Adoptive T-cell therapy - Solutions for T-cell engineering and expansion

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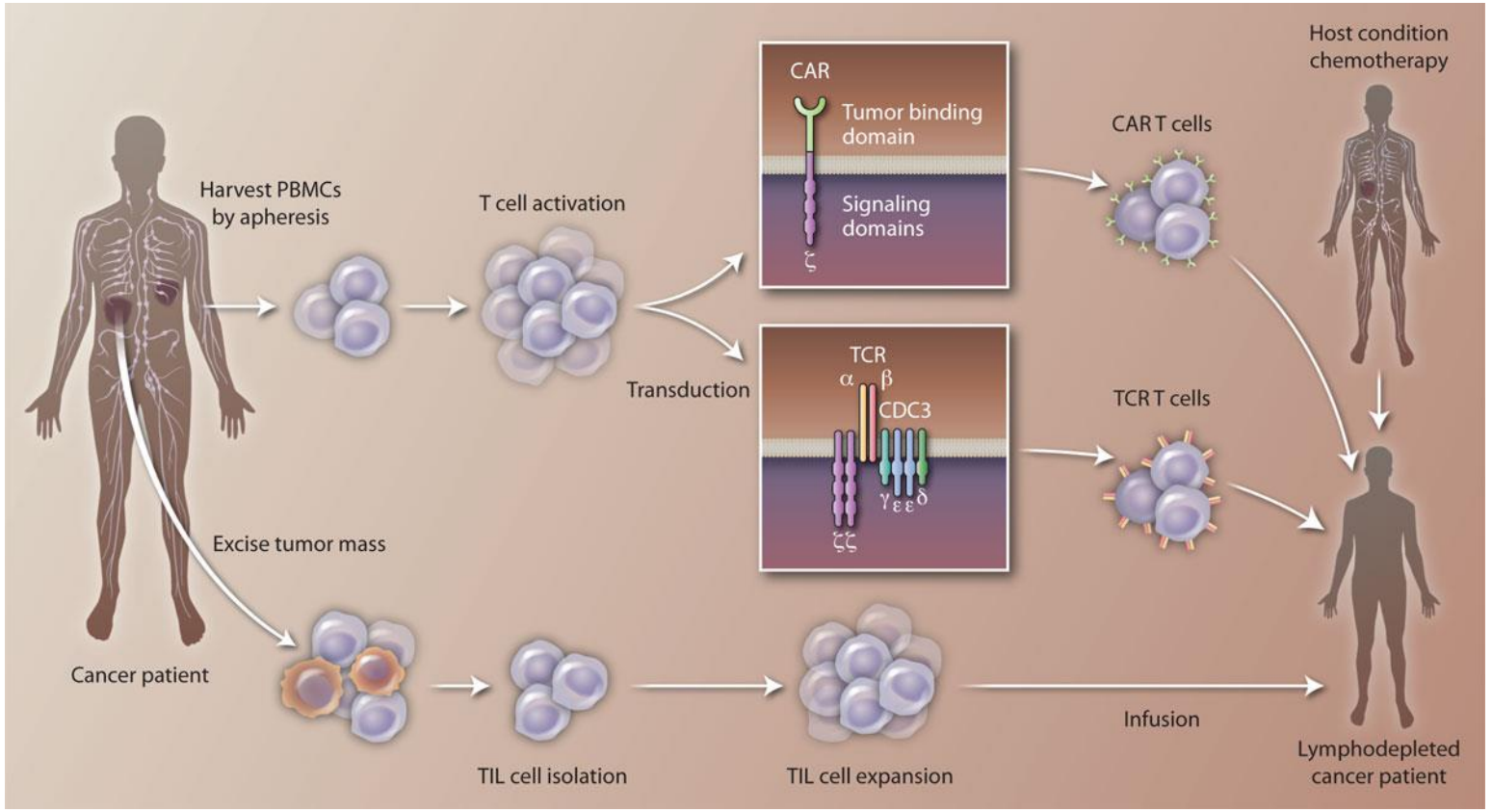
Senior Product Manager & Scientific Support Specialist

Adoptive T cell-based therapies



June CH et al. Science Translational Medicine 2015: Vol. 7, Issue 280, pp. 280ps7

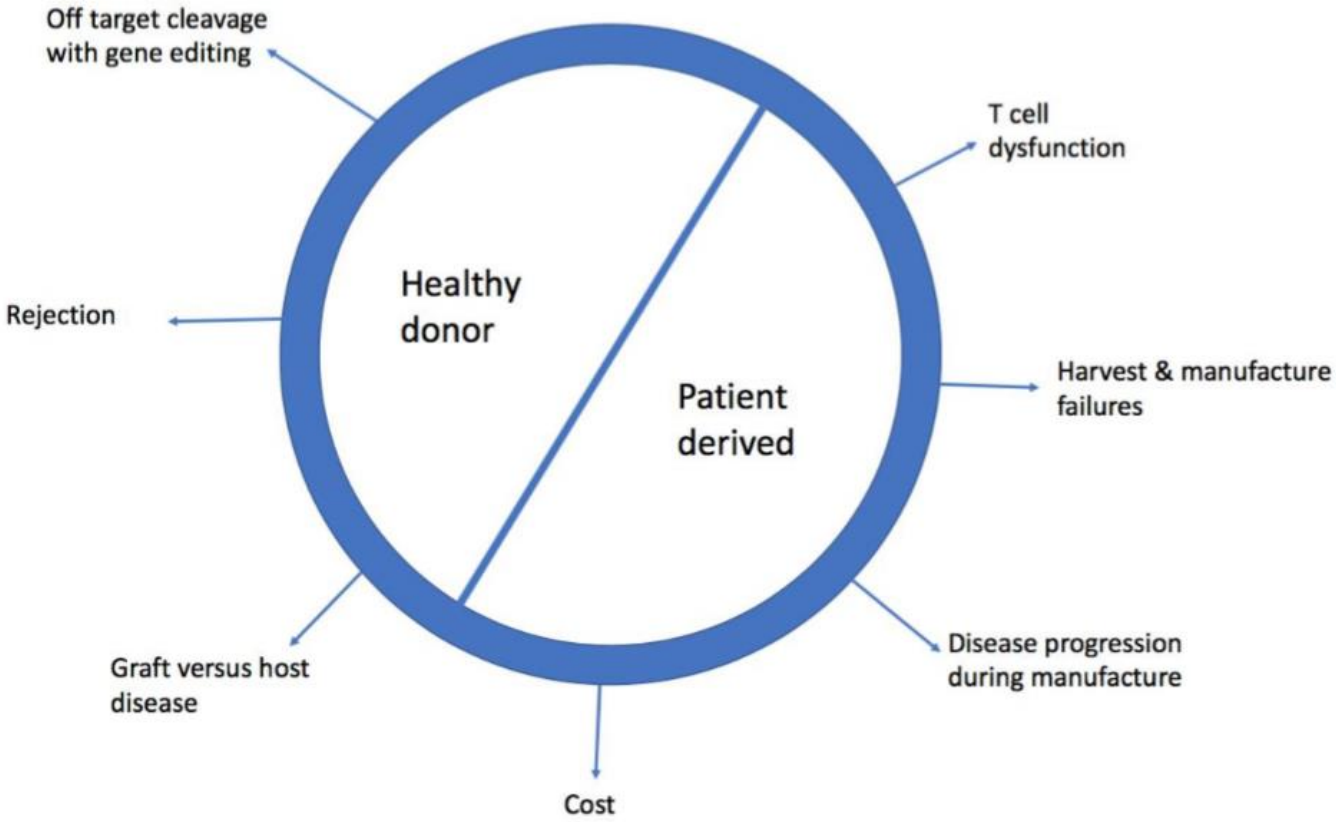
Three main approaches:

- **Expansion/enrichment** of tumor-infiltrating lymphocytes (**TILs**)
- **Engineering** of PBMCs with T-cell receptors (**TCRs**)
- **Engineering** of PBMCs with chimeric antigen receptors (**CARs**)

Autologous vs. Allogeneic cell therapies

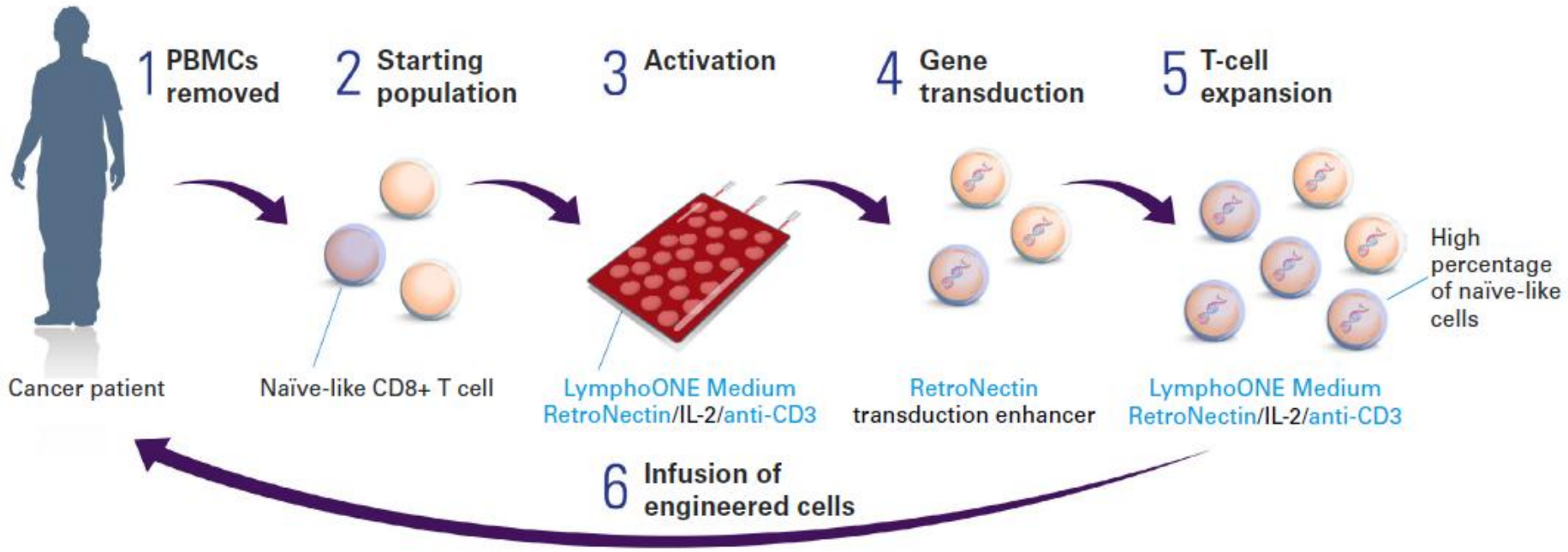
- Autologous treatments: use a person's own cells

- Allogeneic treatments use cells from a healthy donor whose human leukocyte antigens (HLA) match the patient's HLA
  → off the shelf solutions



Graham et al., 2018

Adoptive T-cell therapies



- Although T cells can be isolated from blood and grown *ex vivo*, large-scale T cell expansion is still challenging
- Takara Bio offers a complete solution for T-cell stimulation, transduction and expansion

Tools for T-cell expansion & transduction

- Benefits of the combined use:
  - Highly efficient expansion of T cells
  - Greater proportion of naïve-like T cells (CD45RA$^+$/CCR7$^+$ phenotype) with high therapeutic potential

LymphoONE Medium
WK552S/WK552

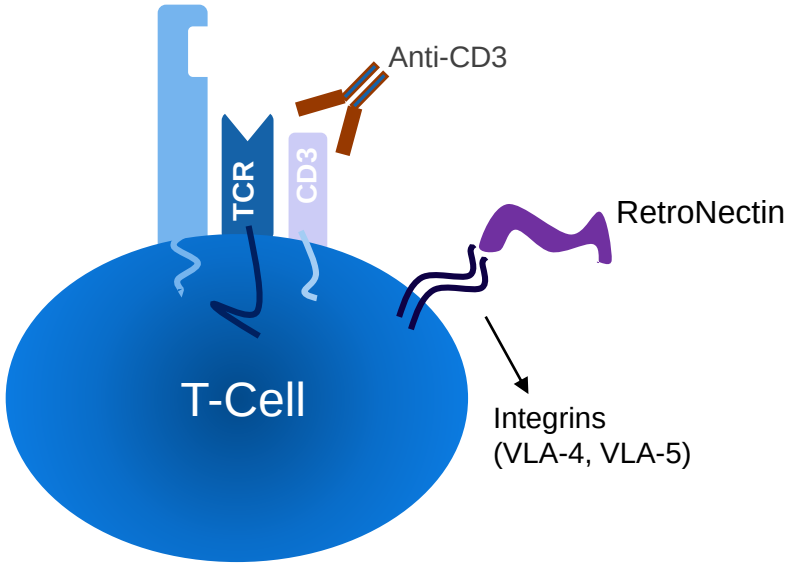
Anti-CD3 antibody
T210

RetroNectin Reagent
T100A/B, T202

CultiLife Culture Bag
FU0005/FU0010

T-cell activation

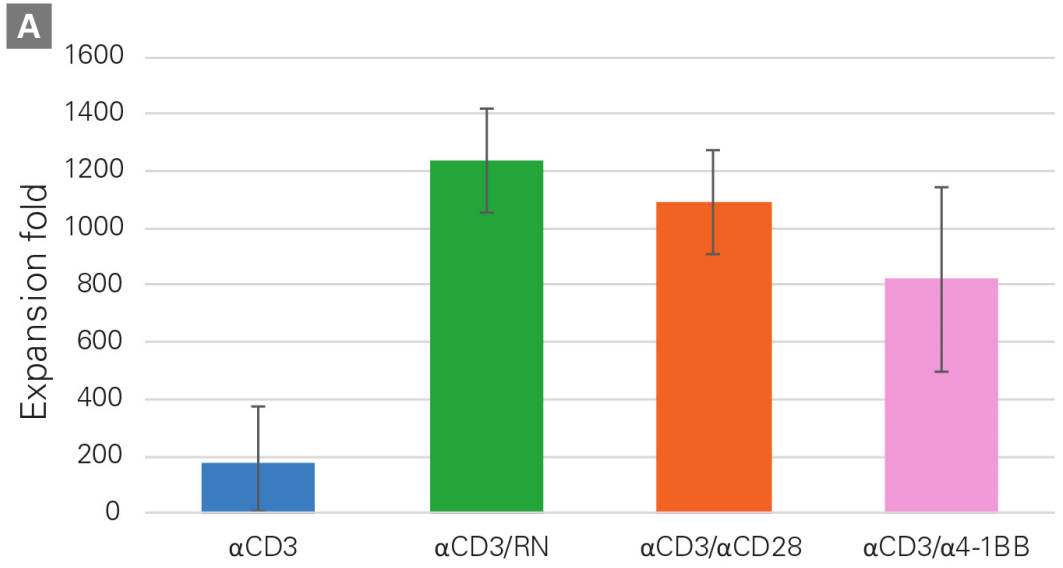
- Anti-CD3 mAb GMP grade
  - Clone OKT3 (mouse IgG2a)
  - GMP-grade, suitable for *ex vivo* cell culture and gene therapy studies



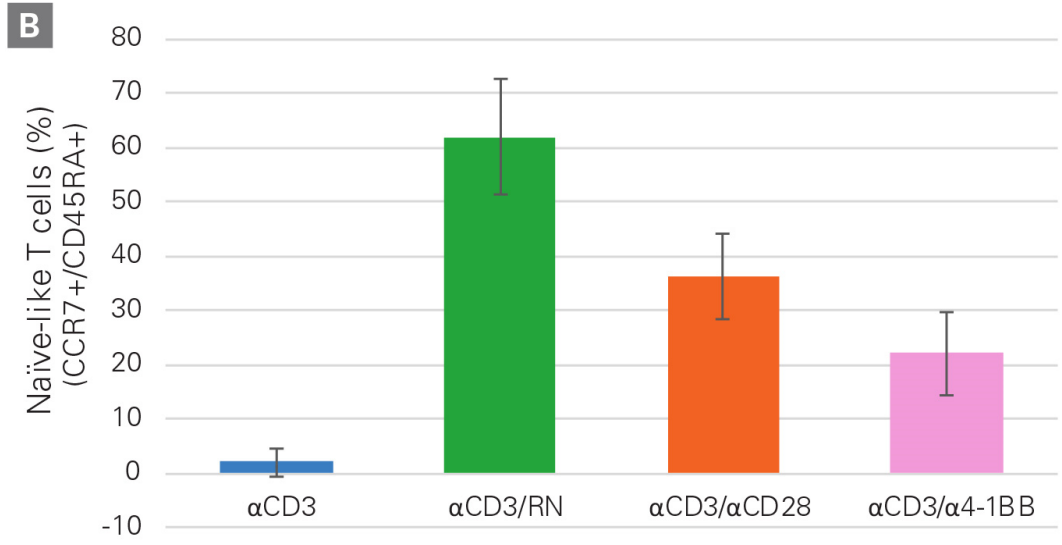
- RetroNectin Reagent:
  - Recombinant human fibronectin fragment (rFN-CH-296)
  - Increases T-cell expansion via binding to integrins (VLA-4, VLA-5) expressed on T cells
  - Research-grade and GMP-grade versions available

Combined RetroNectin/anti-CD3 stimulation

- Higher fold expansion with RN/αCD3 stimulation compared to other protocols
- Greater proportion of naïve-like T cells (CCR7+/CD45RA+ phenotype)

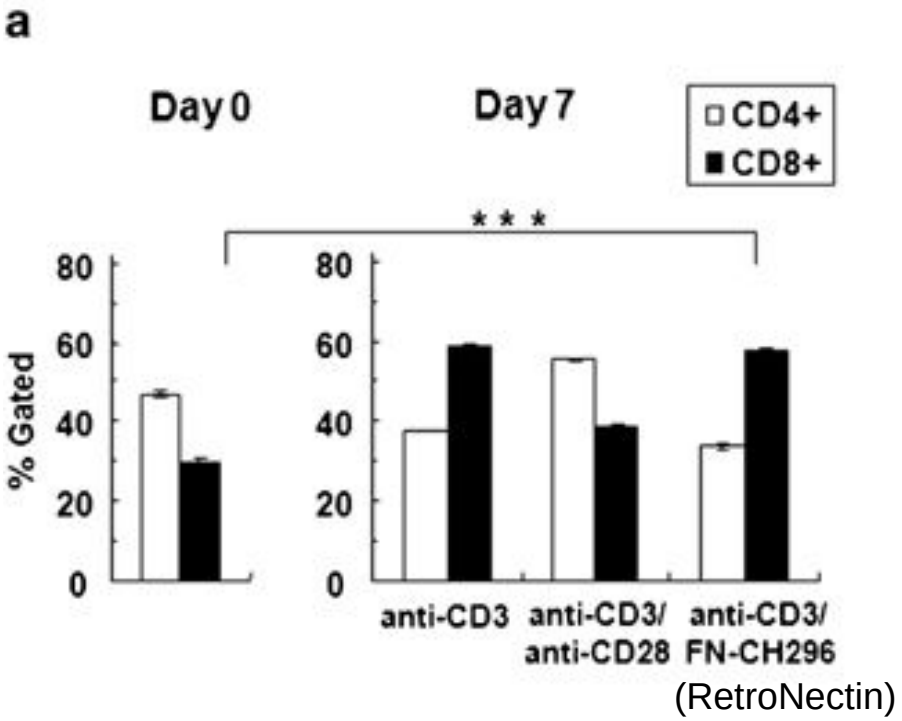


Higher fold expansion



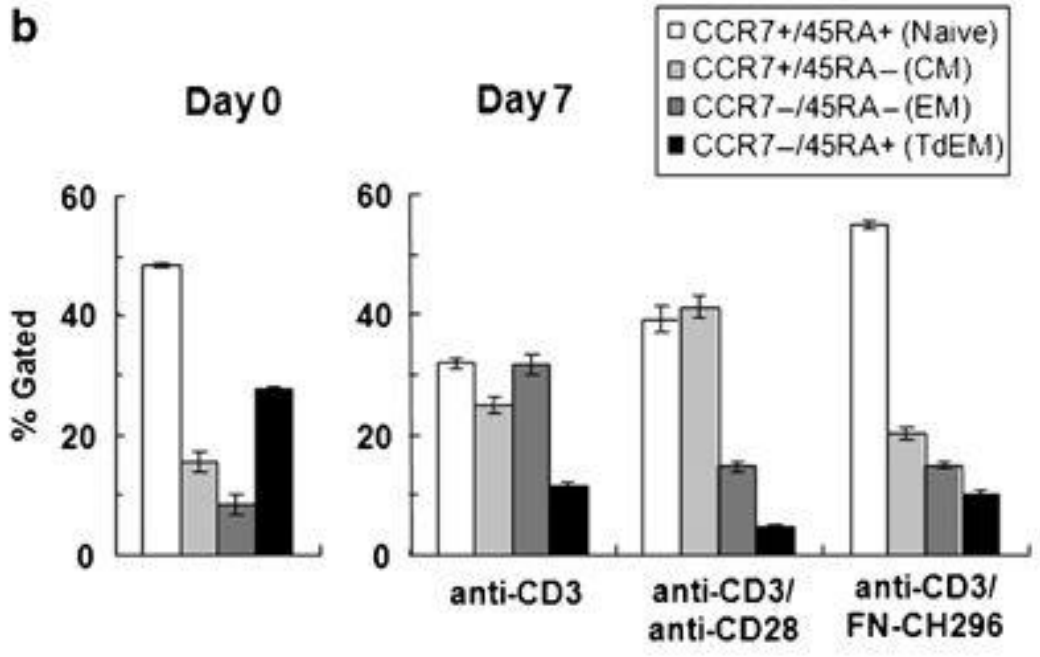
Greater proportion of naïve-like T cells

Preservation of naïve-like phenotype



(RetroNectin)

Increased CD8+ population compared to CD3/CD28 stimulation



(RetroNectin)

Greater proportion of of naïve-like T cells (CCR7+/ CD45RA+)

CM: central memory
EM: effector memory
TdEM: terminally differentiated effector memory

Yu SS et al. *In vivo* persistence of genetically modified T cells generated *ex vivo* using the fibronectin CH296 stimulation method. Cancer Gene Therapy. 2008; 15: 508–516.

In vivo persistence of genetically modified T cells

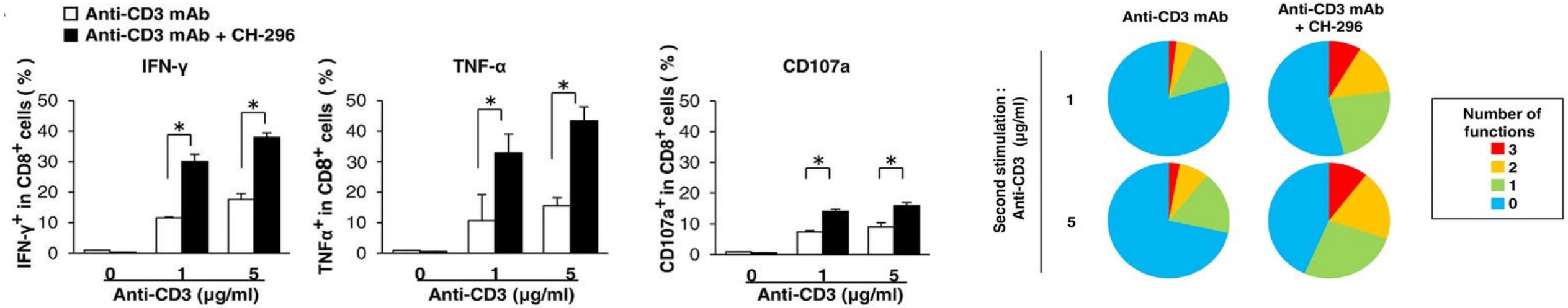
- Engineered T cells were transplanted into NOD/SCID mice, and mice were killed 14 days later. High engraftment efficiency for all groups observed.
- The infiltration of human cells into the bone marrow and thymus was significantly higher with the anti-CD3/FN-CH296 group than that with the anti-CD3 group.

Group	*Days*	*PBL (%)*	*Bone marrow (%)*	*Thymus (%)*	*Spleen (%)*
Anti-CD3	7	90.0 (87.6–93.9)	ND	ND	ND
	14	95.1 (92.2–98.0)	69.5 (63.7–79.9)	67.0 (24.3–94.1)	97.9 (96.3–99.3)
Anti-CD3/anti-CD28	7	92.1 (89.4–95.6)	ND	ND	ND
	14	98.8 (97.9–99.4)	88.4 (81.0–93.6)	89.0 (67.2–96.6)	99.0 (98.7–99.4)
Anti-CD3/FN-CH296	7	94.2 (92.6–95.5)	ND	ND	ND
	14	98.2 (97.0–99.2)	79.3 (69.7–97.5)	83.4 (70.1–96.0)	98.5 (97.8–99.1)

Yu SS et al. *In vivo* persistence of genetically modified T cells generated *ex vivo* using the fibronectin CH296 stimulation method. Cancer Gene Therapy. 2008; 15: 508–516.

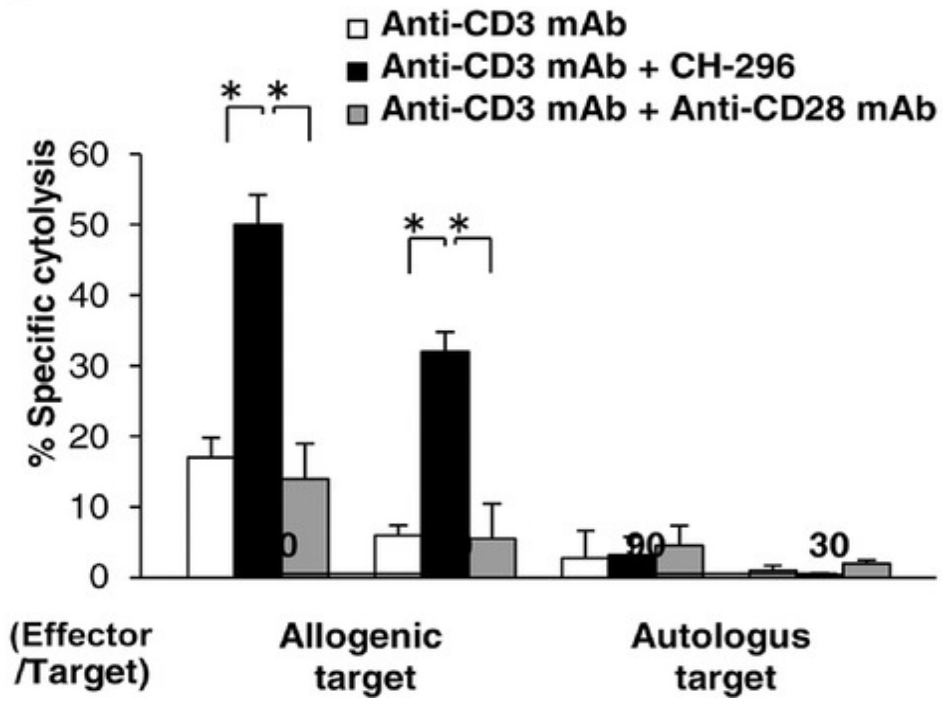
Improved multifunctionality of CD8+ T cells

- RetroNectin stimulation in the initial *in vitro* expansion of tumor-reactive T cells improves CD8+ T-cell (cytotoxic lymphocyte) multifunctionality.



Hosoi H et al. Stimulation through very late antigen-4 and -5 improves the multifunctionality and memory formation of CD8+ T cells. Eur J immunol. 2014; 44:1747-1758

Ag-specific cytotoxicity of CD8+ T cells



After 7 days of stimulation, lymphocytes were mixed with allogeneic or autologous PHA-blast target cells at effector/target ratios of 90 and 30. Cytotoxicity was assessed by a calcein-AM release assay.

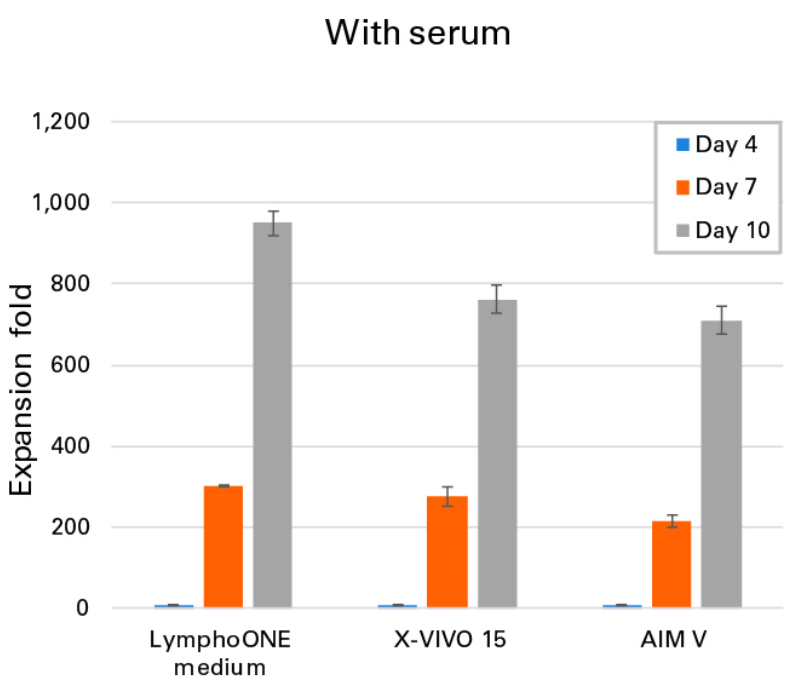
Human T cells stimulated with anti-CD3 mAb plus CH-296 exhibited higher cytotoxicity against allogeneic target cells than T cells stimulated with anti-CD3 mAb alone or anti-CD3 and anti-CD28 mAbs

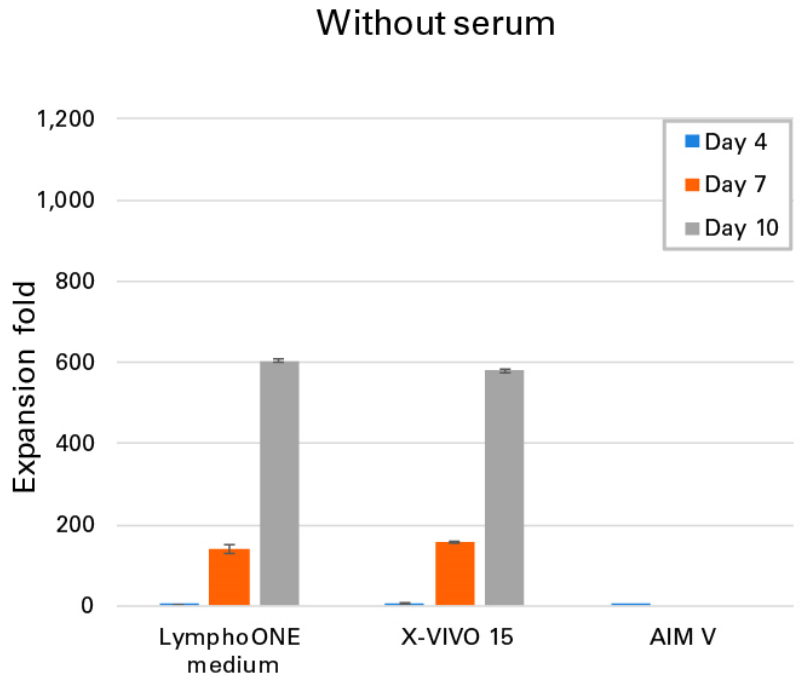
Hosoi H et al. Stimulation through very late antigen-4 and -5 improves the multifunctionality and memory formation of CD8+ T cells. Eur J immunol. 2014; 44:1747-1758

LymphoONE T-Cell Expansion Xeno-Free Medium

- Improved version of GT-T551 medium, specifically formulated for *ex vivo* human T-cell expansion and transduction
- Suitable for serum-free T-cell expansion
- Xeno-free, serum-free, chemically defined medium
- Supplemented with human serum albumin, human insulin, L-glutamine, and streptomycin
- GMP-grade version (w/o antibiotics) expected for Q4, 2019

RetroNectin expansion protocol in LymphoONE medium

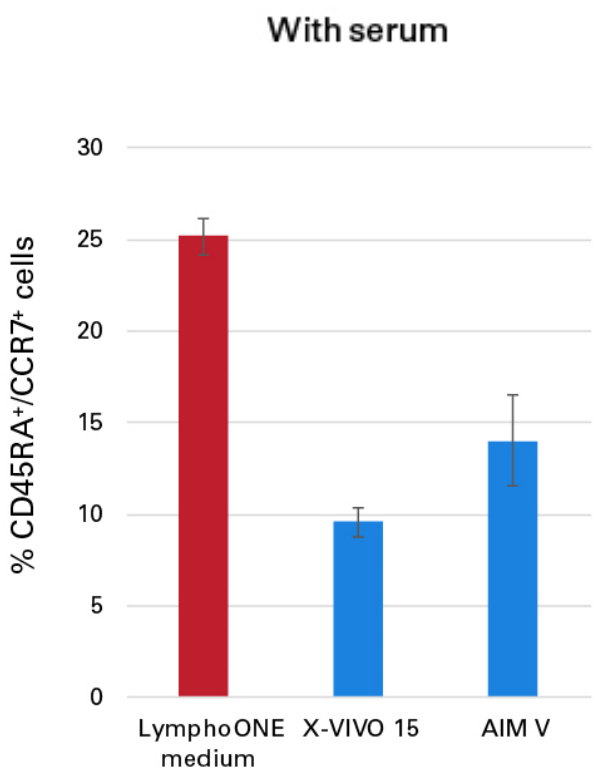


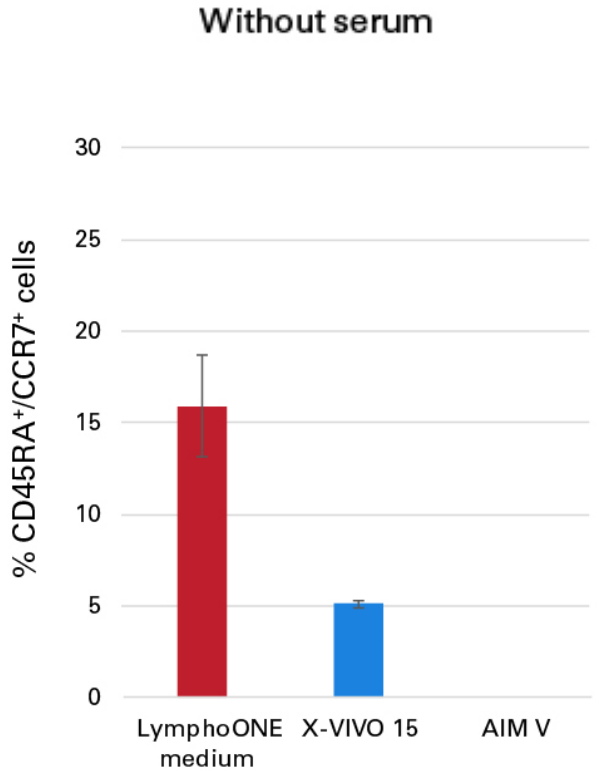


Higher fold expansion in LymphoONE compared to other media

T-cell expansion via stimulation by RetroNectin, anti-CD3 mAb, and IL-2

RetroNectin expansion protocol in LymphoONE medium





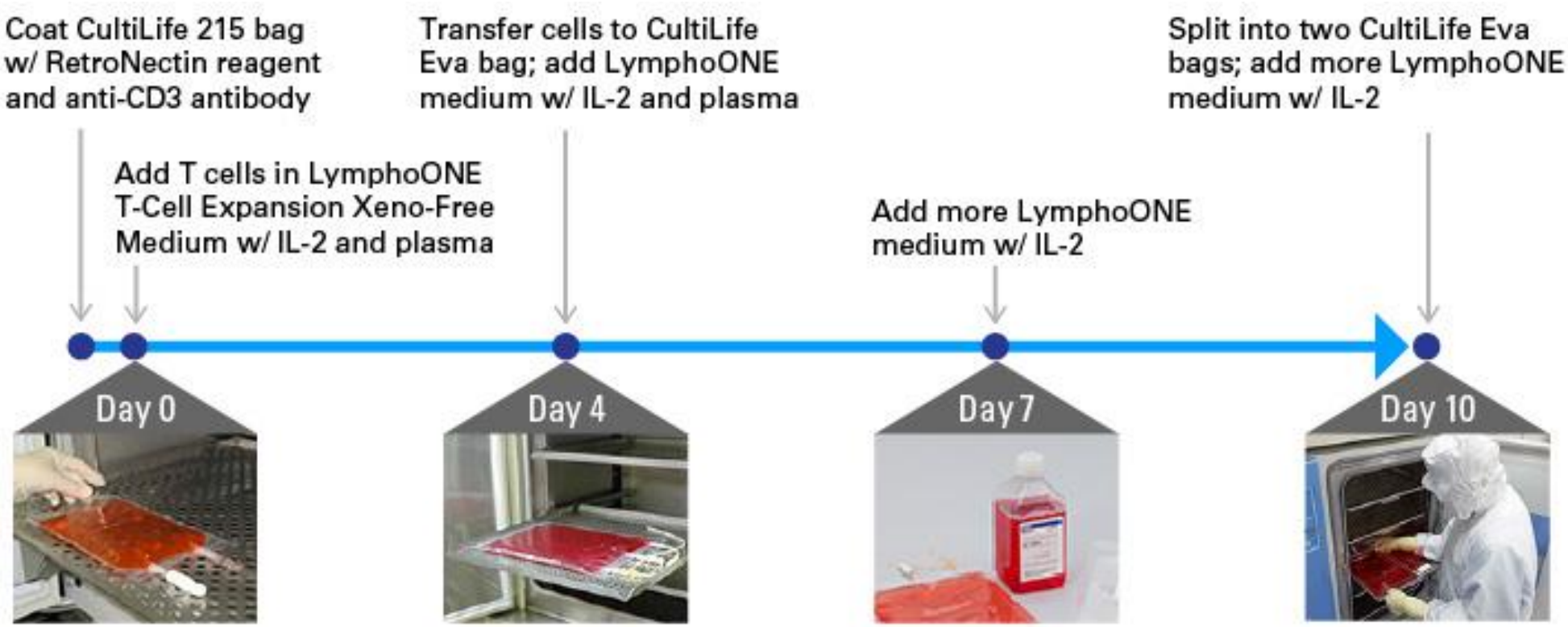
Greater proportion of naïve-like T cells ($CD45RA^+/CCR7^+$ phenotype) compared to other media

T-cell expansion via stimulation by RetroNectin, anti-CD3 mAb, and IL-2

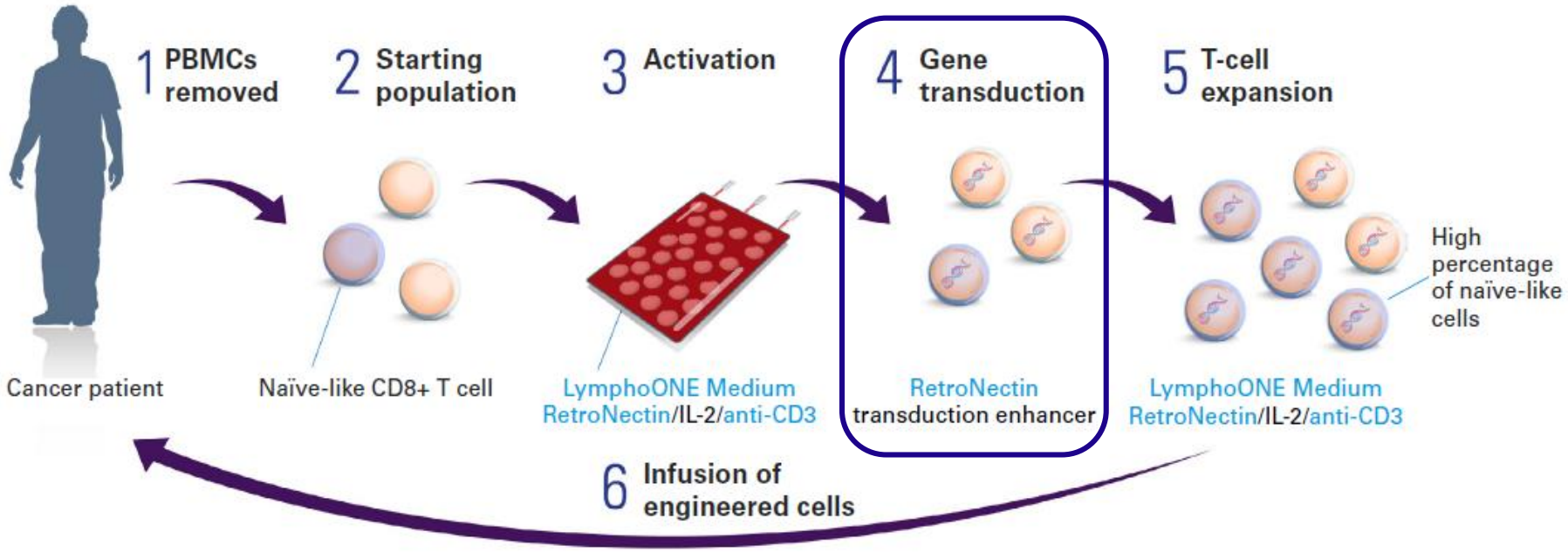
Large-scale expansion using CultiLife culture bags

- A sterile, closed system - reduces the risk of culture contamination and user infection
- Gas permeable and transparent (suitable for visual inspection of cells via microscopy)
- Available in two sizes (215 cm^2 and 640 cm^2)
- CultiLife 215 bags can be coated with RetroNectin for T-cell activation and transduction

RetroNectin Expansion Protocol in culture bags



Adoptive T-cell Therapy

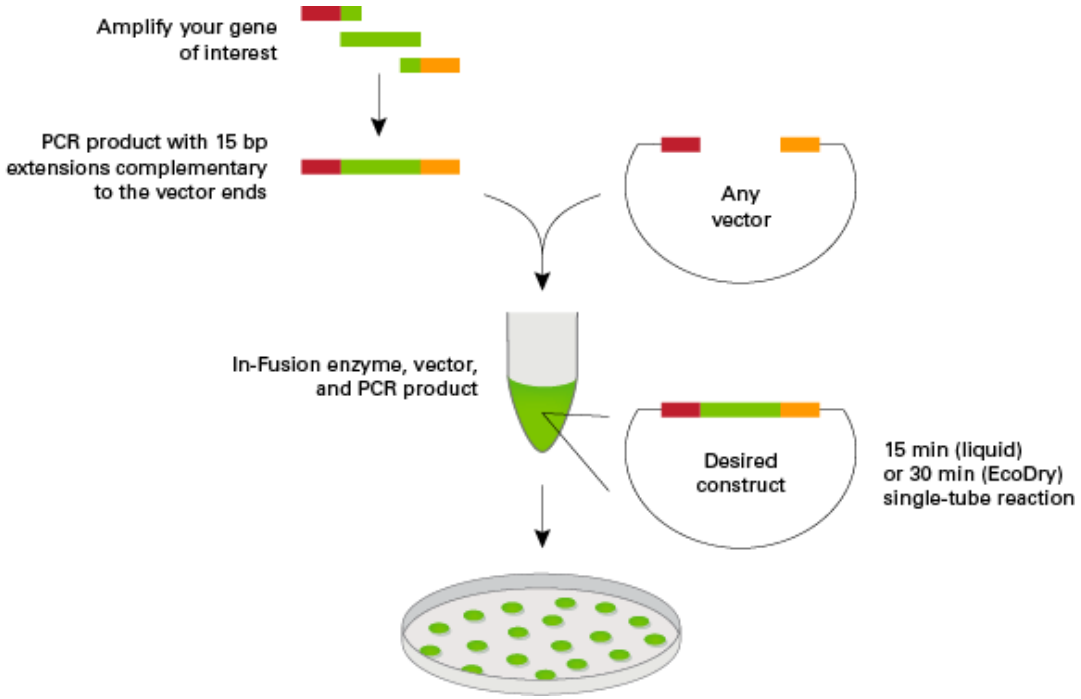


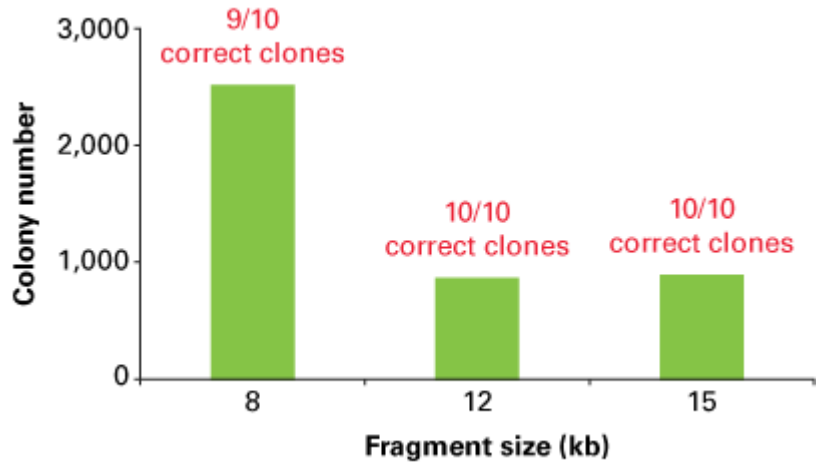
Classical CAR/TCR engineering
- In-Fusion cloning
- Retroviral or lentiviral delivery
- RetroNectin transduction enhancer

Refinement of cell engineering (CRISPR/Cas9)
- Targeted CAR integration
- KO of specific genes
- Footprint-free engineering

T-cell engineering using viral delivery

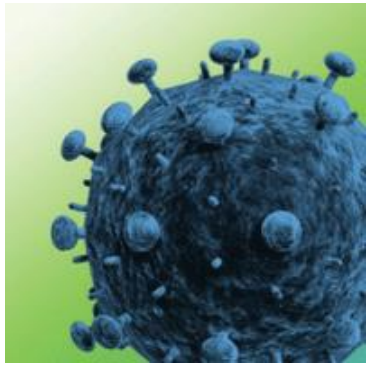
- Cloning into retroviral/lentiviral vectors
  - A broad portfolio of viral vectors
  - High fidelity DNA Polymerases (PrimeSTAR Max, PrimeSTAR GXL)
  - In-Fusion HD technology for highly efficient, seamless cloning





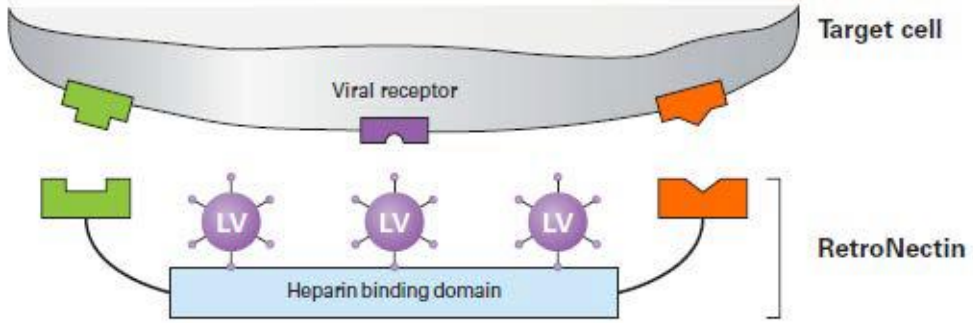
T-cell engineering using viral delivery

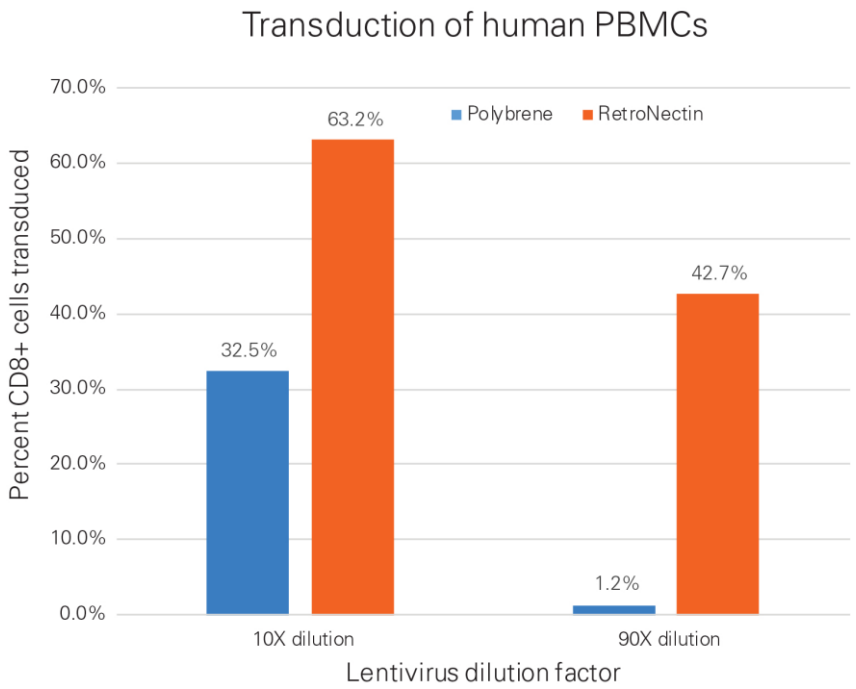
- Production of viral particles (Lenti-X and Retro-X portfolios)
  - **Packaging:**
    - Lenti-X Packaging Single Shots (VSV-G): convenient, lyophilized format - 4th generation
    - Retro-X Universal Packaging System: p10A1, pAmpho, pEco, or pVSV-G envelope
  - **Titration:**
    - Retro-X or Lenti-X qRT-PCR Titration Kit: Accurate titration in 4 hr
    - Lenti-X GoStix Plus: Quantitative output in only 10 minutes
    - Lenti-X p24 Rapid Titer Kit: ELISA-based titration
    - Lenti-X Provirus Quantitation Kit: Integrated copy number quantification via qPCR
  - **Concentration:**
    - Retro-X or Lenti-X Concentrator: 100-fold concentration without ultracentrifugation
  - **Purification:**
    - Lenti-X Maxi Purification Kit: Column-based method, high yield and purity
  - **Integration Site Analysis**
    - Lenti-X or Retro-X Integration Site Analysis Kit: Identify the exact site of integration



Transduction of hematopoietic cells

- RetroNectin Recombinant Human Fibronectin Fragment
  - Enhances lentiviral/retroviral transduction by aiding the colocalization of target cells and viral particles
  - GMP-grade version available





Higher transduction efficiencies in CD8+ cells with RetroNectin compared to Polybrene, especially at low MOI (90X dilution).

Transduction of hematopoietic cells


OPEN ACCESS Freely available online

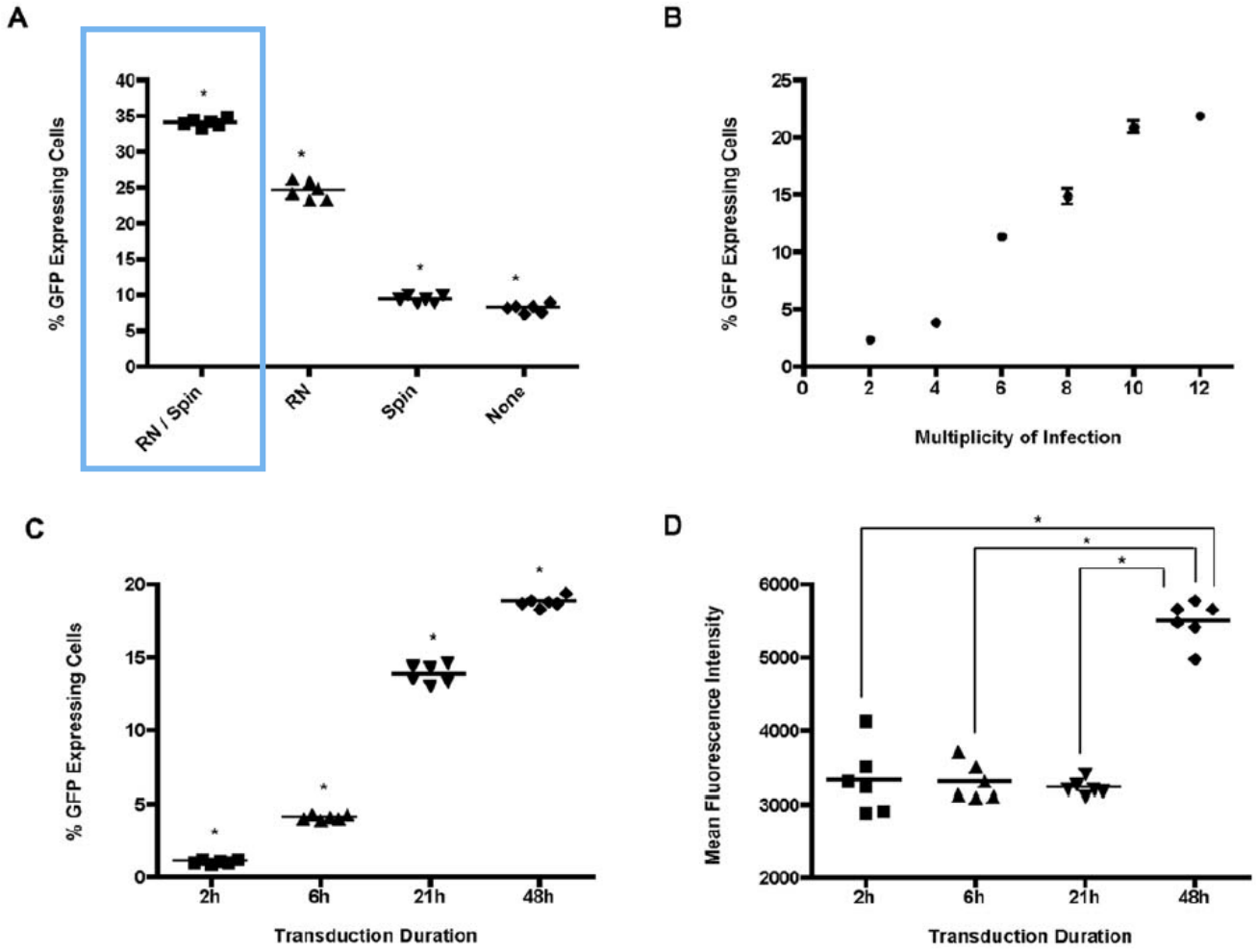
PLOS one

Towards a Clinically Relevant Lentiviral Transduction Protocol for Primary Human CD34$^+$ Hematopoietic Stem/ Progenitor Cells

Michelle Millington, Allison Arndt, Maureen Boyd, Tanya Applegate, Sylvie Shen*

Johnson and Johnson Research Pty Ltd., Eveleigh, New South Wales, Australia

- Retronectin (RN) combined with spinoculation resulted in the highest transduction efficiency (34.1%)
- RetroNectin reagent alone resulted in a statistically significant increase in transduction rate (24.6%)
- No advantage of spinoculation alone (10% of cells transduced)

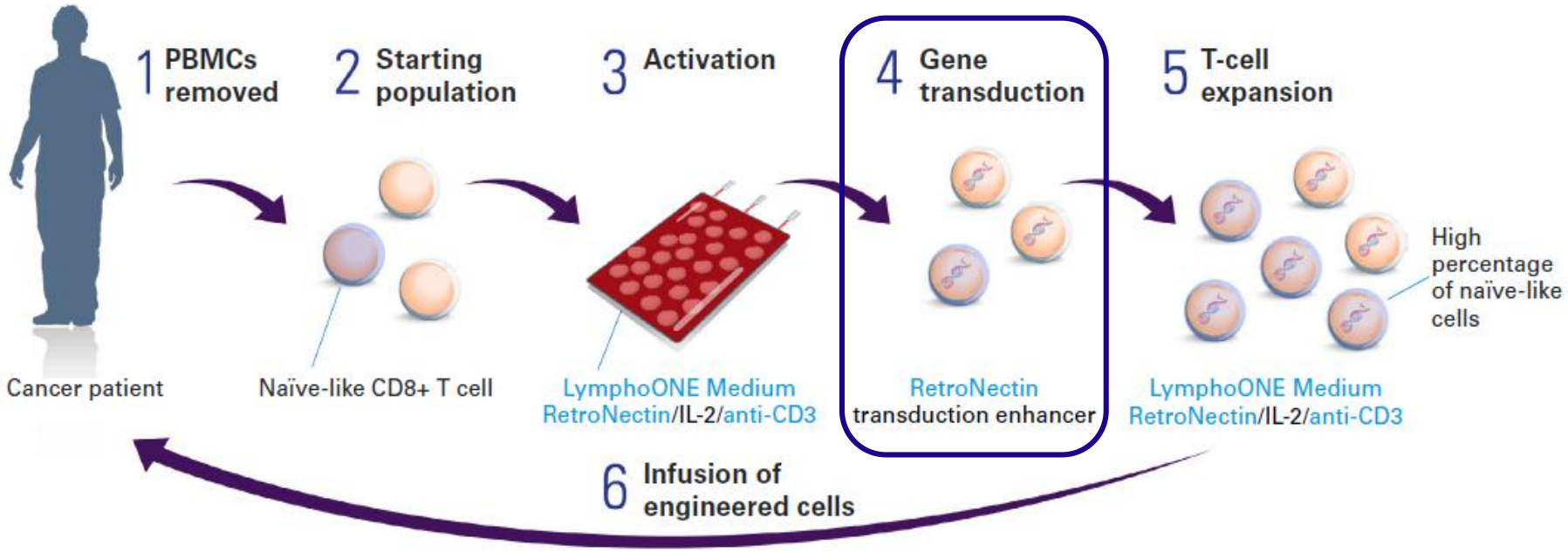


Clontech TaKaRa cellartis

Use of RetroNectin GMP in clinical trials

- Used in over 68 protocols for clinical trials, at 44 institutions worldwide:
  - **Metastatic Synovial Cell Sarcoma and Melanoma:** Transduction of patient-derived lymphocytes with TCR/CAR genes that recognize cancer antigens (*e.g.*, MART-1, gp100, or NY-ESO-1) for therapy (Robbins et al. 2011, J Clin Oncol 29:917-92).
  - **Multiple Myeloma**: Anti-BCMA CAR T cells (Ali, S. A. *et al.* 2016 *Blood* **128,** 1688–1700).
  - **B-cell malignancies (Follicular lymphoma, CLL):** Transduction of patient derived T-cells with anti-CD19-CAR (Kochenderfer et al., 2012, Blood 119(12):2709-20).
  - **B cell acute lymphoblastic leukemia (B-ALL):** Five relapsed B-ALL subjects have been treated with autologous T cells expressing a CD19-specific CD28/CD3ζ second-generation dual-signaling chimeric antigen receptor (CAR) termed 19-28z (Brentjens RJ et al., 2013, Sci Transl Med. 5(177):177ra38)

Adoptive T-cell Therapy



Classical CAR/TCR engineering
- In-Fusion cloning
- Retroviral or lentiviral delivery
- RetroNectin transduction enhancer

Refinement of cell engineering (CRISPR/Cas9)
- Targeted CAR integration
- KO of specific genes
- Footprint-free engineering

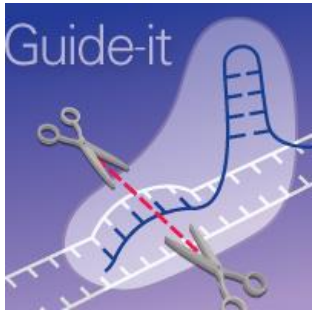
The promise of CRISPR/Cas9 genome editing

- Improve CAR expression / avoid insertional oncogenesis:
  - Site-specific integration of CAR/TCR constructs
- Prevent T-cell exhaustion due to inhibitory receptors (immune checkpoints):
  - Knockout of PD-1, CTLA-4, or LAG-3 receptors
- Prevent graft-versus-host disease (GvHD):
  - Knockout TCR
- Prevent host rejection:
  - Knockout HLA or B2M

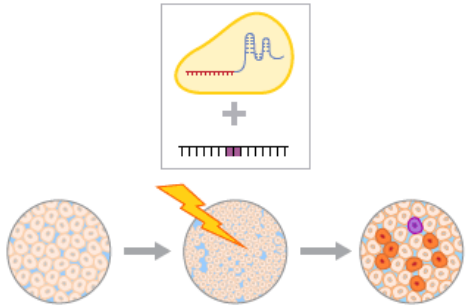
Key requirements for allogeneic, off-the-shelf solutions

Footprint-free T-cell engineering

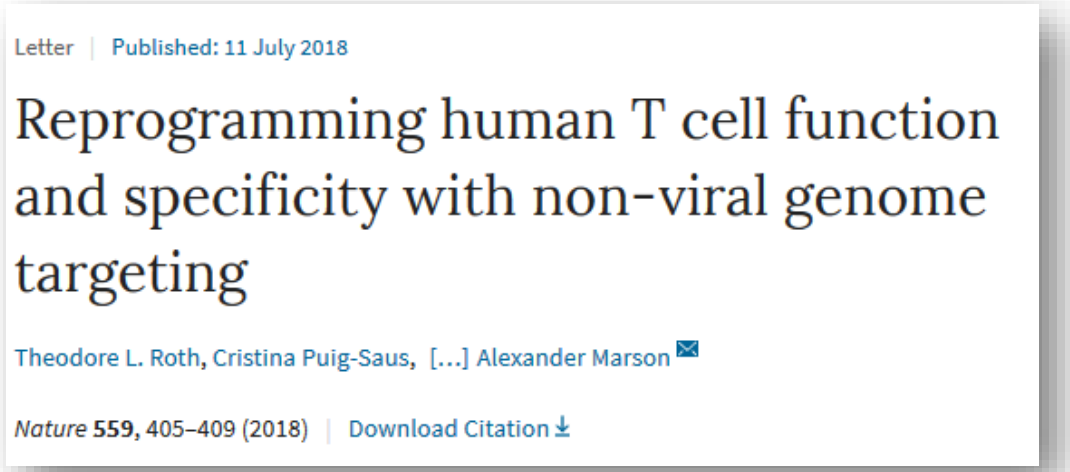
- Guide-it tools for CRISPR/Cas9 gene editing
  - Guide-it Recombinant Cas9 (Electroporation-Ready):
    - High concentration and low glycerol
  - Guide-it sgRNA *In Vitro* Transcription Kit & Screening Kit:
    - Produce sgRNAs using IVT and screen for efficacy
  - Guide-it Long ssDNA Production System
    - Produce ssDNA repair templates up to 5 kb
    - Higher knockin specificity compared to dsDNA templates
    - Lower toxicity compared to dsDNA templates



Electroporation of RNP complexes (and ssDNA HDR template) for KO/KI



Footprint-free T-cell engineering



Letter | Published: 11 July 2018

Reprogramming human T cell function and specificity with non-viral genome targeting

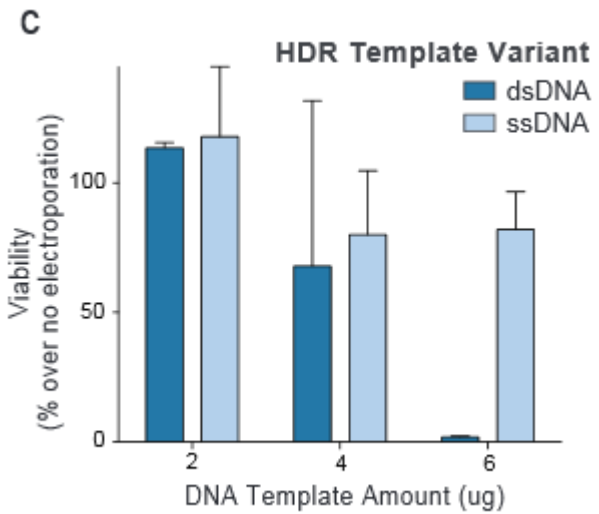
Theodore L. Roth, Cristina Puig-Saus, [...] Alexander Marson

Nature **559**, 405–409 (2018) | Download Citation

- Knockin of sequences encoding a TCR specific to the NY-ESO-1 antigen at the *TCR*α locus using electroporation of RNP complexes
- Evaluation of long ssDNA repair templates vs dsDNA

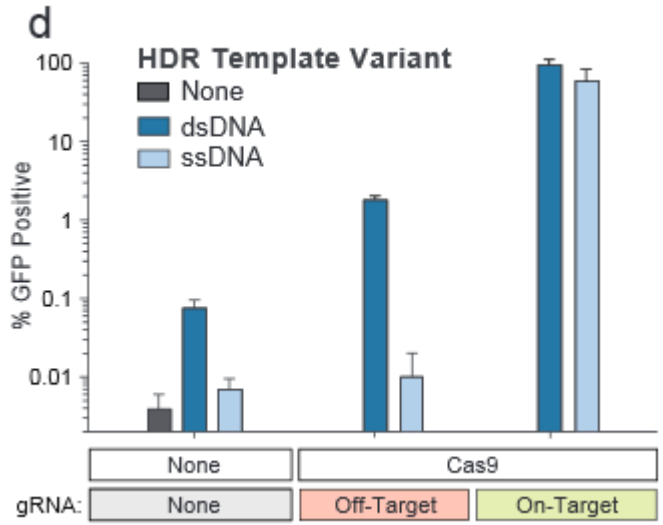
Footprint-free T-cell engineering

ssDNA is less toxic



At 4 days post-electroporation, long ssDNA HDR templates (~1.3 kb) did not show the decreasing viability in CD3+ T cells electroporated with a linear dsDNA HDR template of the same length.

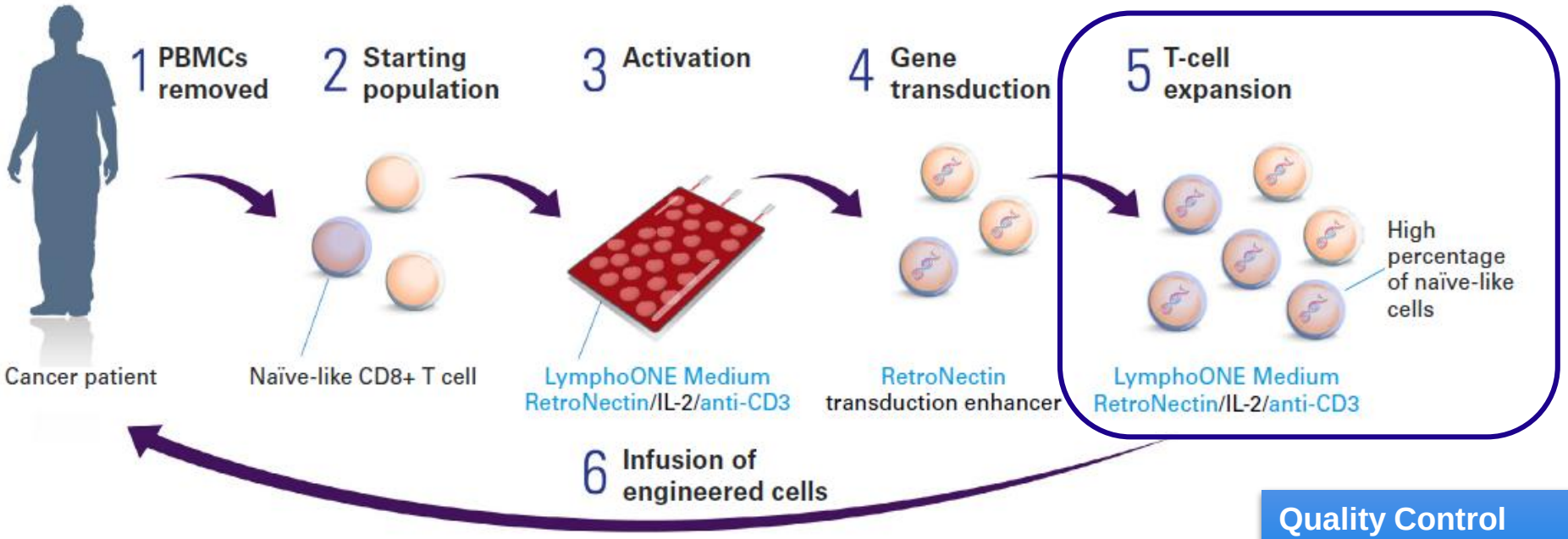
ssDNA is more specific



ssDNA HDR templates reduced functional off-target integrations approximately 100-fold, while maintaining efficient on-target integration.

Roth TL et al., Nature 2018; 559:405–409.

Adoptive T-cell Therapy



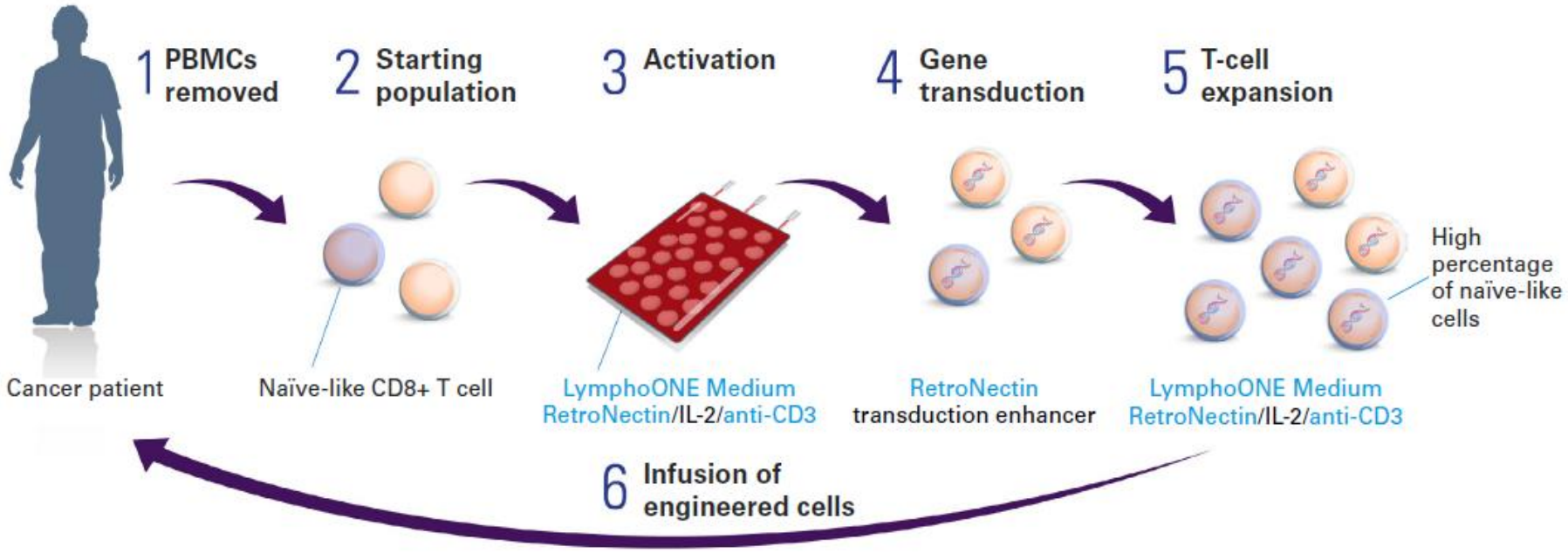
Quality Control

- Mycoplasma
- RetroNectin EIA Kit
- Virus detection (copy number, integration site)

Quality Testing of engineered T cells

- Mycoplasma Detection
  - TaKaRa PCR Mycoplasma Detection Set
- RetroNectin Detection
  - RetroNectin EIA Kit: quantitative determination of release from RetroNectin-coated plates and residual RetroNectin levels in cryopreservation solutions of cells cultured or transduced in the presence of RetroNectin
- Provirus copy number detection (qPCR)
  - Provirus Copy Number Detection Primer Set, Human (for Real Time PCR) & Cycleave PCR Core Kit (MMLV-derived Retrovirus)
  - Lenti-X Provirus Quantitation Kit
- Integration Site Analysis
  - Retro-X Integration Site Analysis Kit
  - Lenti-X Integration Site Analysis Kit

Adoptive T-cell Therapy



Immune repertoire analysis
- SMARTer TCR profiling

Transcriptomic analysis
- SMARTer RNA-seq portfolio
- qPCR portfolio

DNA-seq
- ThruPLEX
- PicoPLEX

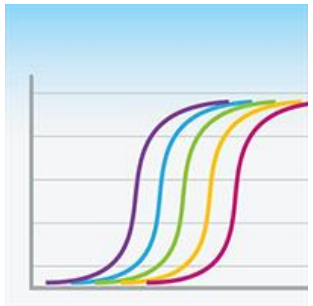
TCR Profiling

- Determining T-cell repertoires in cancer patients
  - Upstream of a CAR-T workflow: biomarker discovery
- Identifying tumor-reactive T-cell clones
- 5' RACE-based approach that captures full-length sequence information for entire variable regions of TCR-α and TCR-β subunits
  - SMARTer Human TCR a/b Profiling Kit
  - SMARTer Human single cell TCR a/b Profiling Kit
  - SMARTer Mouse TCR a/b Profiling Kit

Transcriptional Profiling / DNA-seq

- SMART-Seq kits
  - Single-cell mRNA-seq: solutions for mRNA-seq with ultra low inputs
  - Total RNA-seq: identification of both non-coding and coding RNAs
  - Small RNA-seq: miRNAs, siRNAs, piRNAs, snpRNAs

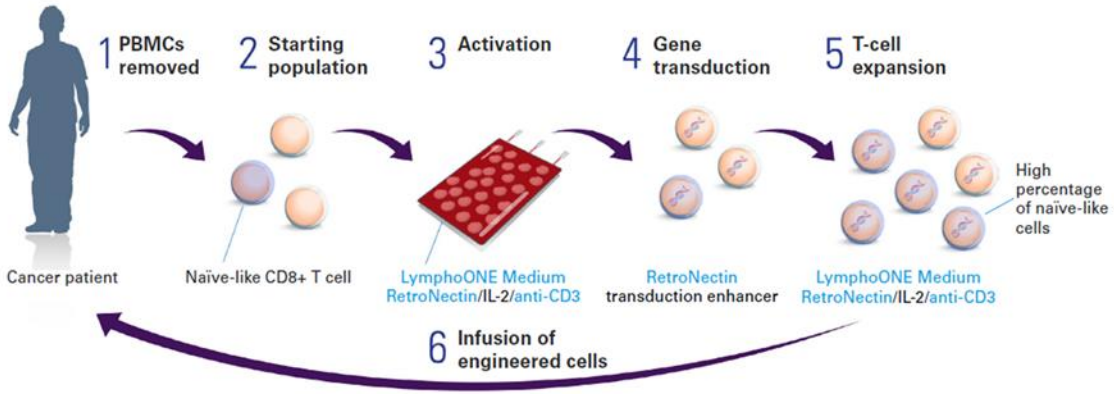
- qRT-PCR portfolio
  - One-step or two-step RT-qPCR kits
  - Probe-based or TB Green dye-based



- DNA-seq kits
  - ThruPLEX Plasma-seq / ThruPLEX Tag-seq Kits: cell-free DNA sequencing
  - PicoPLEX Gold: analysis of tumor burden (CTCs)

Summary – T-cell stimulation & expansion

- Benefits of the combined use:
  - Highly efficient expansion of T cells
  - Greater proportion of naïve-like T cells (CD45RA$^+$/CCR7$^+$ phenotype) with high therapeutic potential



LymphoONE Medium
WK552S/WK552

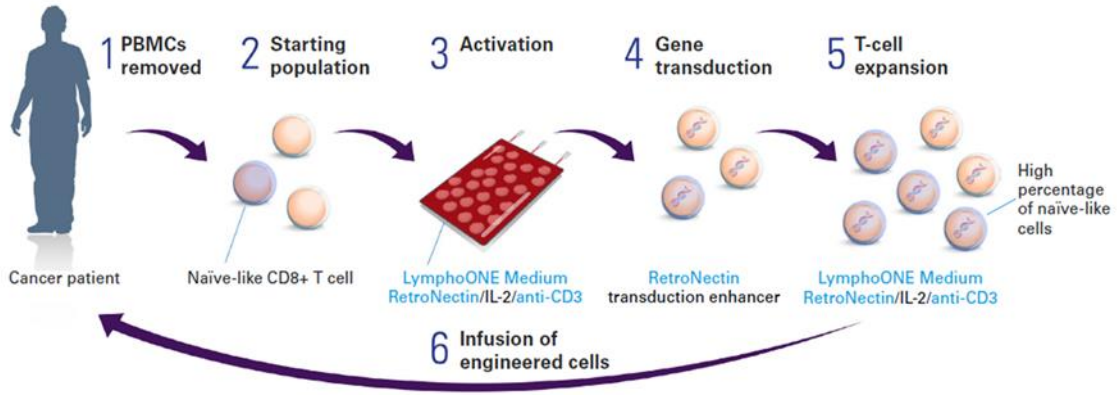
Anti-CD3 antibody
T210

RetroNectin Reagent
T100A/B, T202

CultiLife Culture Bag
FU0005/FU0010

Additional tools for T-cell engineering and analysis

- TCR/CAR vector construction:
  - Viral vectors
  - In-Fusion cloning
  - PrimeSTAR Max Polymerase
- Viral transduction:
  - Packaging, Titration
  - Concentration, Purification
  - RetroNectin transduction enhancer
  - Integration Site Analysis
- CRISPR-mediated T-cell engineering:
  - Recombinant Cas9
  - sgRNA IVT Kit
  - Long ssDNA Production System



- TCR profiling:
  SMARTer Human TCR a/b Profiling Kit
  SMARTer Human scTCR a/b Profiling Kit
- Transcriptional profiling:
  - SMART-Seq kits
  - qPCR portfolio
- DNA-seq
  - ThruPLEX, PicoPLEX Gold

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