

YMTHE, Volume 28

Supplemental Information

TEG001 Insert Integrity from Vector

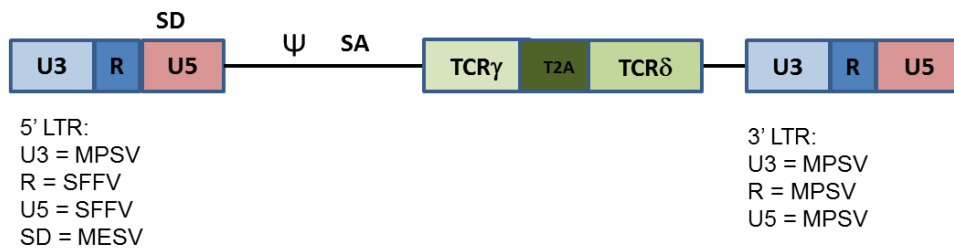
Producer Cells until Medicinal Product

Trudy Straetemans, Anke Janssen, Koen Jansen, Ruud Doorn, Tineke Aarts, Anna D.D. van Muyden, Marieke Simonis, Judith Bergboer, Moniek de Witte, Zsolt Sebestyen, and Jurgen Kuball

Supplemental Information

Figure S1. TEG001 transgene in the manufacturing process

A



B

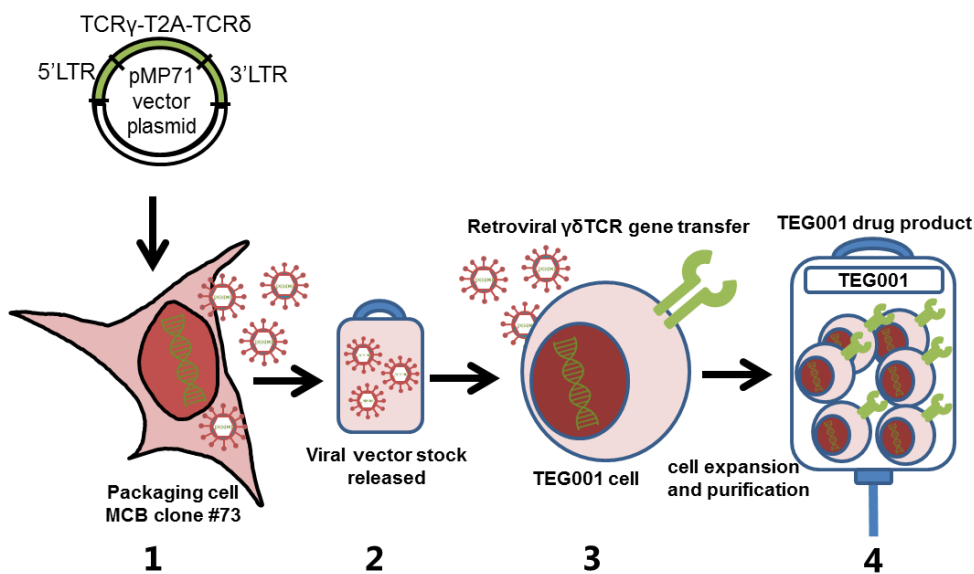


Figure S1. TEG001 transgene in the manufacturing process

A. Schematic picture of the TEG001 transgene DNA. LTR = Long Terminal Repeat; SD = Splice Donor site; SA = Splice Acceptor; Ψ = Packaging signal; PBS = endogenous primer binding site for initiation of retroviral inverse transcription

MPSV= Myeloproliferative Sarcoma Virus; SFFV= Spleen Focus Forming Virus; MESV= Murine Embryonic Stem cell Virus; T2A = 2A ribosomal skipping sequence derived from *Thosea Asigna* Virus. **B.** TEG001 transgene in the manufacturing chain.

1. TEG001 transgene from pMP71-based retroviral vector is stably integrated into the 293VecRD114 packaging cell genome followed by selection of a single cell clone to expand and produce the master cell bank (MCB).

2. From the MCB a GMP-lot vector supernatant was produced and released at a dedicated manufacturing site. Viral particles contain TEG001 proviral DNA.

3. Patient derived T cells are activated and transduced with viral particles to insert the TEG001 transgene and express the tumor-specific $\gamma\delta$ TCR, followed by expansion and purification.

4. TEG001 drug product is formulated and released to be infused into the patient.

Figure S2. Targeted Locus Amplification

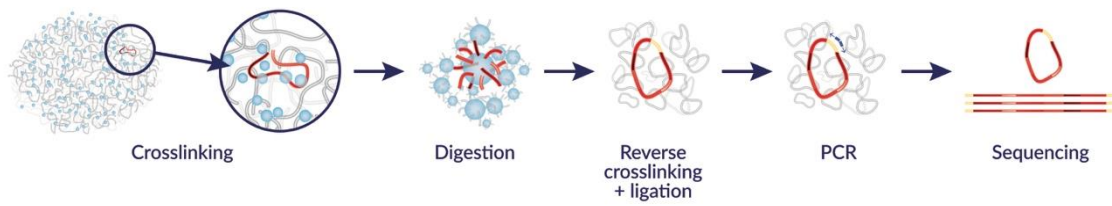
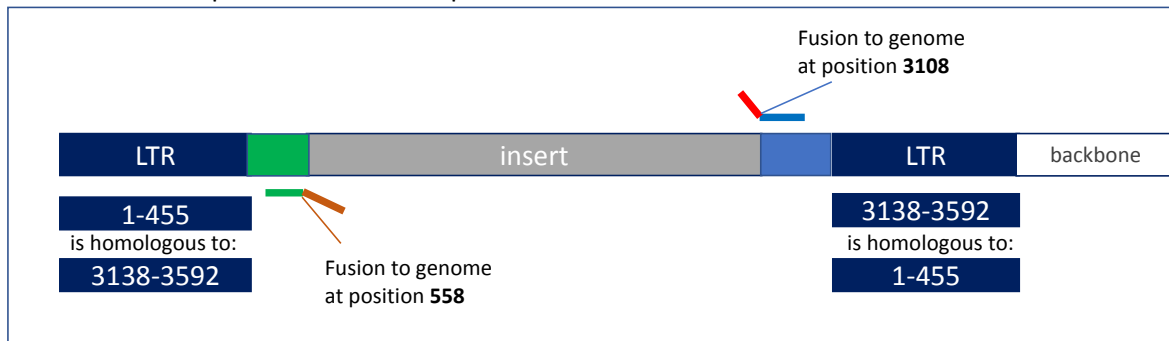


Figure S2. Schematic representation of TLA technology

First step in the TLA protocol is cross-linking of the DNA in the cells. Next the cross-linked DNA is digested, ligated and isolated. Overall, these steps lead to reshuffling of the DNA in the input material, which are millions of cells. In this reshuffling step the original physical proximity of DNA sequences is a key factor in this random process. Therefore, a large region can be targeted and sequenced specifically. The specific region of interest, in this case the transgene sequence, is amplified by PCR using transgene specific primer sets. Finally, NGS sequencing provides the data sets of interest.

Figure S3. TEG001 integration

A. Schematic representation of the plasmid reference



B. Schematic representation of the integration in the genome

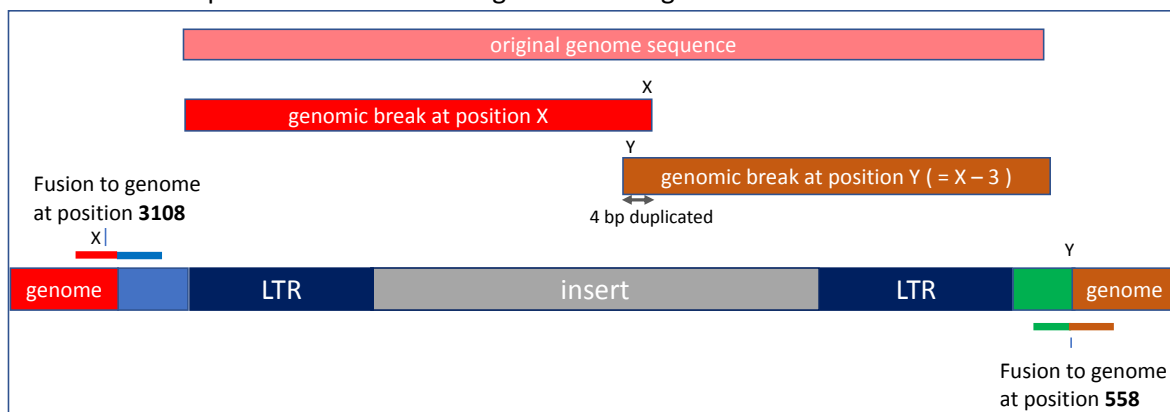


Figure S3. Illustration of TEG001 insert integration

A. Schematic representation of the plasmid reference sequence indicating the positions that fuse to the genome. As a result of retroviral integration and replication process from viral RNA into the genome, the fusion sites are not at the 5' and 3' outer ends of the sequence. **B.** All genome fusions are found with the same two positions within the TEG001 transgene, namely positions 558 and 3108 and how these fusion sites in the transgenic sequence correspond to a genomic integration is shown. 4 bp of the human host genome were duplicated at the site of integration.

Figure S4. TEG001 medicinal product contains central memory and effector memory T cells

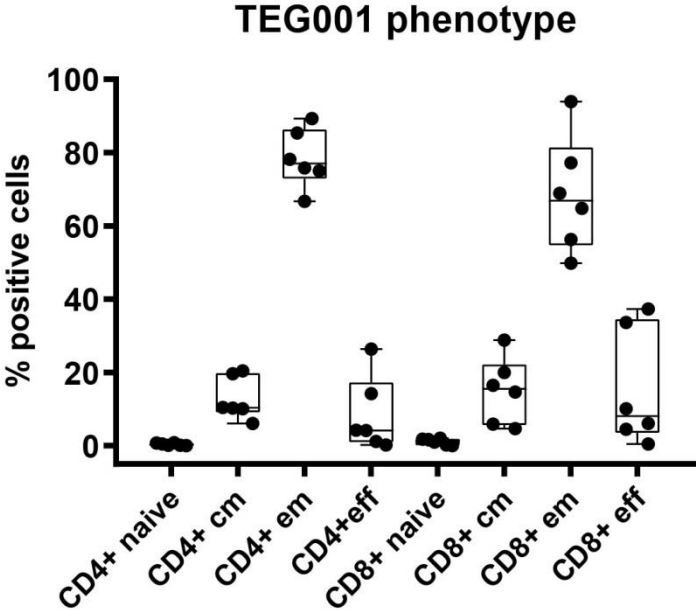


Figure S4. Differentiation phenotype of TEG001 medicinal product
TEG001 cells have a predominant effector-memory phenotype. The phenotype of TEG001 medicinal product derived from five different healthy donors was determined by measuring CD45RO in combination with CD62L expression. CD45RO-/CD62L+ is considered as naive, CD45RO+/CD62L+ as central memory (cm), CD45RO+/CD62L- as effector memory (em), and CD45RO-/CD62L- as effector (eff).

Figure S5. TEG001 qPCR quality controls

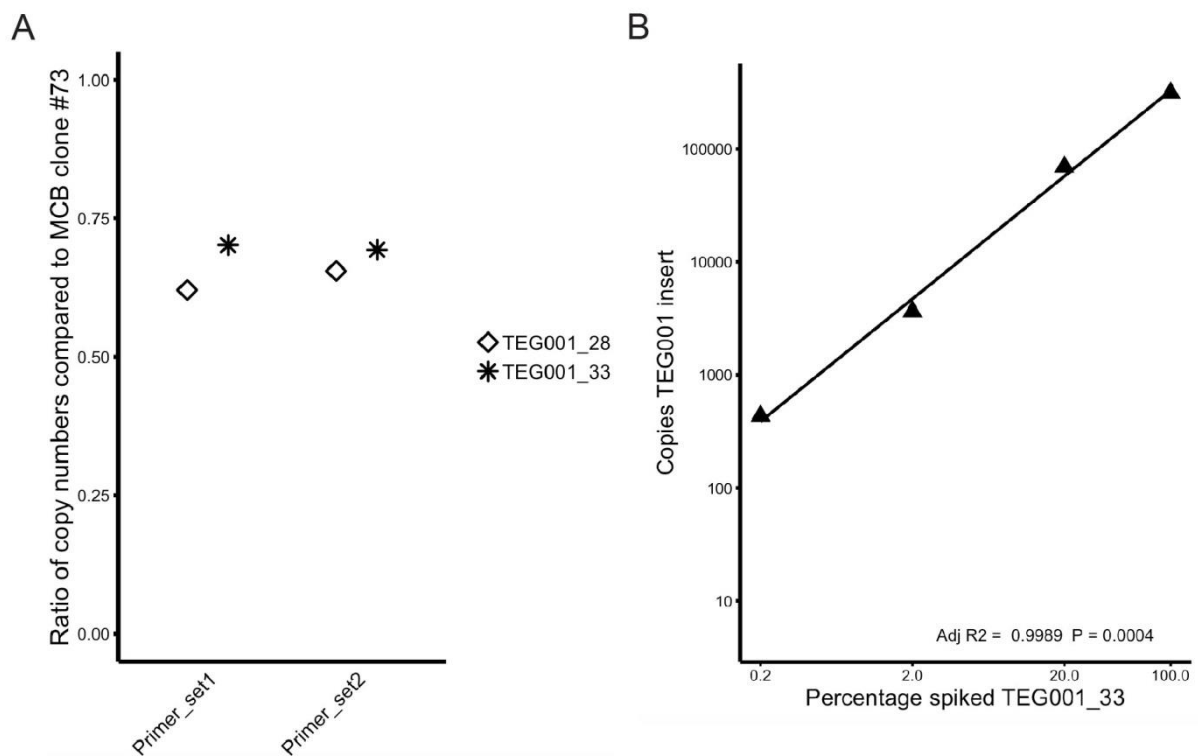


Figure S5. Quality controls for gene specific qPCR to detect copy numbers TEG001 transgene

A. Copy numbers per unit/DNA are compared to copy number per unit/DNA of MCB clone #73 for two different primer sets for qPCR are shown for TEG001_28 and TEG001_33 cell product.

Experiment was performed in triplicates, mean copy number per primer set is shown. **B.** TEG001_33 cells were spiked in different concentrations (0.2%, 2%, 20% and 100%) into a background of healthy donor PBMC's. Relation between the percentage of spiked TEG001 cells and the copy number of TEG001 insert per ng DNA is shown. Experiment was performed in duplicates, mean copy number per frequency is shown.

Figure S6

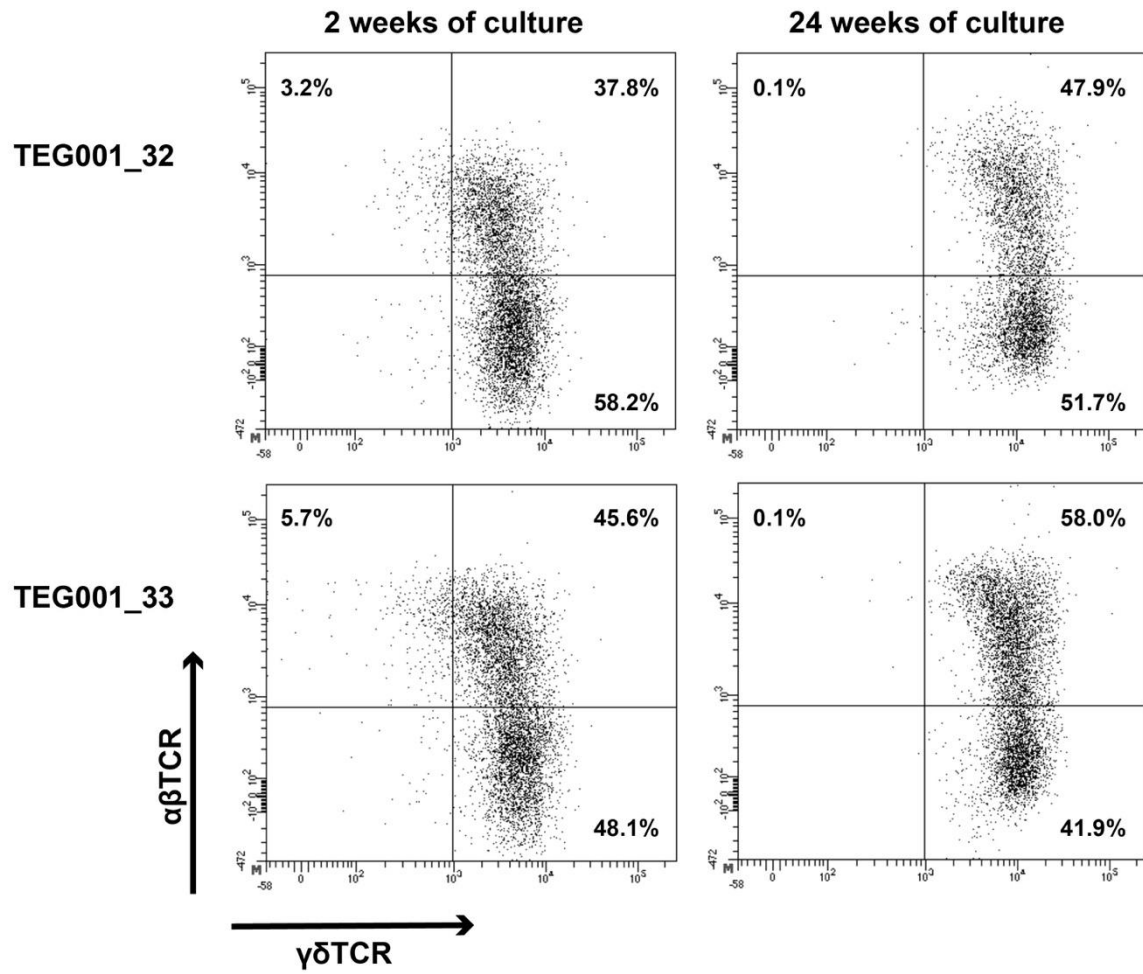


Figure S6. Stable expression of $\gamma\delta$ TCR protein in cultured TEG001 cells

T cells from healthy donor apheresis material was used to produce TEG001 cells according to ¹⁶. Long-term $\gamma\delta$ TCR expression was measured by flow cytometry after 24 weeks of *in vitro* culture using a rapid expansion protocol, including a bi-weekly stimulation with feeder cells and cytokines.

Table S1 Comprehensive overview of molecular characterization of CAR T and TCR engineered T cell products

retro = gamma retroviral vector, lenti = lentiviral vector, transient = transient lentiviral transfection of GMP packaging cell line, nr = not reported, na = not applicable, X = Reported, RC = Release Criterion for medicinal product

Product	Clinical trial identifier	Vector carrier	Master cell bank				Medicinal Infusion Product			Reference
			Transgene Integrity	Vector Copy Numbers	Insertion Site	Reference to GMP production	Transgene Integrity	Vector Copy Numbers	Insertion Site	
CAR T cell clinical trial reports										
CD19-28z	NCT00924326 NCT01087294	retro	nr	nr	nr	1	nr	nr	nr	2-5
CD19-28z	NCT01593696	retro	nr	nr	nr	1, 6	nr	nr	nr	7
CD19-28z	NCT02348216	retro	nr	nr	nr	-	nr	nr	nr	8, 9
CD19-28z	NCT00466531	retro	Reported - data not shown	Reported - data not shown	nr	10	nr	nr	nr	11
CD19-28z	NCT01044069	retro	Reported - data not shown	Reported - data not shown	nr	10	nr	X individual VCN/cell	nr	12, 13
CD4z	NCT0101341, 14 and 15	retro	nr	nr	nr	14	nr	X individual VCN/cell	X	14, 16
CTL019 CD19-41BBz	NCT01029366	lenti	na	na	na		nr	X individual VCN/cell	nr	17, 18
CTL019 CD19-41BBz	NCT01029366	lenti	na	na	na		nr	X Mean VCN/cell of released products	nr	19
CTL019 CD19-41BBz	NCT01626495	lenti	na	na	na		nr	nr	nr	20
CTL019 CD19-41BBz	NCT01626495 NCT01029366	lenti	na	na	na		nr	RC: 0.02-4 VCN/cell, not per individual product	nr	21
CTL019 CD19-41BBz	NCT01029366	lenti	na	na	na		X	nr	X	22
CTL019 CD19-41BBz	NCT01626495	lenti	na	na	na		X in leukemic cells	nr	X	23
CTL019 CD19-41BBz	NCT02435849	lenti	na	na	na		nr	nr	nr	24
CART-meso-41BBz	NCT02159716	lenti	na	na	na		nr	nr	nr	25
CD19-CD28TM-41BBz	NCT01865617	lenti	na	na	na		nr	nr	nr	26, 27
CD20-CD28TM-41BBz	NCT00621452	electoporation	na	na	na		nr	nr	nr	28

GD2 CAR	NCT00085930	retro	Reported - data not shown	nr	nr	29	nr	nr	nr	29, 30
BCMA 41BBz	NCT02658929	lenti	na	na	na		nr	nr	nr	31
TCR T cell clinical trial reports										
NY-ESO ^{c259} TCR	NCT01352286	lenti	na	na	na		nr	nr	nr	32, 33
NY-ESO TCR	NCT00670748	retro	nr	nr	nr	34	nr	nr	nr	35
Mart-1 TCR gp100 TCR	NCI-07-C-0174 NCI-07-C-0175	retro	nr	nr	nr		nr	nr	nr	34, 36, 37
MAGE-A3 TCR	NCT0135040 NCT01352286	lenti	na	na	na		nr	X VCN/cell of individual products	nr	38, 39
WT-1 TCR	NCT01640301	lenti	na	na	na		nr	RC: < 5 VCN/cell Not per individual product reported	nr	40
Pre-clinical reports										
CD20 CAR	-	lenti	na	na	na		nr	X	nr	41
CD19-41BBz CAR	-	lenti	na	na	na		nr	X mean and maximum VCN/cell, not per individual product	nr	42
CD19 CAR	IND #14645	lenti	na	na	na		nr	X VCN/cell per individual products	nr	43
BCMA CAR	NCT02658929	lenti	na	na	na		nr	X	nr	44
Mart-1 1D3 TCR	NTR3539	retro	Reported - data not shown	nr	nr		nr	nr	nr	45
mF5Mart-1 TCR F5Mart-1 TCR CD19 CAR CEA CAR 2G1 TCR	-	retro	nr	nr	nr	46	nr	nr	nr	46, 47
This report										
TEG001	NTR6541	retro	X	X	X	This report	X	X	X	This report

Table S1 references

1. Kochenderfer, J.N., et al., *Construction and Preclinical Evaluation of an Anti-CD19 Chimeric Antigen Receptor*. Journal of Immunotherapy, 2009. **32**(7): p. 689-702.
2. Kochenderfer, J.N., et al., *Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation*. Blood, 2013. **122**(25): p. 4129-4139.
3. Kochenderfer, J.N., et al., *B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells*. Blood, 2012. **119**(12): p. 2709-2720.
4. Kochenderfer, J.N., et al., *Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19*. Blood, 2010. **116**(20): p. 4099-4102.
5. Kochenderfer, J.N., et al., *Lymphoma Remissions Caused by Anti-CD19 Chimeric Antigen Receptor T Cells Are Associated With High Serum Interleukin-15 Levels*. Journal of Clinical Oncology, 2017. **35**(16).
6. Tumaini, B., et al., *Simplified process for the production of anti-CD19-CAR-engineered T cells*. Cytotherapy, 2013. **15**(11): p. 1406-1415.
7. Lee, D.W., et al., *T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial*. Lancet, 2015. **385**(9967): p. 517-528.
8. Neelapu, S.S., et al., *Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma*. New England Journal of Medicine, 2017. **377**(26): p. 2531-2544.
9. Locke, F.L., et al., *Phase 1 Results of ZUMA-1: A Multicenter Study of KTE-C19 Anti-CD19 CAR T Cell Therapy in Refractory Aggressive Lymphoma*. Molecular Therapy, 2017. **25**(1): p. 285-295.
10. Hollyman, D., et al., *Manufacturing validation of biologically functional T cells targeted to CD19 antigen for autologous adoptive cell therapy*. J Immunother, 2009. **32**(2): p. 169-80.
11. Brentjens, R.J., et al., *Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias*. Blood, 2011. **118**(18): p. 4817-4828.
12. Brentjens, R.J., et al., *CD19-Targeted T Cells Rapidly Induce Molecular Remissions in Adults with Chemotherapy-Refractory Acute Lymphoblastic Leukemia*. Science Translational Medicine, 2013. **5**(177).
13. Davila, M.L., et al., *Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia*. Sci Transl Med, 2014. **6**(224): p. 224ra25.
14. Mitsuyasu, R.T., et al., *Prolonged survival and tissue trafficking following adoptive transfer of CD4 zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects*. Blood, 2000. **96**(3): p. 785-793.
15. Deeks, S.G., et al., *A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy*. Molecular Therapy, 2002. **5**(6): p. 788-797.
16. Scholler, J., et al., *Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells*. Sci Transl Med, 2012. **4**(132): p. 132ra53.
17. Kalos, M., et al., *T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia*. Sci Transl Med, 2011. **3**(95): p. 95ra73.
18. Porter, D.L., et al., *Chimeric Antigen Receptor-Modified T Cells in Chronic Lymphoid Leukemia*. New England Journal of Medicine, 2011. **365**(8): p. 725-733.
19. Porter, D.L., et al., *Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia*. Science Translational Medicine, 2015. **7**(303).
20. Grupp, S.A., et al., *Chimeric Antigen Receptor-Modified T Cells for Acute Lymphoid Leukemia*. New England Journal of Medicine, 2013. **368**(16): p. 1509-1518.
21. Maude, S.L., et al., *Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia*. New England Journal of Medicine, 2014. **371**(16): p. 1507-1517.
22. Fraietta, J.A., et al., *Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells*. Nature, 2018. **558**(7709): p. 307-312.
23. Ruella, M., et al., *Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell*. Nat Med, 2018. **24**(10): p. 1499-1503.
24. Maude, S.L., et al., *Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia*. New England Journal of Medicine, 2018. **378**(5): p. 439-448.
25. Haas, A.R., et al., *Phase I Study of Lentiviral-Transduced Chimeric Antigen Receptor-Modified T Cells Recognizing Mesothelin in Advanced Solid Cancers*. Mol Ther, 2019.
26. Turtle, C.J., et al., *CD19 CAR-T cells of defined CD4(+): CD8(+) composition in adult B cell ALL patients*. Journal of Clinical Investigation, 2016. **126**(6): p. 2123-2138.
27. Turtle, C.J., et al., *Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8(+) and CD4(+) CD19-specific chimeric antigen receptor-modified T cells*. Science Translational Medicine, 2016. **8**(355).
28. Till, B.G., et al., *CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results*. Blood, 2012. **119**(17): p. 3940-3950.
29. Pule, M.A., et al., *Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma*. Nature Medicine, 2008. **14**(11): p. 1264-1270.
30. Louis, C.U., et al., *Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma*. Blood, 2011. **118**(23): p. 6050-6056.
31. Raje, N., et al., *Anti-BCMA CAR T-Cell Therapy bb2121 in Relapsed or Refractory Multiple Myeloma*. New England Journal of Medicine, 2019. **380**(18): p. 1726-1737.
32. Rapoport, A.P., et al., *NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma*. Nature Medicine, 2015. **21**(8): p. 914-921.
33. Stadtmayer, E.A., et al., *Long-term safety and activity of NY-ESO-1 SPEAR T cells after autologous stem cell transplant for myeloma*. Blood Advances, 2019. **3**(13): p. 2022-2034.
34. Johnson, L.A., et al., *Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen*. Blood, 2009. **114**(3): p. 535-546.
35. Robbins, P.F., et al., *A Pilot Trial Using Lymphocytes Genetically Engineered with an NY-ESO-1-Reactive T-cell Receptor: Long-term Follow-up and Correlates with Response*. Clinical Cancer Research, 2015. **21**(5): p. 1019-1027.

36. Chodon, T., et al., *Adoptive Transfer of MART-1 T-Cell Receptor Transgenic Lymphocytes and Dendritic Cell Vaccination in Patients with Metastatic Melanoma*. *Clinical Cancer Research*, 2014. **20**(9): p. 2457-2465.
37. Morgan, R.A., et al., *Cancer regression in patients after transfer of genetically engineered lymphocytes*. *Science*, 2006. **314**(5796): p. 126-129.
38. Cameron, B.J., et al., *Identification of a Titin-Derived HLA-A1-Presented Peptide as a Cross-Reactive Target for Engineered MAGE A3-Directed T Cells*. *Science Translational Medicine*, 2013. **5**(197).
39. Linette, G.P., et al., *Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma*. *Blood*, 2013. **122**(6): p. 863-871.
40. Chapuis, A.G., et al., *T cell receptor gene therapy targeting WT1 prevents acute myeloid leukemia relapse post-transplant*. *Nat Med*, 2019. **25**(7): p. 1064-1072.
41. Lock, D., et al., *Automated Manufacturing of Potent CD20-Directed Chimeric Antigen Receptor T Cells for Clinical Use*. *Hum Gene Ther*, 2017. **28**(10): p. 914-925.
42. Blaeschke, F., et al., *Induction of a central memory and stem cell memory phenotype in functionally active CD4(+) and CD8(+) CAR T cells produced in an automated good manufacturing practice system for the treatment of CD19(+) acute lymphoblastic leukemia*. *Cancer Immunol Immunother*, 2018. **67**(7): p. 1053-1066.
43. Wang, X., et al., *Phenotypic and functional attributes of lentivirus-modified CD19-specific human CD8+ central memory T cells manufactured at clinical scale*. *J Immunother*, 2012. **35**(9): p. 689-701.
44. Friedman, K.M., et al., *Effective Targeting of Multiple B-Cell Maturation Antigen-Expressing Hematological Malignancies by Anti-B-Cell Maturation Antigen Chimeric Antigen Receptor T Cells*. *Hum Gene Ther*, 2018. **29**(5): p. 585-601.
45. Gomez-Eerland, R., et al., *Manufacture of gene-modified human T-cells with a memory stem/central memory phenotype*. *Hum Gene Ther Methods*, 2014. **25**(5): p. 277-87.
46. Feldman, S.A., et al., *Rapid Production of Clinical-Grade Gammaretroviral Vectors in Expanded Surface Roller Bottles Using a "Modified" Step-Filtration Process for Clearance of Packaging Cells*. *Human Gene Therapy*, 2011. **22**(1): p. 107-115.
47. Lu, T.Y.L., et al., *A Rapid Cell Expansion Process for Production of Engineered Autologous CAR-T Cell Therapies*. *Human Gene Therapy Methods*, 2016. **27**(6): p. 209-218.

Table S2. Overview of the exact locations of the 9 integrations of the TEG001 transgene in genome of the MCB.

sequence 1	position 1	orientation 1	sequence 2	position 2	orientation 2
pMP71_TCRγ5-T2A-δ5	558	tail	chr1	28970905	head
pMP71_TCRγ5-T2A-δ5	3108	head	chr1	28971527	tail
pMP71_TCRγ5-T2A-δ5	559	tail	chr1	234508872	tail
pMP71_TCRγ5-T2A-δ5	3108	head	chr1	234508871	head
pMP71_TCRγ5-T2A-δ5	558	tail	chr11	66667547	tail
pMP71_TCRγ5-T2A-δ5	3108	head	chr11	66667544	head
pMP71_TCRγ5-T2A-δ5	558	tail	chr2	69533753	head
pMP71_TCRγ5-T2A-δ5	3108	head	chr2	69533748	tail
pMP71_TCRγ5-T2A-δ5	558	tail	chr22	17857891	tail
pMP71_TCRγ5-T2A-δ5	3108	head	chr22	17857887	head
pMP71_TCRγ5-T2A-δ5	558	tail	chr5	99110063	head
pMP71_TCRγ5-T2A-δ5	3108	head	chr5	99110066	tail
pMP71_TCRγ5-T2A-δ5	558	tail	chr6	108183211	head
pMP71_TCRγ5-T2A-δ5	3108	head	chr6	108183215	tail
pMP71_TCRγ5-T2A-δ5	558	tail	chr6	132721404	tail
pMP71_TCRγ5-T2A-δ5	3108	head	chr6	132721407	head
pMP71_TCRγ5-T2A-δ5	558	tail	chr9	83990726	head
pMP71_TCRγ5-T2A-δ5	3108	head	chr9	83990729	tail