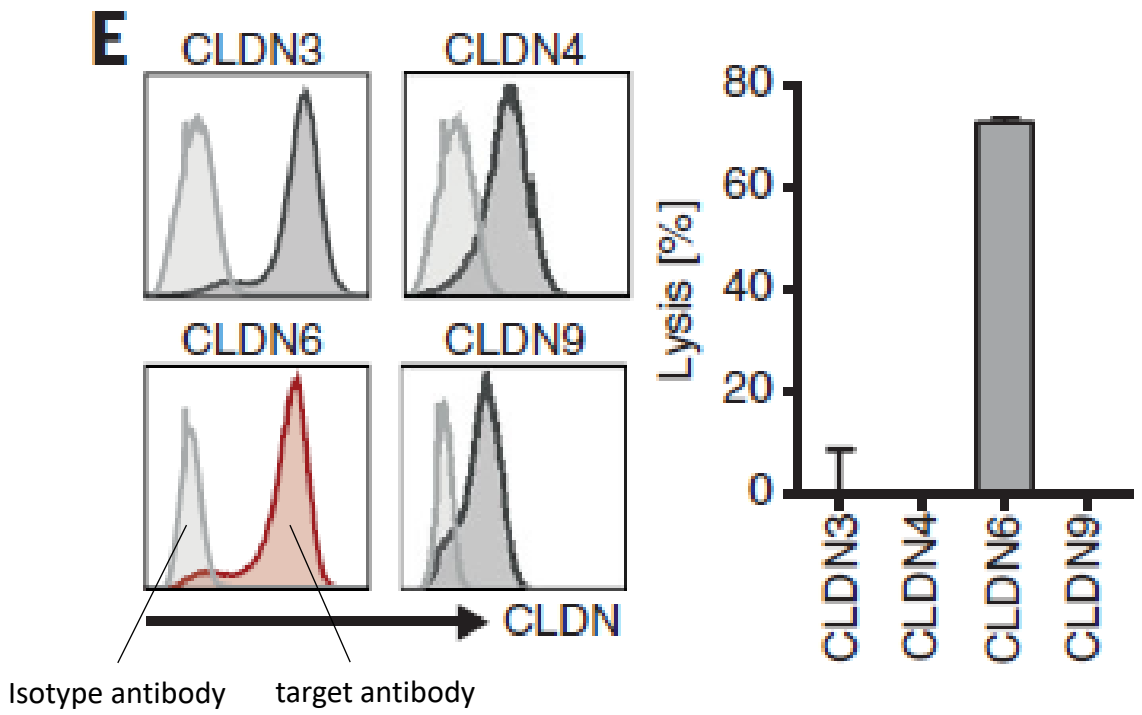
High-binding affinity in nanomolar range

CLDN6neg human COLO-699N lung carcinoma cells were transfected with increasing amounts of CLDN6 RNA and assessed for killing by CAR-T cells: High lysis even at low expression levels.



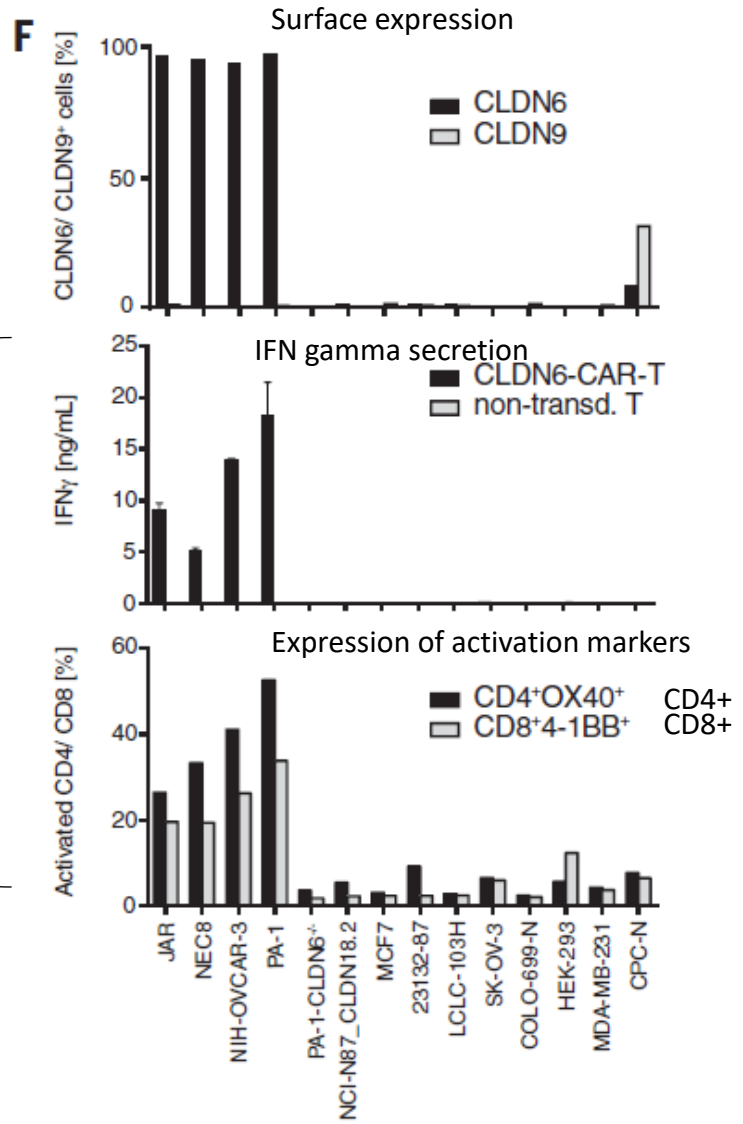
Assessing the specificity of the CLD6-CAR

- CLDN3, 4, 9: expressed in ‘normal’ tissue.
- Homology between first extracellular loop (target) and CLDN3, 4, 9:
  - 81%
  - 85%
  - 98%
  - **Cross-reactivity can be a risk!**
- However, only CLDN6 transfected cells were killed: **precise targeting by CLDN6-CAR-T cells**.



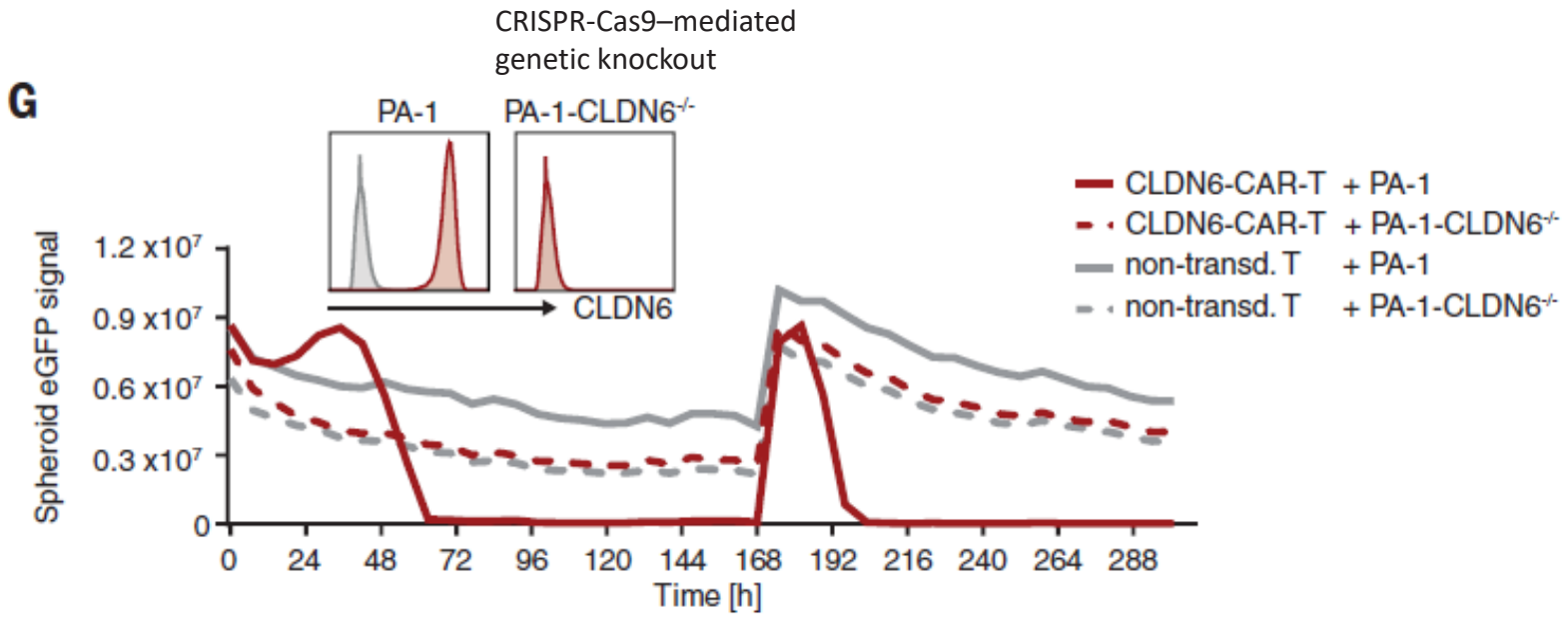
Measuring immune activation

Co-culture with CLDN6-CAR or nontransduced T cells



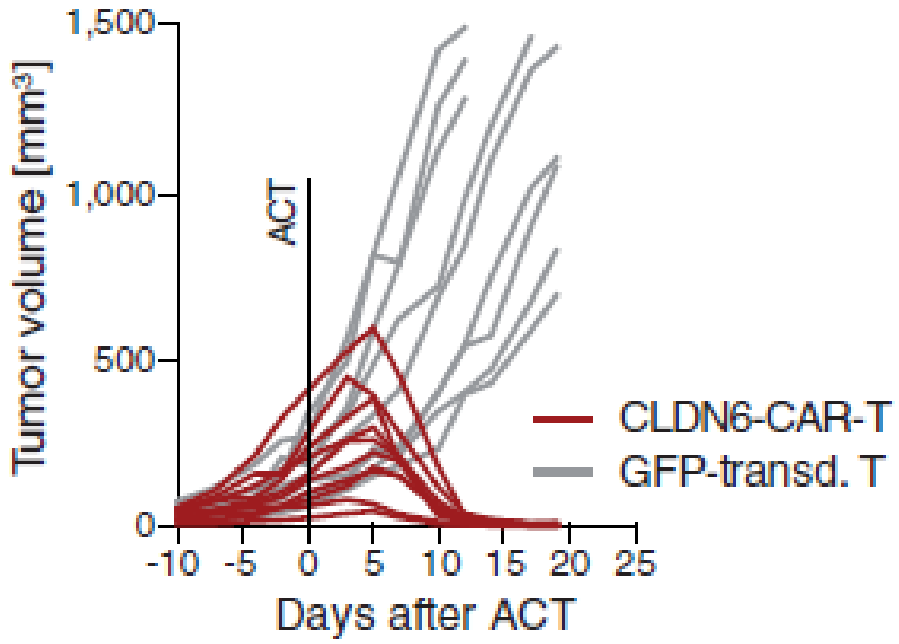
IFN gamma secretion and upregulation of activation markers upon coculture with CLDN6+ but not with CLDN6- targets

Killing of ovarian carcinoma spheroids



- CRISPR-Cas9–mediated genetic knockout completely abrogated CAR-T cell recognition of PA-1 spheroids, confirming target specificity.
- Effective clearance of CLDN6-pos spheroids.

In vivo antitumour activity of human CLDN6-CAR-T cells in mice xenografted subcutaneously with human tumour cell lines



- Mouse is not suitable species in general as binding affinity to mouse CLDN6 ortholog is 15x lower.
- Mouse expresses CLDN6 in some postembryonic somatic tissues.
- Still, all mice undergoing adaptive cell therapy with single CLDN6-CAR-T cell dose experienced complete tumour regression, compared with rapid tumour progression in controls.

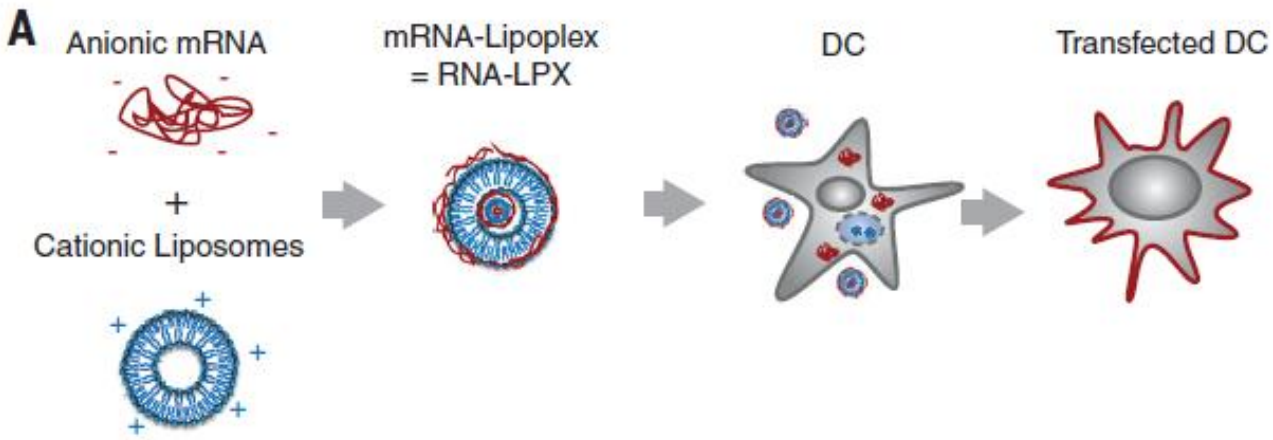
➡ Powerful system to target solid tumours.

Limitations for solid tumours

- Engraftment **and persistence** of transferred CAR-T cells are known to be critical for their clinical effect.
- In hematological malignancies, CAR-T cells are directed against lineage antigens of B cells (usually CD19) and encounter their targets on the host's normal and malignant B cells.
- **These act as antigen-presenting cells that provide strong proliferation signals and promote persistence of CAR-T cells**.
- However, in the solid-tumor setting, the frequency of CAR-T cells typically declines rapidly.
  - impaired accessibility of CAR-T cells to tumor cells within solid lesions.
  - absence of proliferation signals when CAR-T cells encounter the target in an immunosuppressive tumour microenvironment.

Surface expression of CLDN6 on APCs

- Usage of intravenously administered liposomal antigen-encoding RNA (RNA-LPX).
- This nanoparticulate vaccine delivers antigen to APCs in the spleen, lymph nodes, and bone marrow.
- CAR-T cell–amplifying RNA vaccine (**CARVac**).



Published: 01 June 2016

Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy

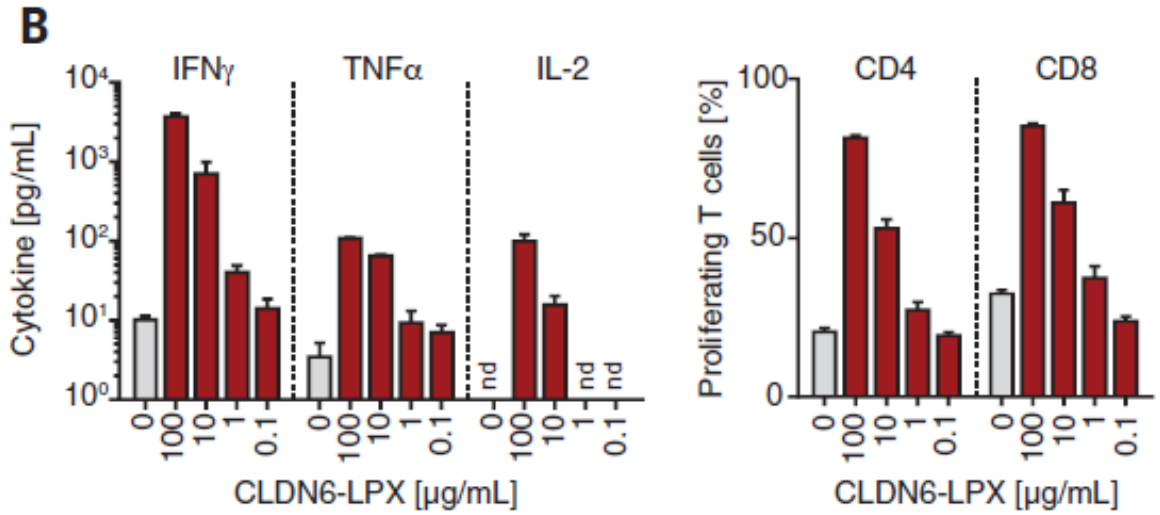
Lena M. Kranz, Mustafa Diken, Heinrich Haas, Sebastian Kreiter, Carmen Loquai, Kerstin C. Reuter, Martin Meng, Daniel Fritz, Fulvia Vascotto, Hossam Hefesha, Christian Grunwitz, Mathias Vormehr, Yves Hüsemann, Abderraouf Selmi, Andreas N. Kuhn, Janina Buck, Evelyna Derhovanessian, Richard Rae, Sebastian Attig, Jan Diekmann, Robert A. Jabulowsky, Sandra Heesch, Jessica Hassel, Peter Langguth, Stephan Grabbe, Christoph Huber, Özlem Türeci & Ugur Sahin -Show fewer authors

Nature **534**, 396–401(2016) | Cite this article

- Systemic delivery of vaccine antigens into dendritic cells (DCs) is hampered by various technical challenges.
- Here we show that DCs can be targeted precisely and effectively *in vivo* using intravenously administered RNA-lipoplexes (RNA-LPX).
- The LPX protects RNA from extracellular ribonucleases and mediates its efficient uptake and expression of the encoded antigen by DC populations and macrophages in various lymphoid compartments.

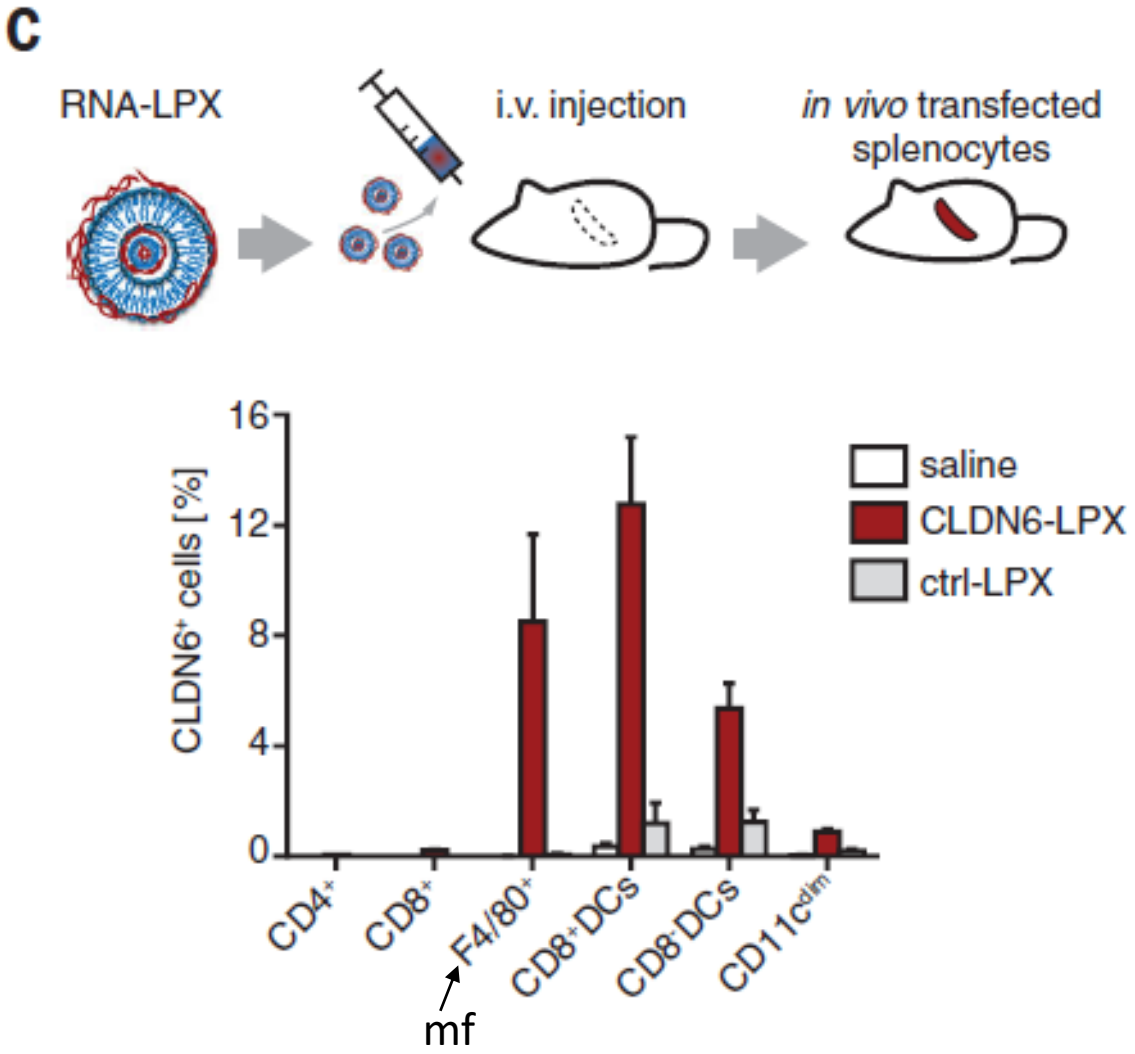
DC expressing CLDN6 induces stimulation and proliferation

- Expression of CLDN6 on DCs induced stimulation and cytokine secretion.
- And induced proliferation of co-cultured CLDN6-CAR-T cells in dose-dependent manner.



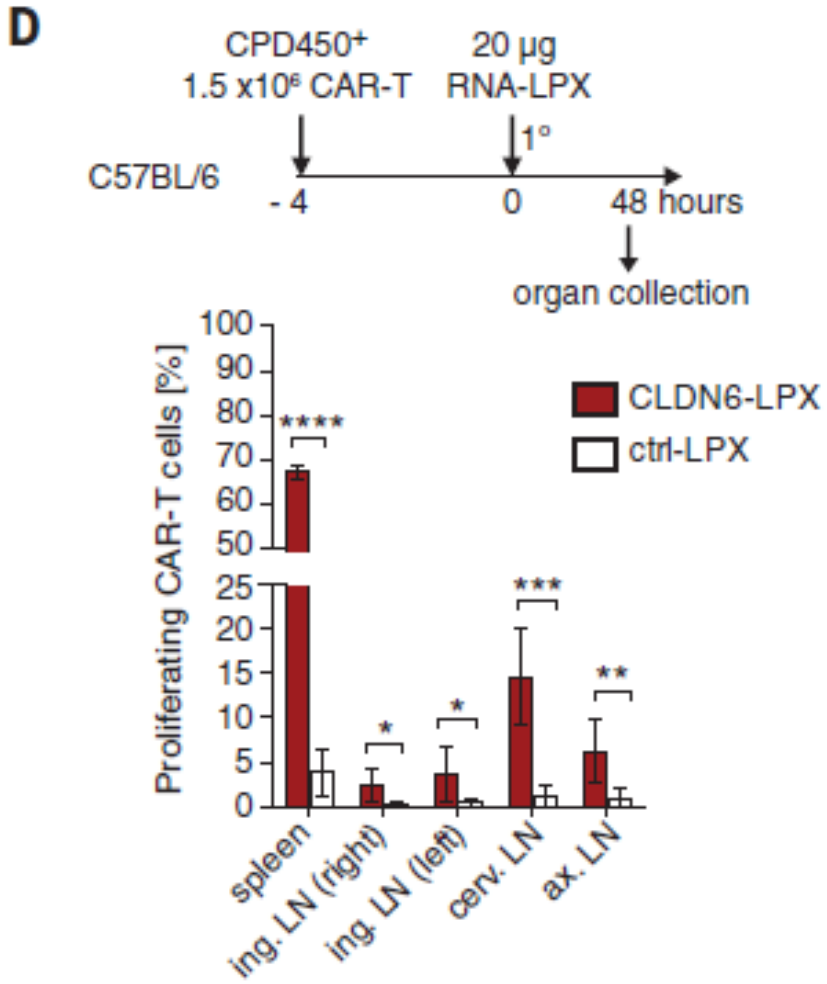
Surface expression of CLDN6 on splenic immune cell populations of BALB/c mice

- BALB/c mice injected with i.v. with CLDN6-LPX.
- CLDN6 surface expression on splenic DCs and mf but not on lymphocytes.



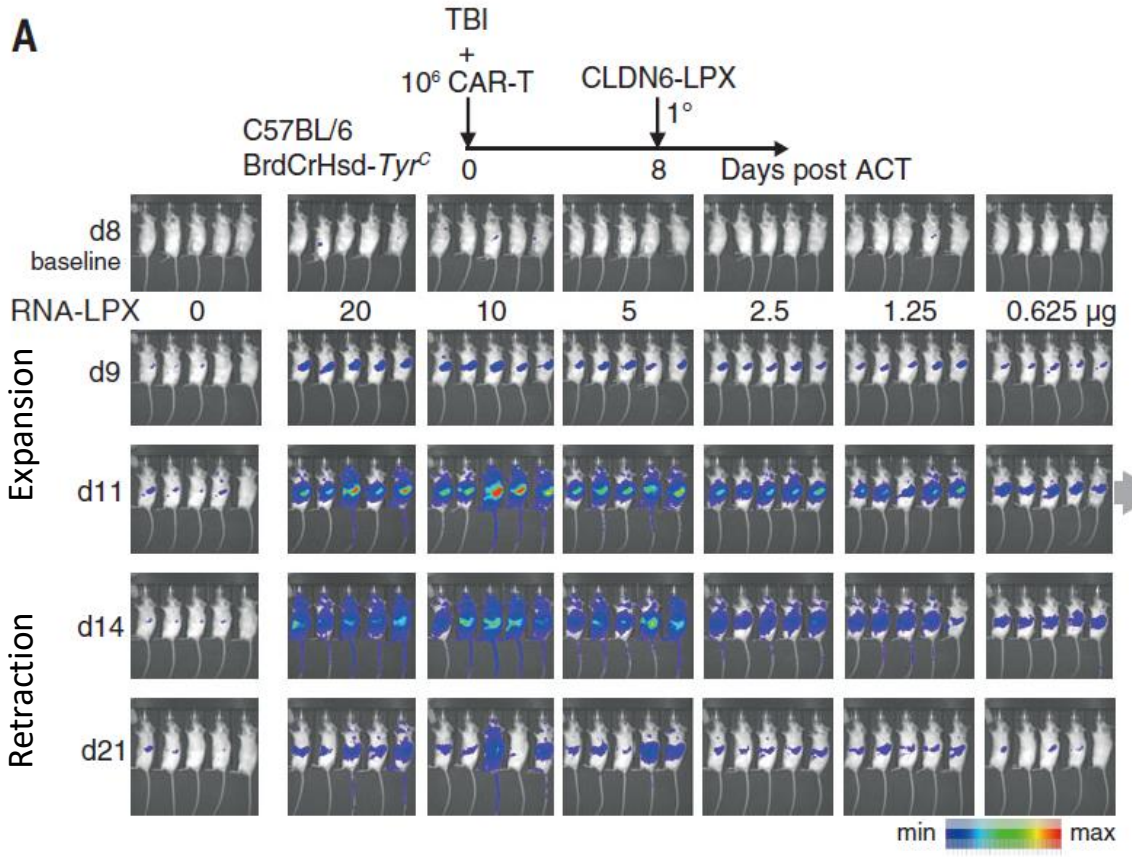
Body-wide functional expression of CAR antigen within lymphoid compartments

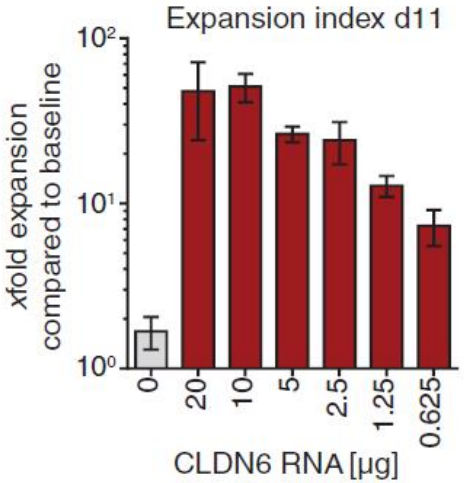
- Next, naïve C57BL/6 mice were engrafted with CLDN6-CAR-T cells labeled with a cell proliferation dye and vaccinated with CLDN6- or control-LPX.
- Spleen and lymph nodes from all major body regions resected from CLDN6- LPX–vaccinated, but not control-treated mice, displayed significantly increased proportions of proliferating CLDN6-CAR-T cells.



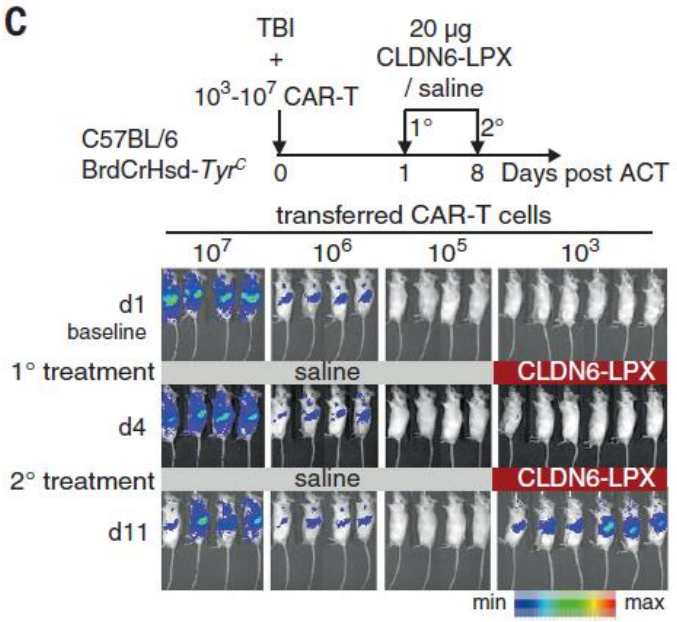
CLDN6 expression leads to massive expansion of CLDN6-CAR-T cells

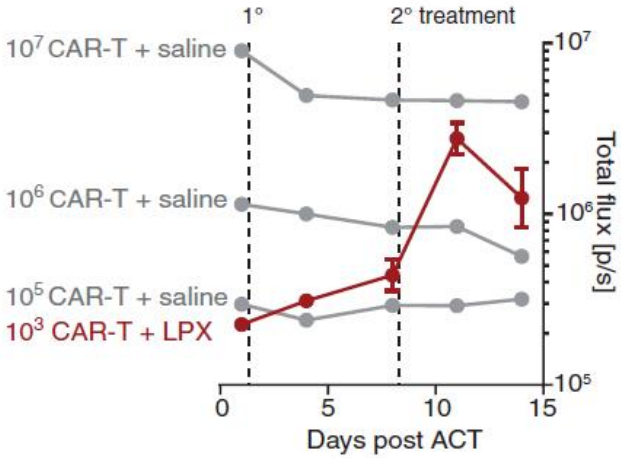
- Thy1.2+ mice underwent total body irradiation → lymphodepletion.
- Engraftment of congenic Thy 1.1+ CLDN6-CAR-T cells coexpressing LUC and GFP.
- Vaccination with CLDN6-LPX.
- Substantial expansion of circulating CLDN-6-CAR T cells even at lowest dose.
- Expansion followed by retraction phase.

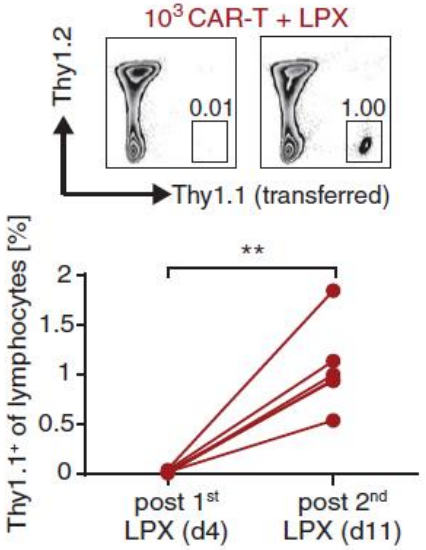




CARVac leads to CAR-T cell expansion irrespective of starting dose

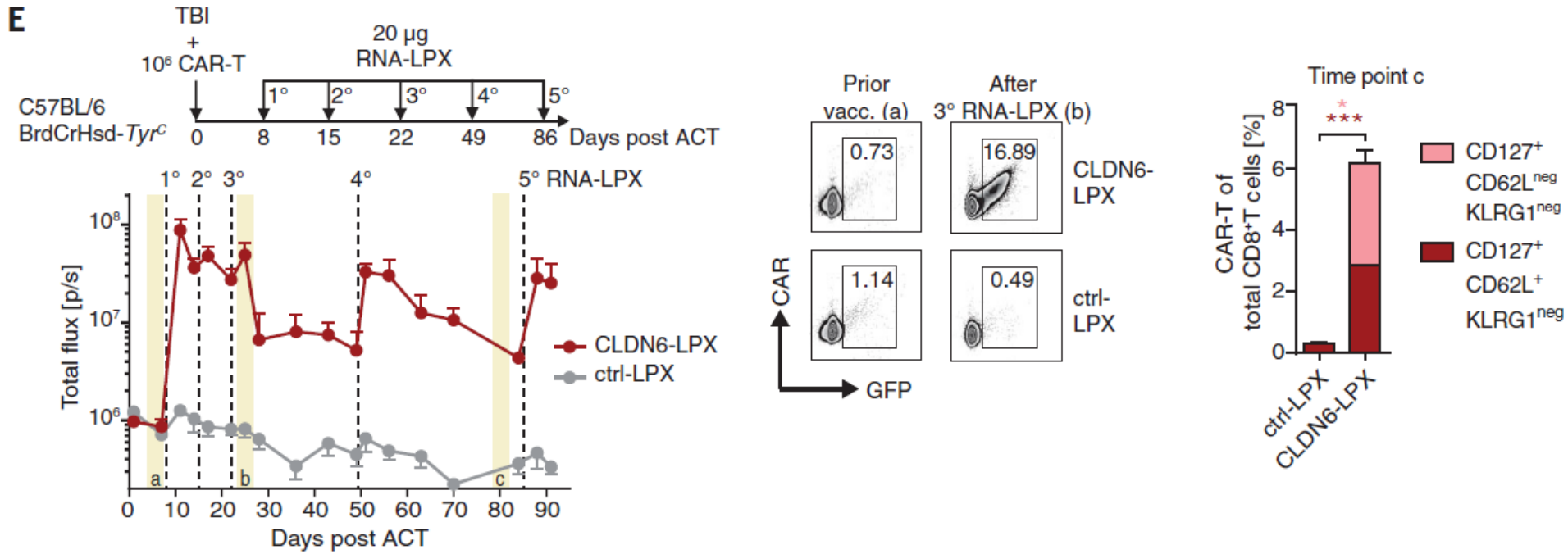






- TBI, different dose of CAR-T cells, untreated or vaccine.
- Non-vaccinated: Linear to number of engrafted cells, stable or slight decline.
- With CARVac, CAR-T cells expanded irrespective of CAR-T starting dose.

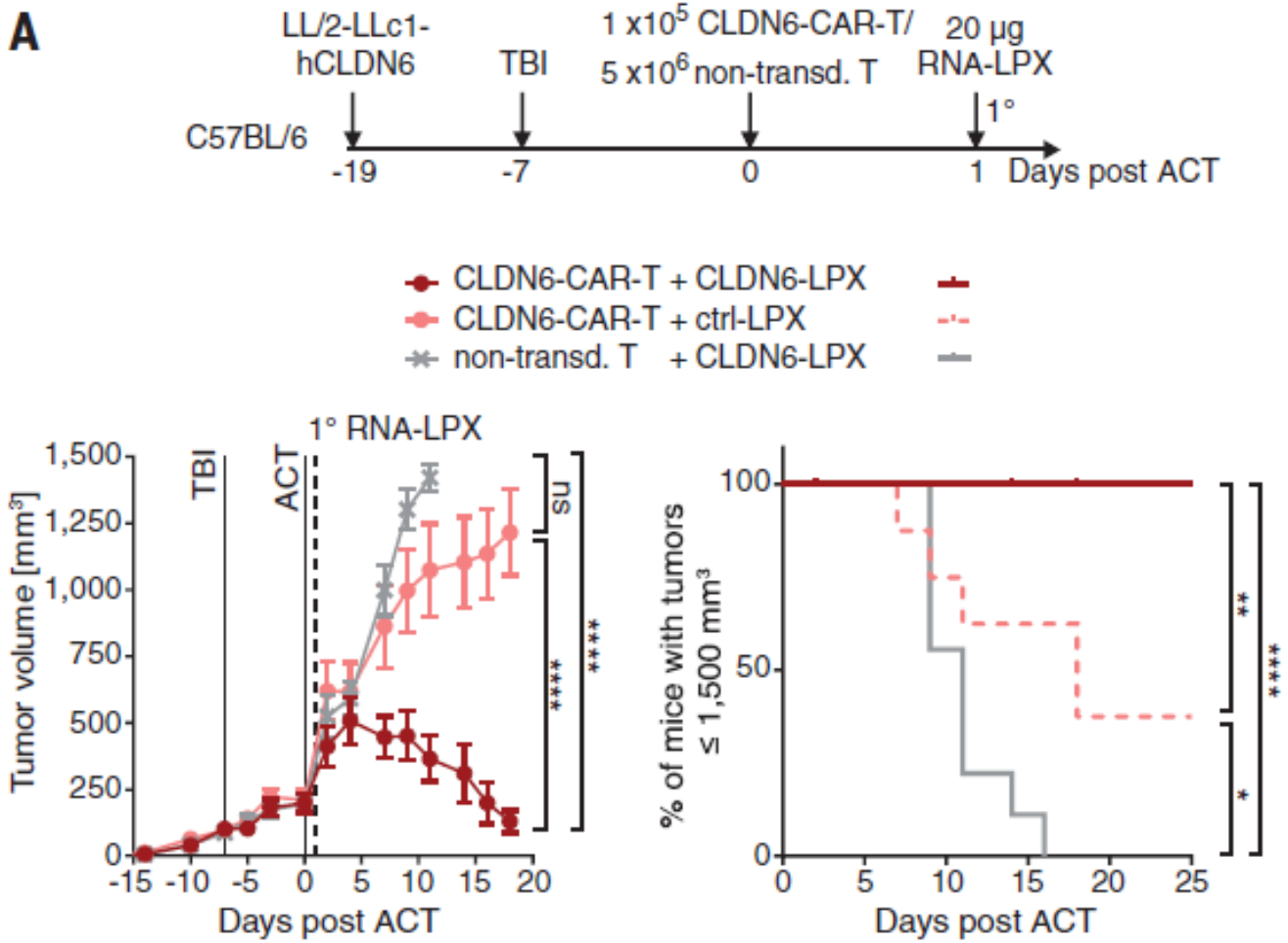
Impact of repetitive RNA-LPX vaccination on long-term persistence of CAR-T cells



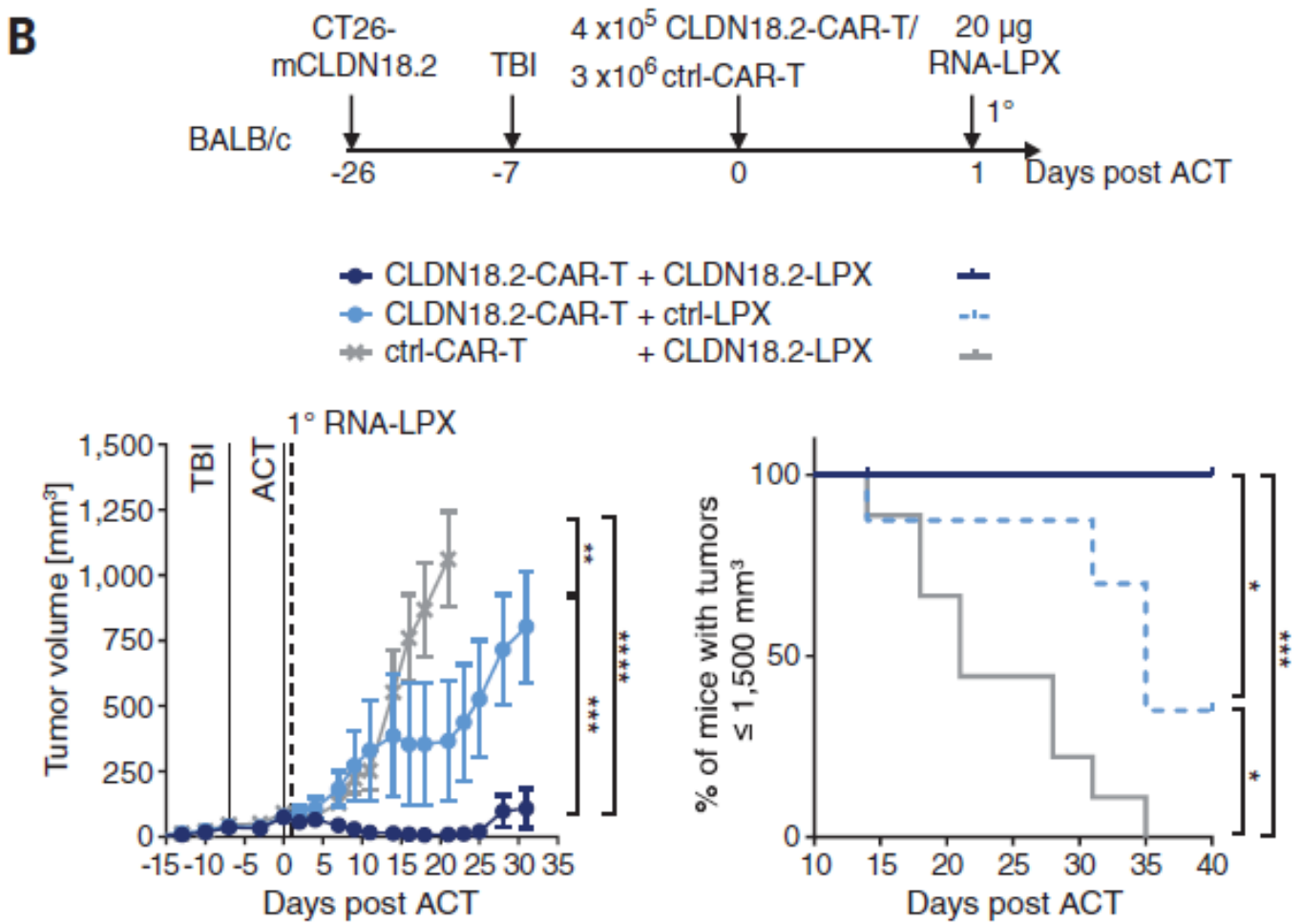
- TBI, CAR-T engraftment, weekly/monthly dose of RNA-LPX.
- 1st dose: rapid amplification of CAR-T cells by more than two orders of magnitude.
- Maintenance at high level.
- Re-expansion after treatment indicative of memory function:
  - Effector memory: CD127+,CD62Lneg, KLRG1neg
  - Central memory" CD127+, CD62L+, KLRG1neg

Impact of RNA-LPX on therapeutic efficacy of CLDN6-CAR-T cells in tumour-bearing mice

- Lewis lung tumour (209 mm^3) with high CLDN6 expression , lymphodepletion, CAR-T cell engraftment, single-dose vaccination.
- CAR-T cells alone only suboptimal reduction of tumour volume, tumour growth delayed.
- 6 of 10 mice receiving CAR-T cells together with CLDN6-LPX vaccination showed complete rejection of large tumors, with a significantly higher median survival.

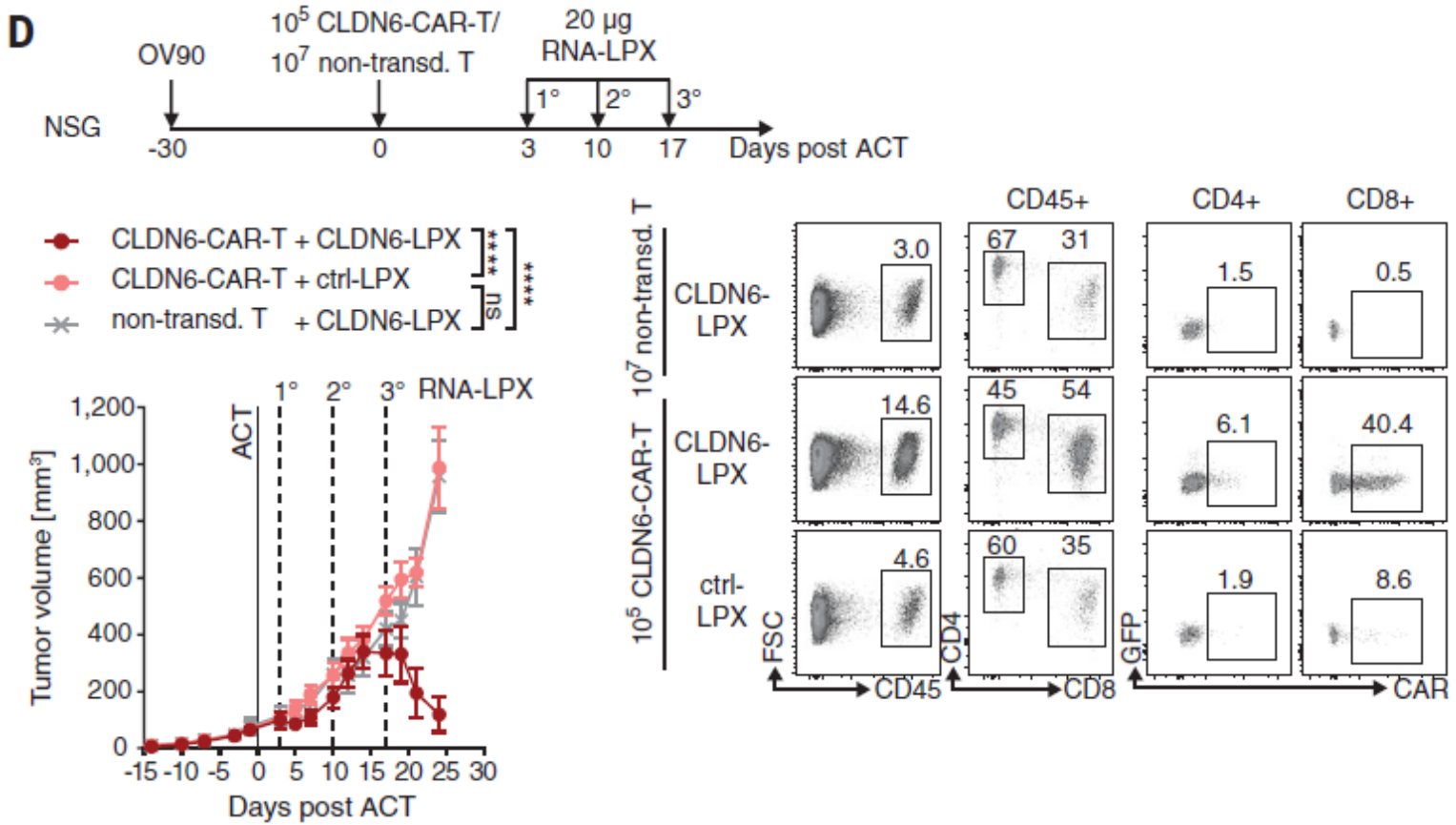


Repetition of same experiment with BALB/c mice with colon carcinoma



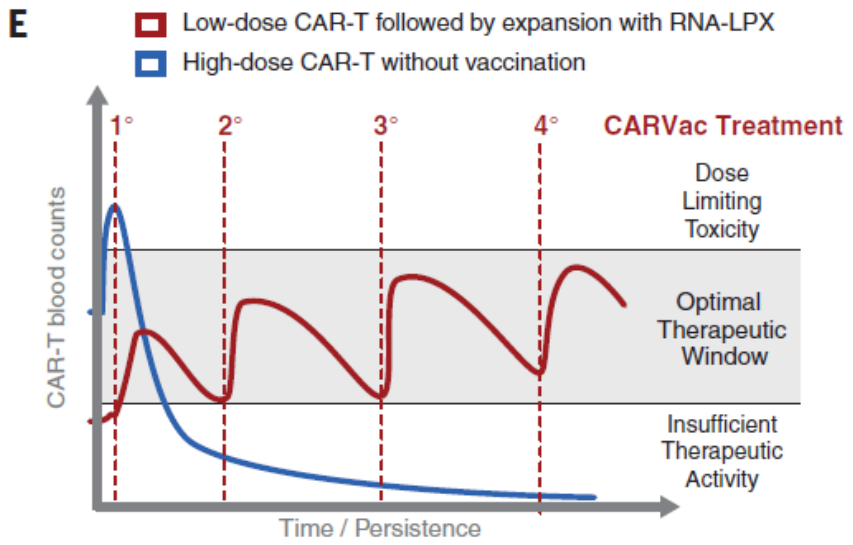
Using **human CLDN6-CAR-T cells** in NOD scid gamma mouse model

- NSG mice bearing advanced tumours.
- CAR-T engraftment (subtherapeutic dose).
- RNA-LPX CARVac.



Conclusions

- First key finding: CLDN6 is oncofetal cell-surface antigen that appears suitable for CAR-T cell targeting.
- Second key finding: CARVac strategy as an approach to improve antitumour efficacy of CAR-T cells.
- CARVac is a universally applicable approach.
- Therapeutic tumour control at lower CAR-T cell dose.



CANCER

Effective combination immunotherapy using oncolytic viruses to deliver CAR targets to solid tumors

Anthony K. Park[1,2,3], Yuman Fong[3]*, Sang-In Kim[3], Jason Yang[1], John P. Murad[1,2], Jianming Lu[3], Brook Jeang[1], Wen-Chung Chang[1], Nanhai G. Chen[3], Sandra H. Thomas[4], Stephen J. Forman[1,5]*, Saul J. Priceman[1,5]*†

- Effective CAR-engineered therapy for solid tumours limited by lack of 1) tumour-restricted, 2) homogenously expressed tumour antigens.
- Considerable safety concerns.
- CD19 has been ideal target for CAR-T cells against haematological malignanices:
  - Highly restricted expression on B cells
  - Acceptable off-tumour and on-target properties.
- FDA approval for patients with B cell malignancies.
- New approaches to introduce validated targets to tumour cells could broaden applicability of CAR-T cell therapy.

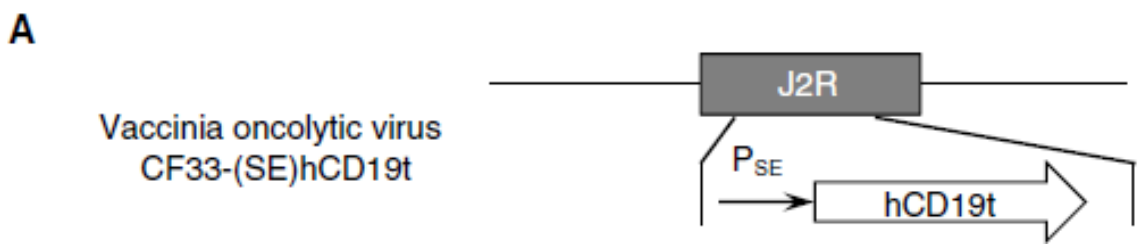
Oncolytic viruses

Published: 01 September 2015

Oncolytic viruses: a new class of immunotherapy drugs

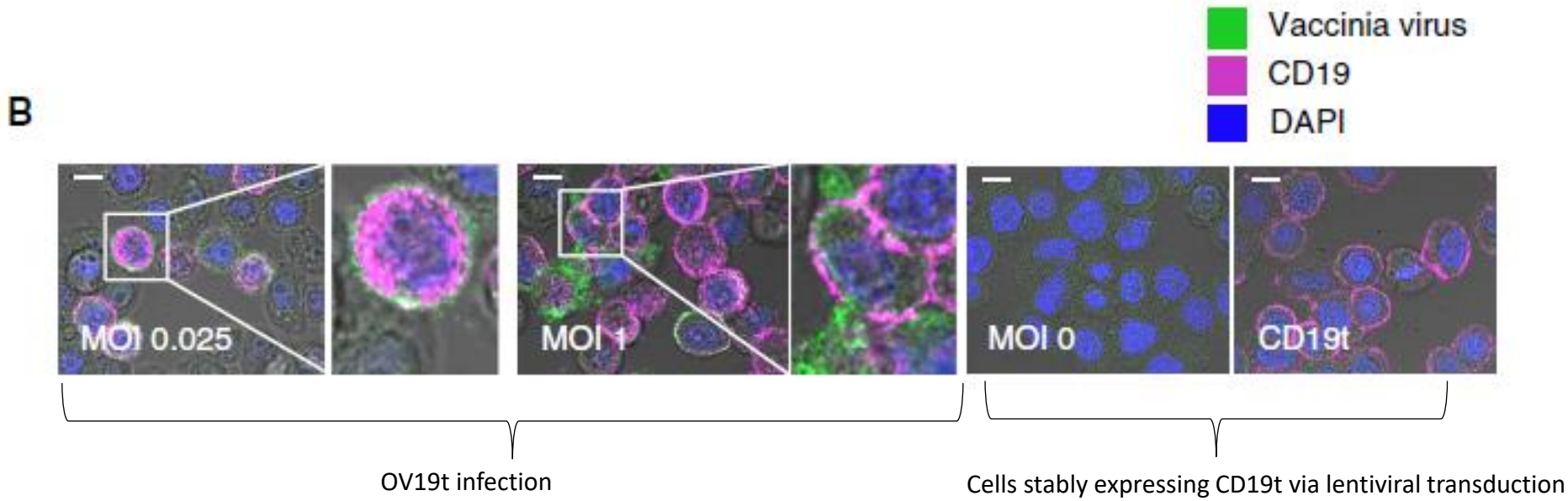
Howard L. Kaufman, Frederick J. Kohlhapp & Andrew Zloza

Nature Reviews Drug Discovery **14**, 642–662(2015) | Cite this article



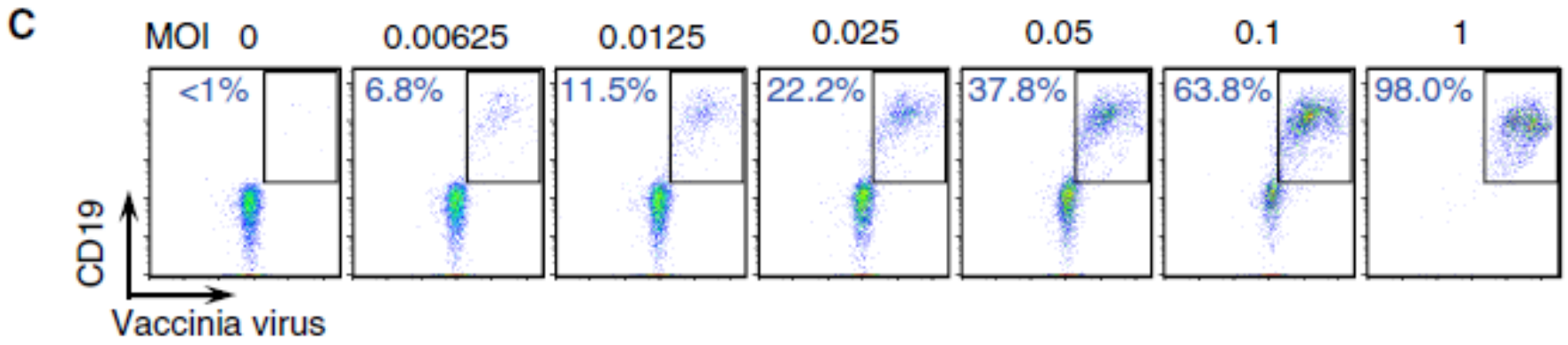
- Oncolytic viruses display tumour cell selectivity and desirable immunogenic properties.
- Targeted transgene delivery to tumour cells.
- Talimogene laherparepvec (TVEC) genetically modified type I herpes simplex virus that expresses granulocyte-macrophage colony-stimulating factor is first clinically approved oncolytic virus.
- Here, transgene delivery of truncated nonsignaling variant of CD19 (CD19t) packaged into vaccinia oncolytic virus.

Immunofluorescence microscopy of MDA-MB-468 cells infected for 24 hours with OV19t at multiplicity of infection (MOI)



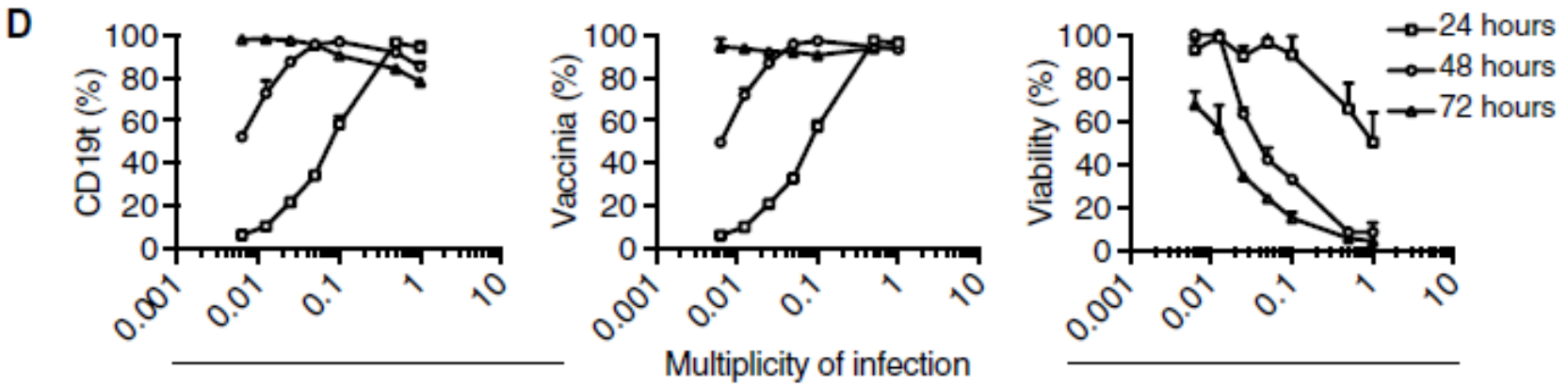
- Ability of OV to infect and generate CD19t at cell surface of tumour cells, infect human triple-negative breast cancer line with OV19t for 16 hours at varying MOI.

Efficiency of CD19t delivery is MOI-dependent



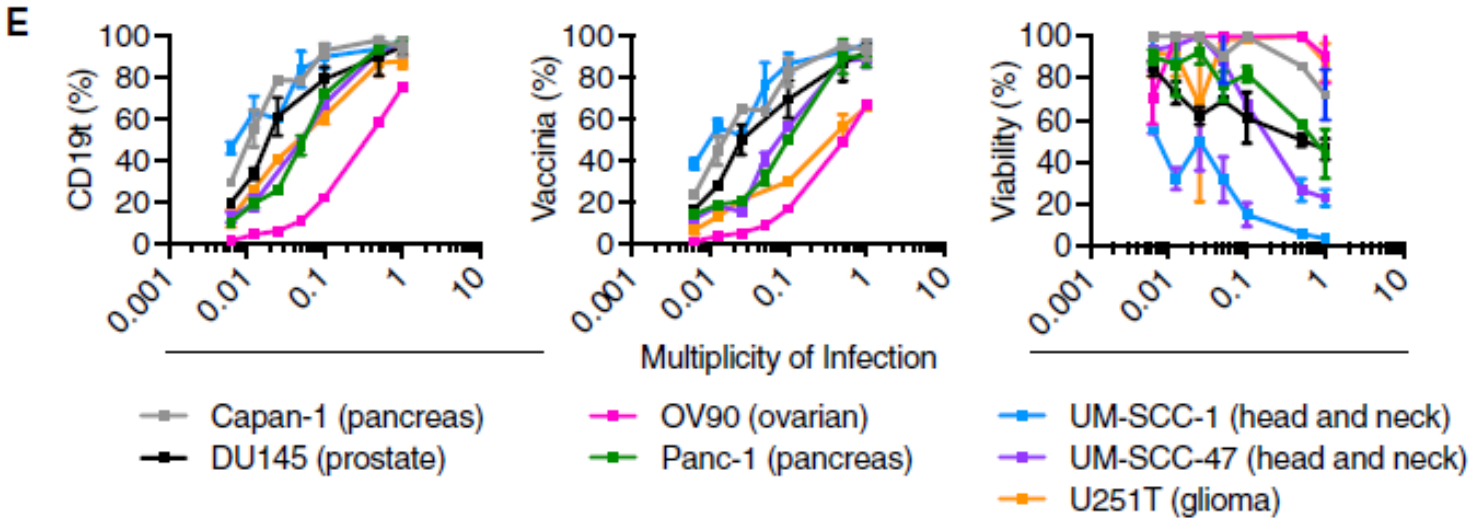
- Flow cytometry showed an MOI-dependent increase in the percent of tumor cells positive for CD19t and vaccinia 24-hour post exposure.

Tumour cells get infected and can be targeted by CAR-T cells



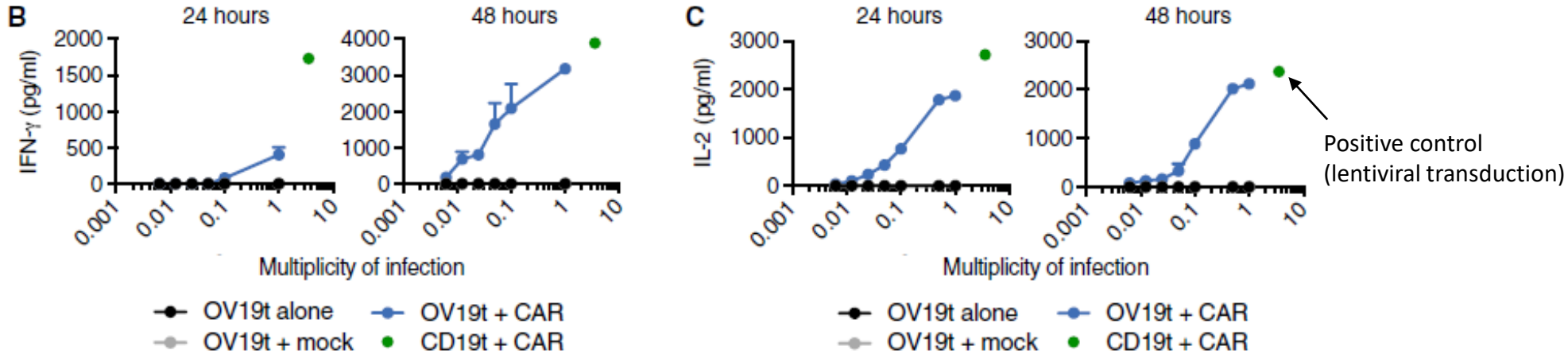
- Almost 100% CD19t-positive tumour cells after 24 hours for MOI = 1 and after 72 hours for lower MOI.
- More than 50% of tumour cells viable after 72 hours with lower MOI, identification of window of opportunity for targeting by CD19-specific CAR-T cells.

Observation is valid for multiple tumour types



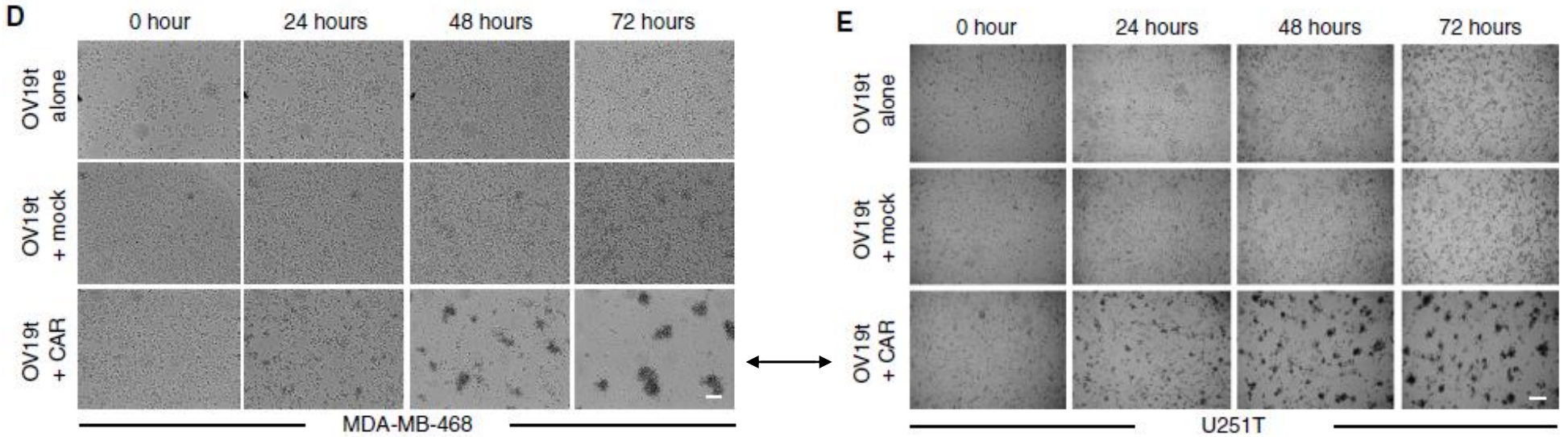
- Repeat experiment in multitude of tumour types.

In vitro activity and cytotoxicity of CD19-CAR-T cells upon OV19t infection



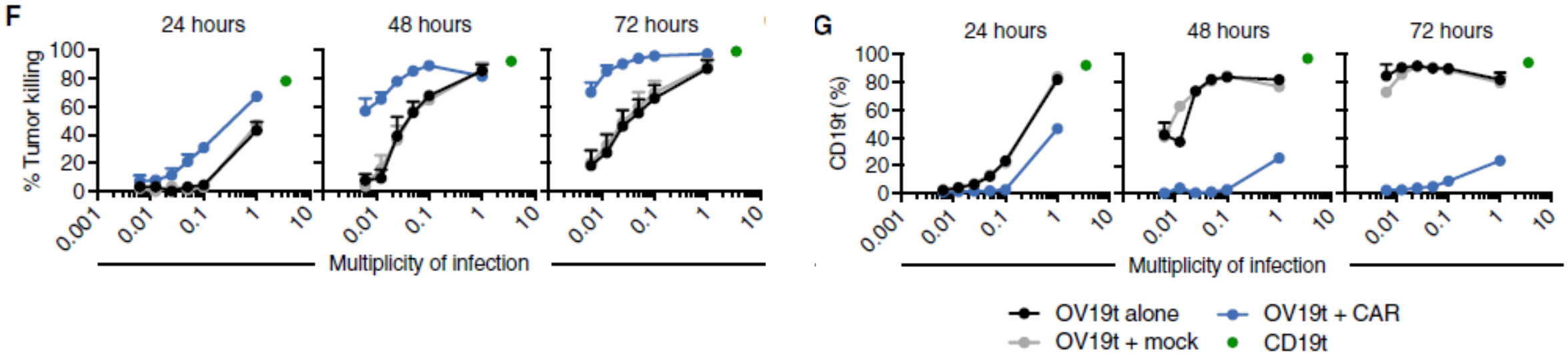
- Co-cultures tumour cells infected with OV19t and CD19-CAR-T cells.
- Supernatants from co-cultures at varying MOIs to evaluate cytokine production.
- CAR-T cells as active upon OV19t as with positive control.

Does CD19-CAR-T cell activity lead to killing of OV19t infected tumour cells (phase contrast)?



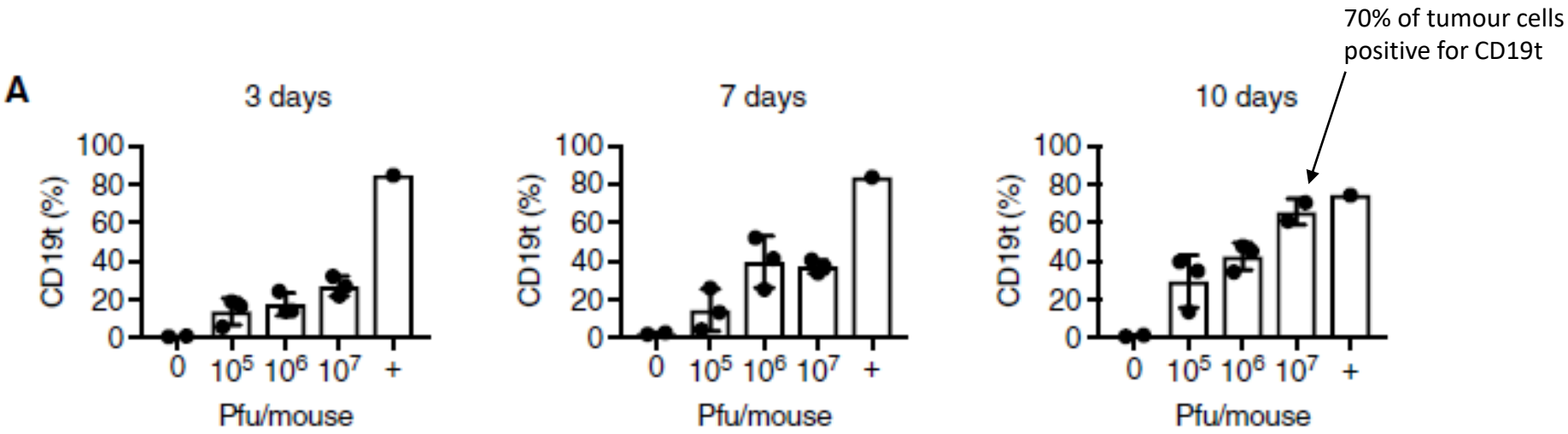
Greater killing when co-cultered with CAR

Does CD19-CAR-T cell activity lead to killing of OV19t infected tumour cells (flow cytometry)?



- OV19t alone is oncolytic in itself.
- But greater killing in combination with CAR-T cells.
- Lower number of CD19t-positive cells, consistent with killing of tumour cells.

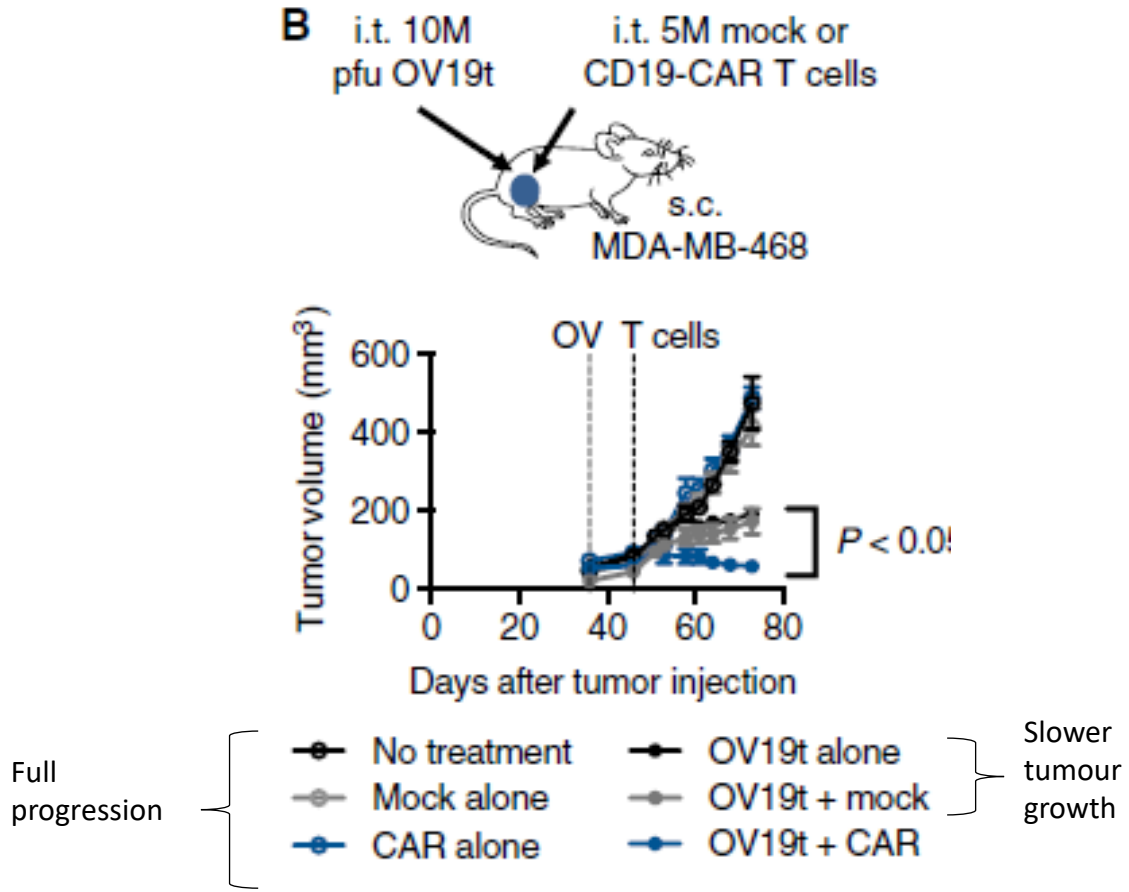
In vivo antitumour efficacy



- Evaluate the antitumor activity of OV19t and the dynamics of CD19t induction in infected tumors.
- Treated mice bearing subcutaneous MDA-MB-468 tumors with a single intratumoral injection of OV19t.
- Varying doses [10^5, 10^6, and 10^7 plaque-forming units (pfu) per mouse] to determine infection efficiency and CD19t positivity in tumors at 3, 7, and 10 days after injection of OV19t.

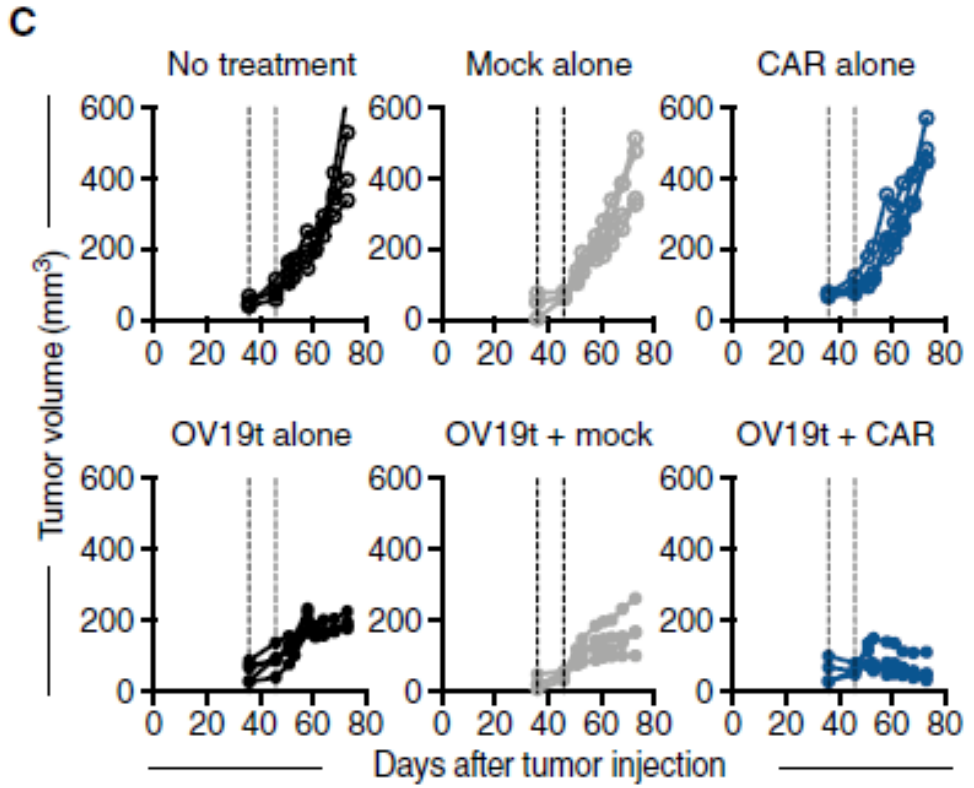
Combination therapy

- Intratumoral delivery of OV19t at 10^7 pfu per mouse.
- 10 d later, intratumoral delivery of 5 x 10^6 mock T cells or CD19-CAR-T cells.



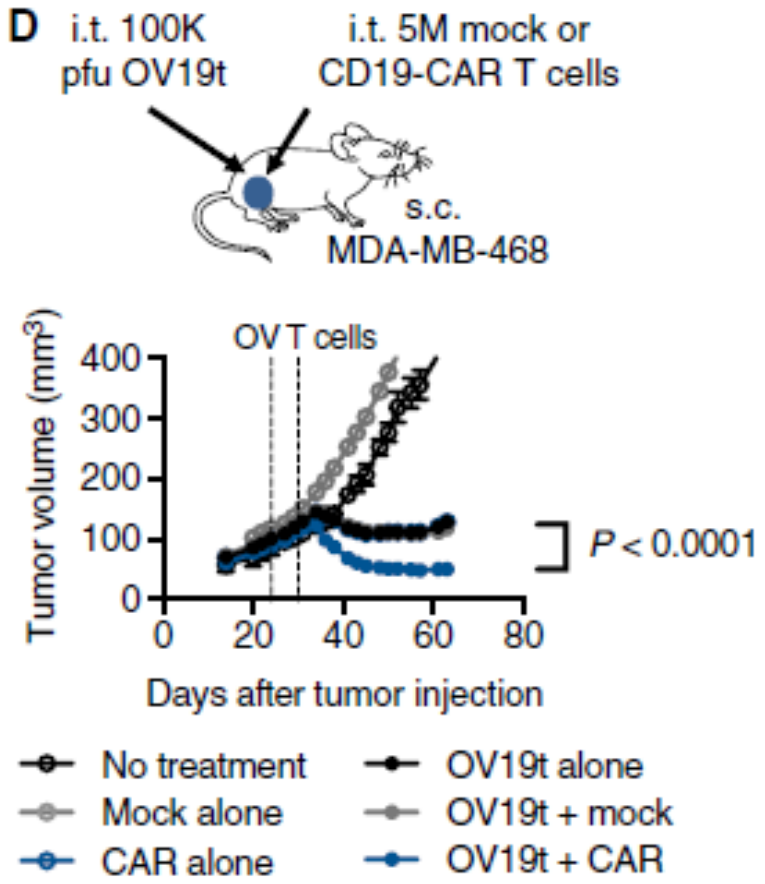
Combination therapy

- Marked tumour regression upon combination of OV19t with CAR-T cells.

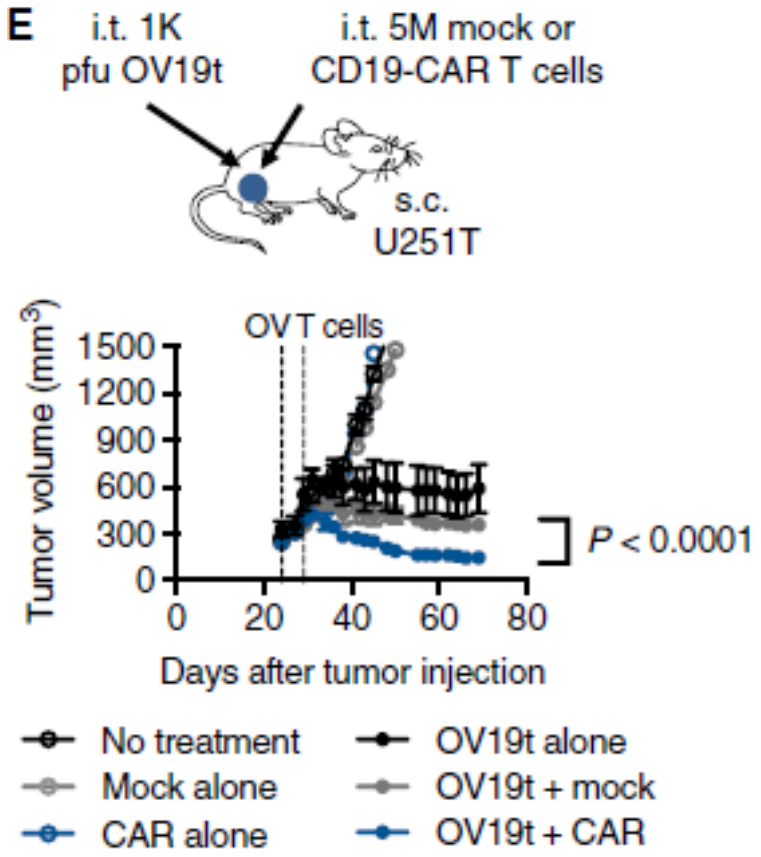


Confirmation of finding using lower dose of OV19t

- Intratumoral delivery of OV19t at **10^5** pfu per mouse confirms result seen before.

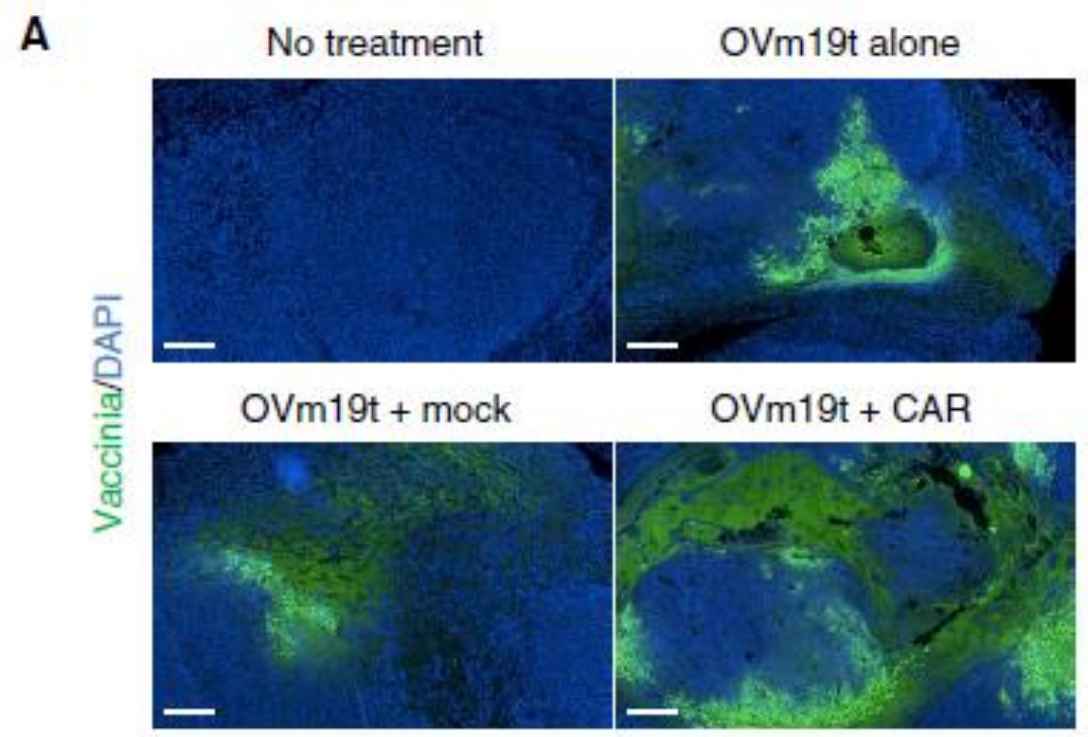


Result confirmed in mice bearing U251T glioma tumour



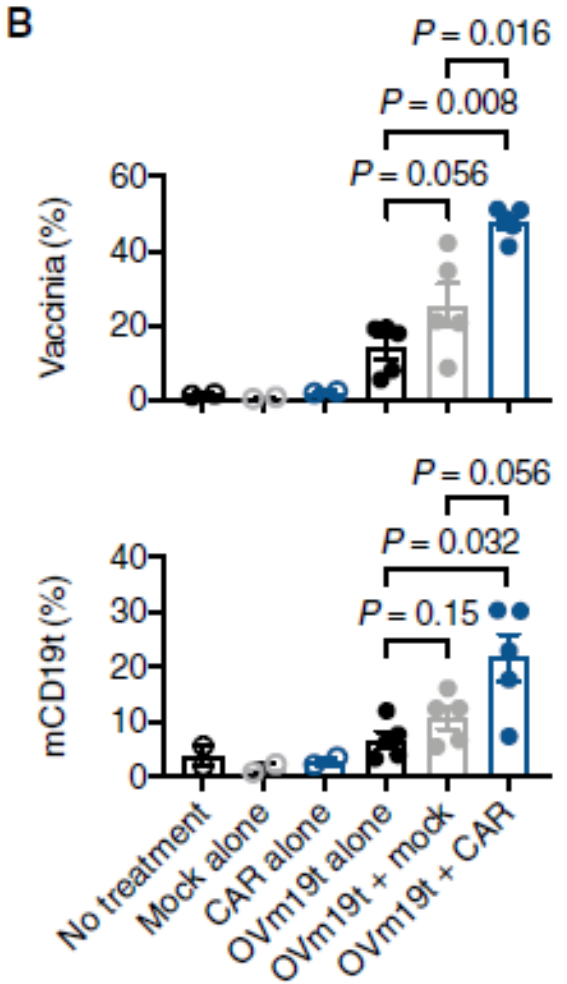
CD19-CAR T cell–mediated tumor killing promotes the release of OV19t

- One of the potential pitfalls of using OV to deliver CAR T cell target antigens to tumor cells is the potential for suboptimal or nonuniform infection of solid tumors.
- Evaluation of the effects of mCD19-CAR T cells on the presence of OVm19t in subcutaneous MC38 tumors.
- Greater spread of vaccinia after CAR treatment.

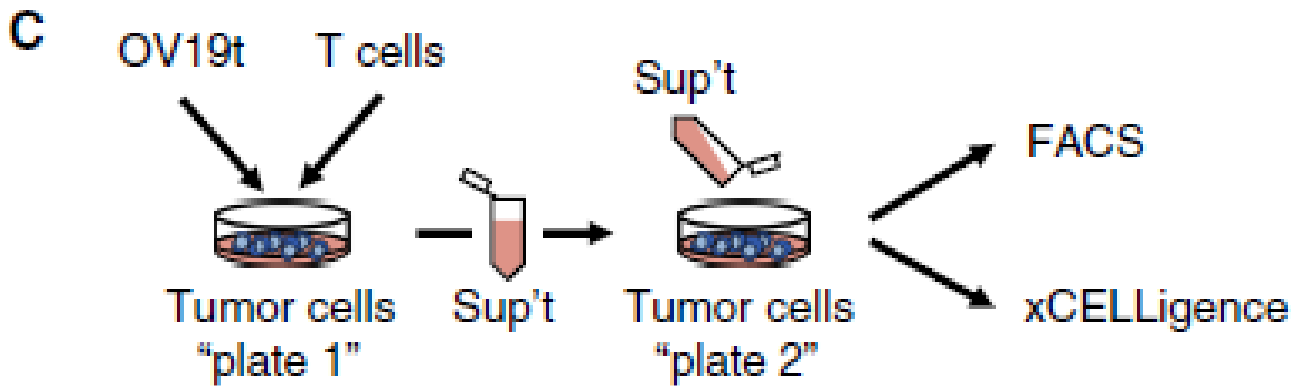


CD19-CAR T cell–mediated tumor killing promotes the release of OV19t

- Cells isolated from tumours and analysed with flow cytometry.
- Increased vaccinia infection and CD19t positivity).



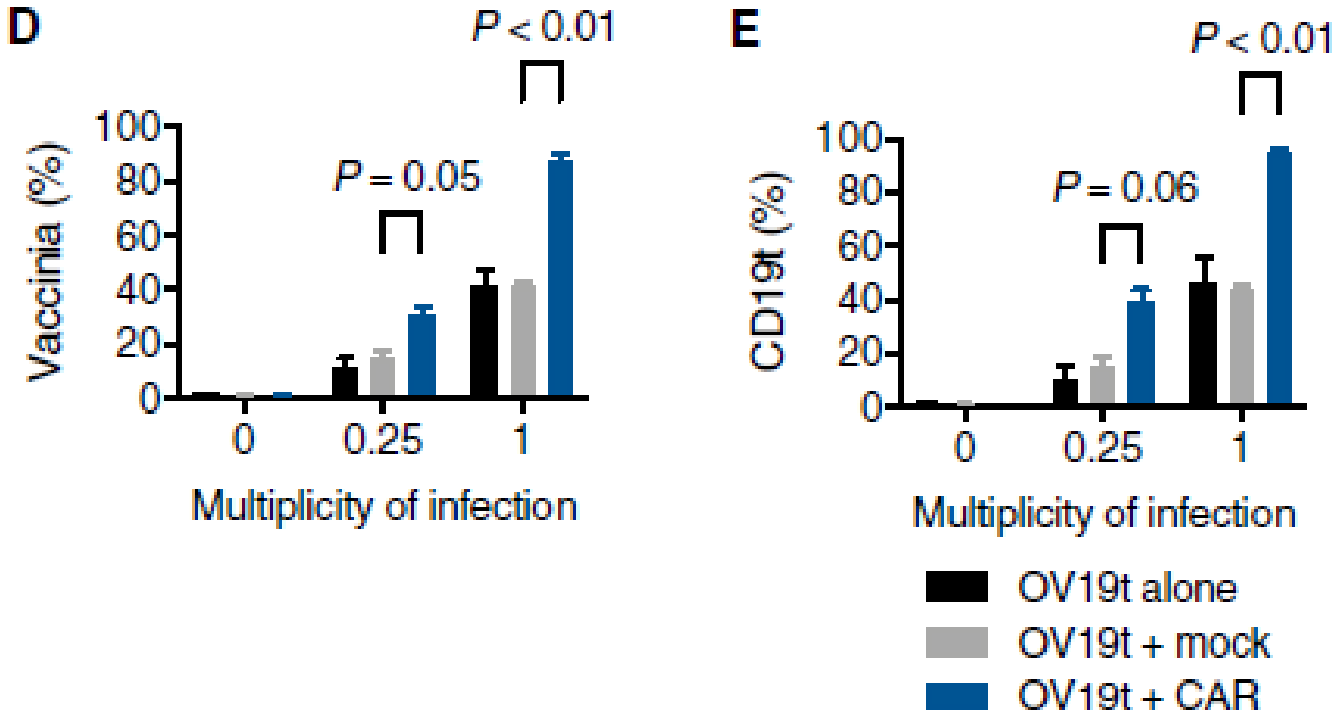
Does CAR T cell–mediated killing of virally infected tumor cells enhance the release of oncolytic virus particles for subsequent infection of surrounding tumor cells?



- MDA-MB-468 tumor cells were first infected with varying MOIs of OV19t and then incubated with mock T cells or CD19-CAR T cells.
- Supernatants were collected and added to newly plated tumor cells that had not been exposed to either T cells or OV19t.

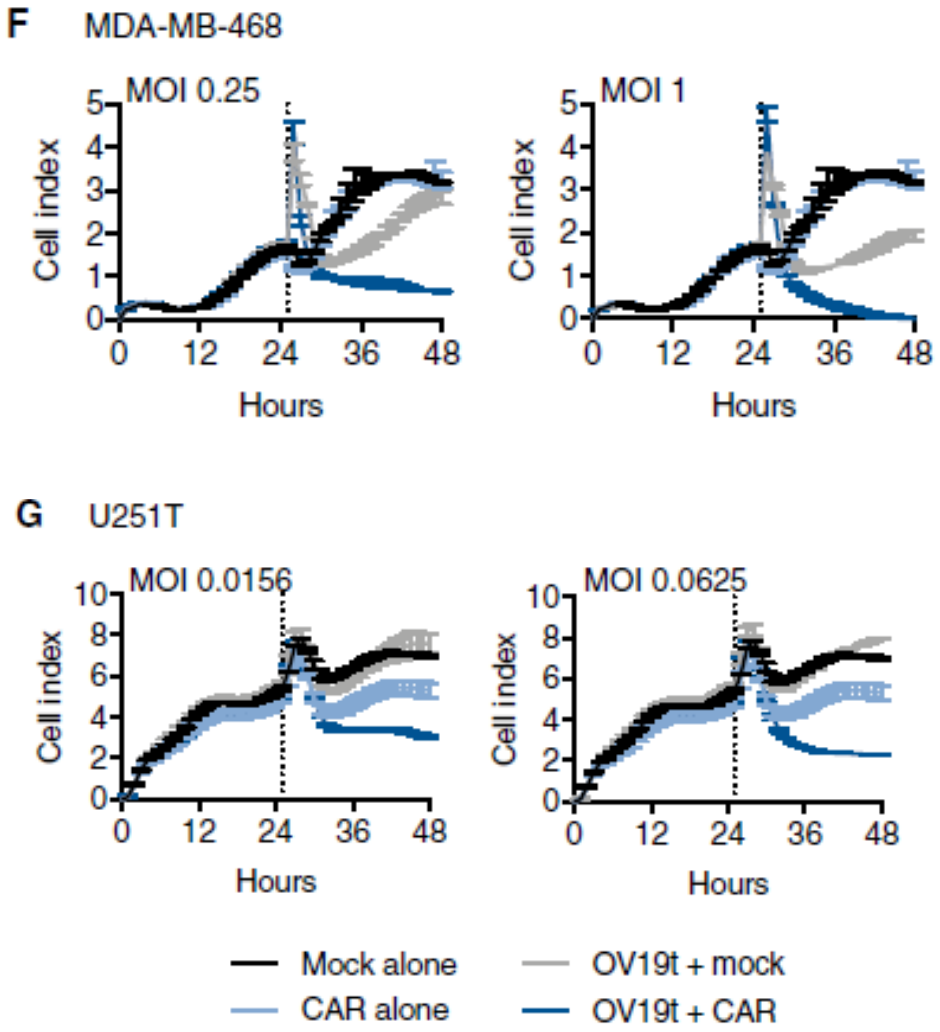
Increased infection…

- Supernatants collected from tumor cells exposed to both OV19t and CD19-CAR T cells resulted in increased infection of naive tumor cells…



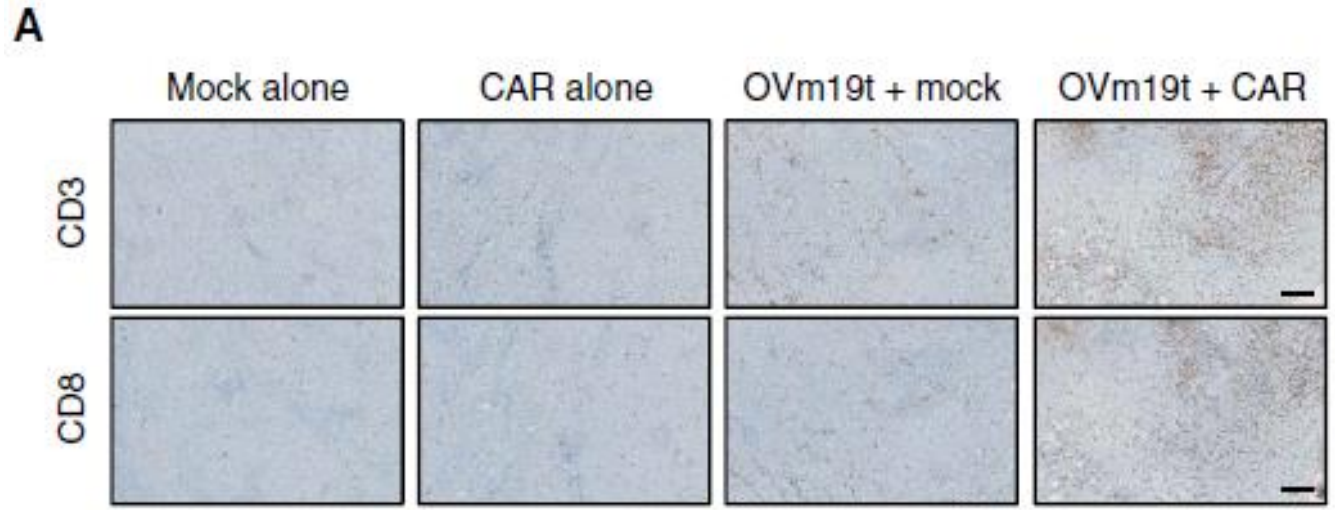
… enhanced killing

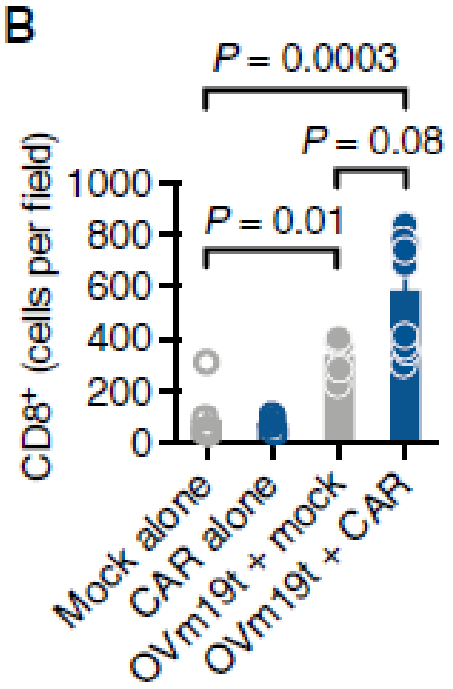
- … resulting in significantly enhanced killing, independent of initial MOI chosen.
- Shown with two different tumour types.



OV + CAR for more pronounced endogenous anti-tumour immunity

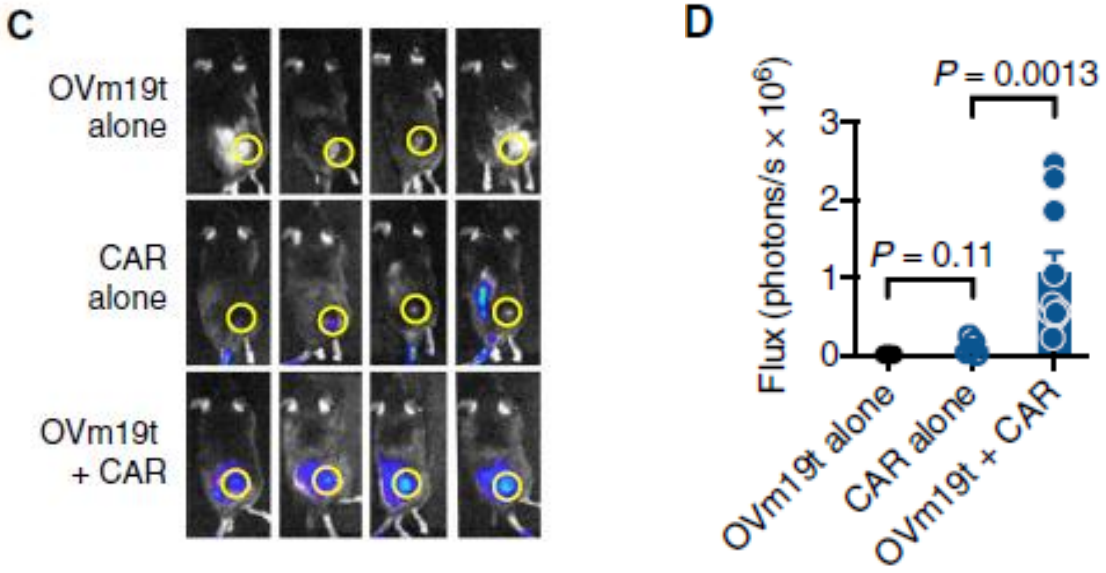
- OV has gained attention for its ability to induce endogenous antitumor immunity and recruit T cells to tumors.
- Assessed the infiltration of T cells into the tumor after OVm19t alone and in combination with mCD19-CAR T cells in the subcutaneous MC38 tumor model.
- Amplification of endogenous CD3+/CD8+ cytotoxic T cell recruitment.





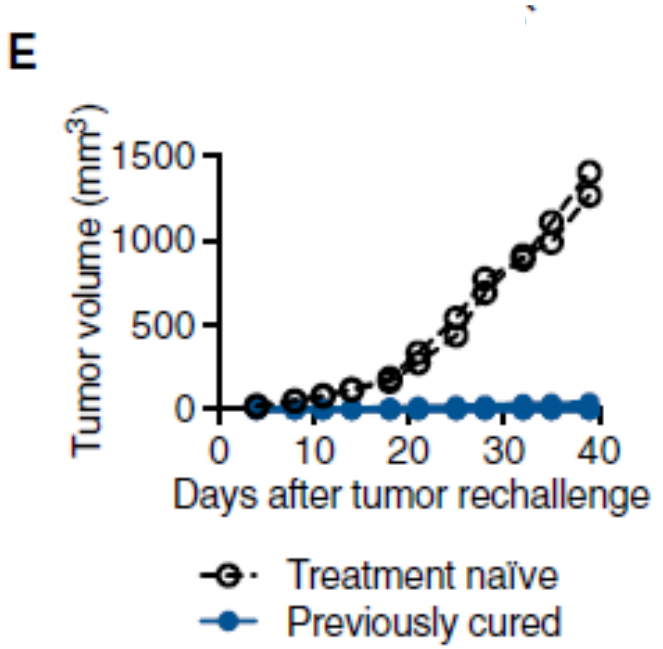
Impact of OV19t on biodistribution of CD19-CAR-T cells expressing luciferase

- Enhanced CD19-CAR-T cell tumour localization upon OV19t infection.



Memory formation

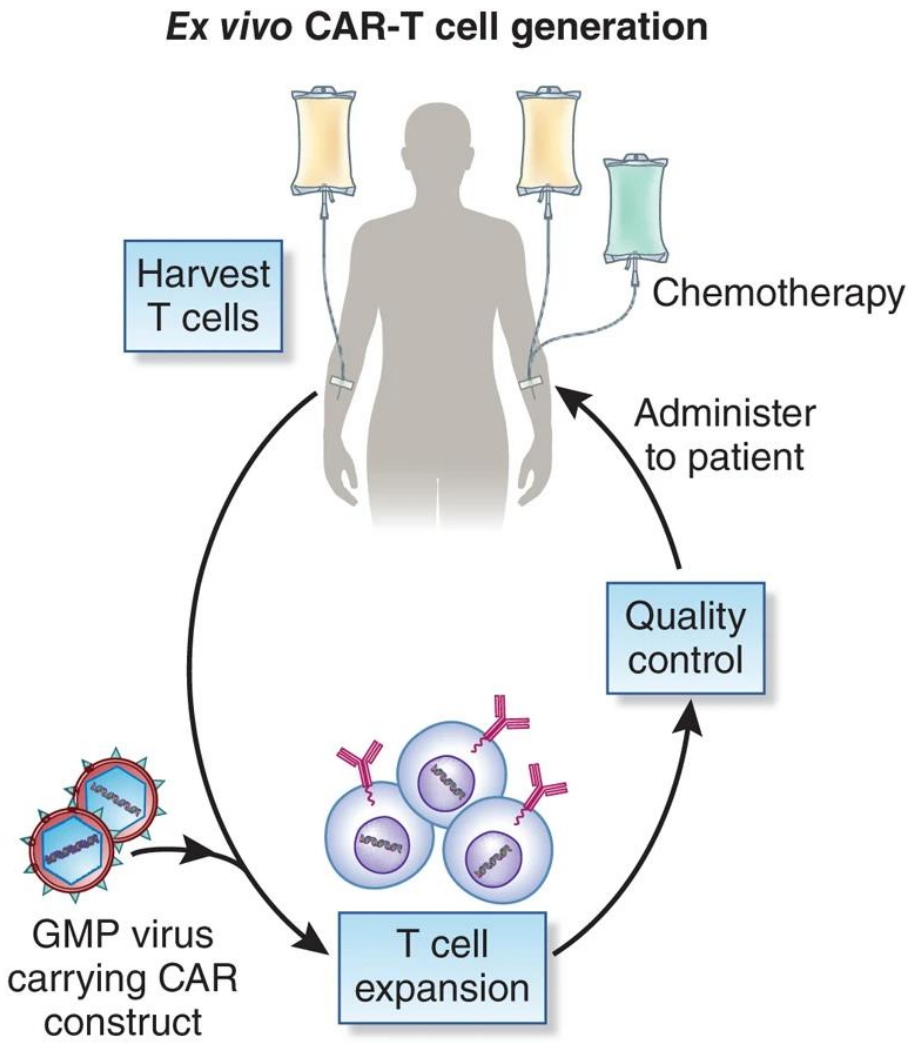
- Rechallenge cured mice by injecting tumour cells into opposite flank.
- Tumours grew with predictable kinetics in treatment-naive but failed to grow in mice previously cured.
- Indication for OV19t-promoted endogenous antitumour immunity and recruitment of CD19-CAR-T cells.



Overall conclusions

- To establish a CAR-T cell based therapy for solid tumours, use recipe:
  - Paper 1: How can we use our RNA vaccines? Combine with CAR-T cell.
  - Paper 2: How can we use our oncolytic viruses? Combine with CAR-T cell.
- Identify tumour-specific target/generate tumour-specific target via oncolytic viruses.
- Design the respective CAR-T cells.
- Make adoptive cell therapy persistent over time (e.g. via RNA vaccine to express antigen in DCs if not presented anyway).

Overall conclusions



Olewus. Nat. Biotechn. 2017.

Question time

THANK YOU FOR YOUR ATTENTION