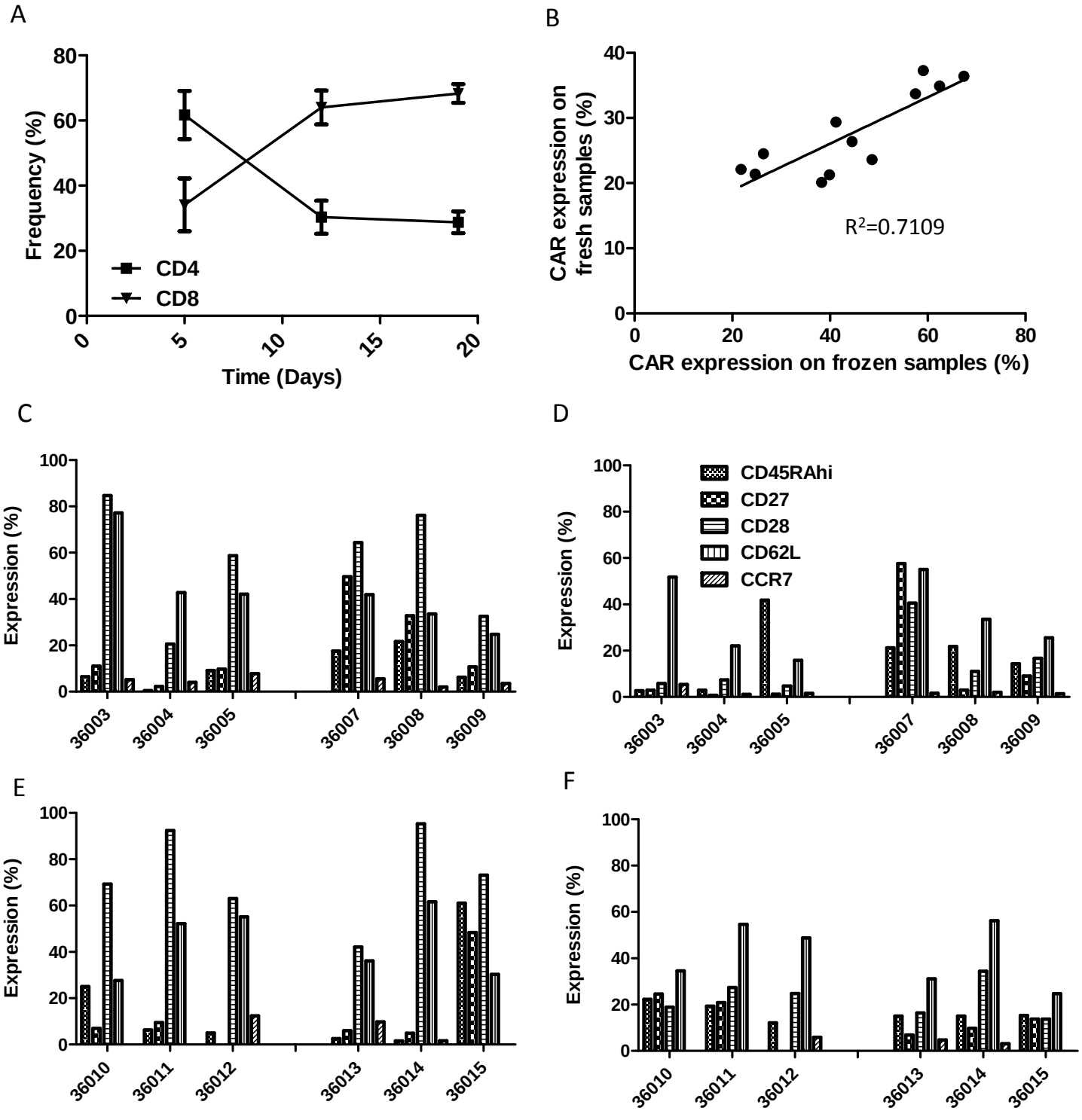
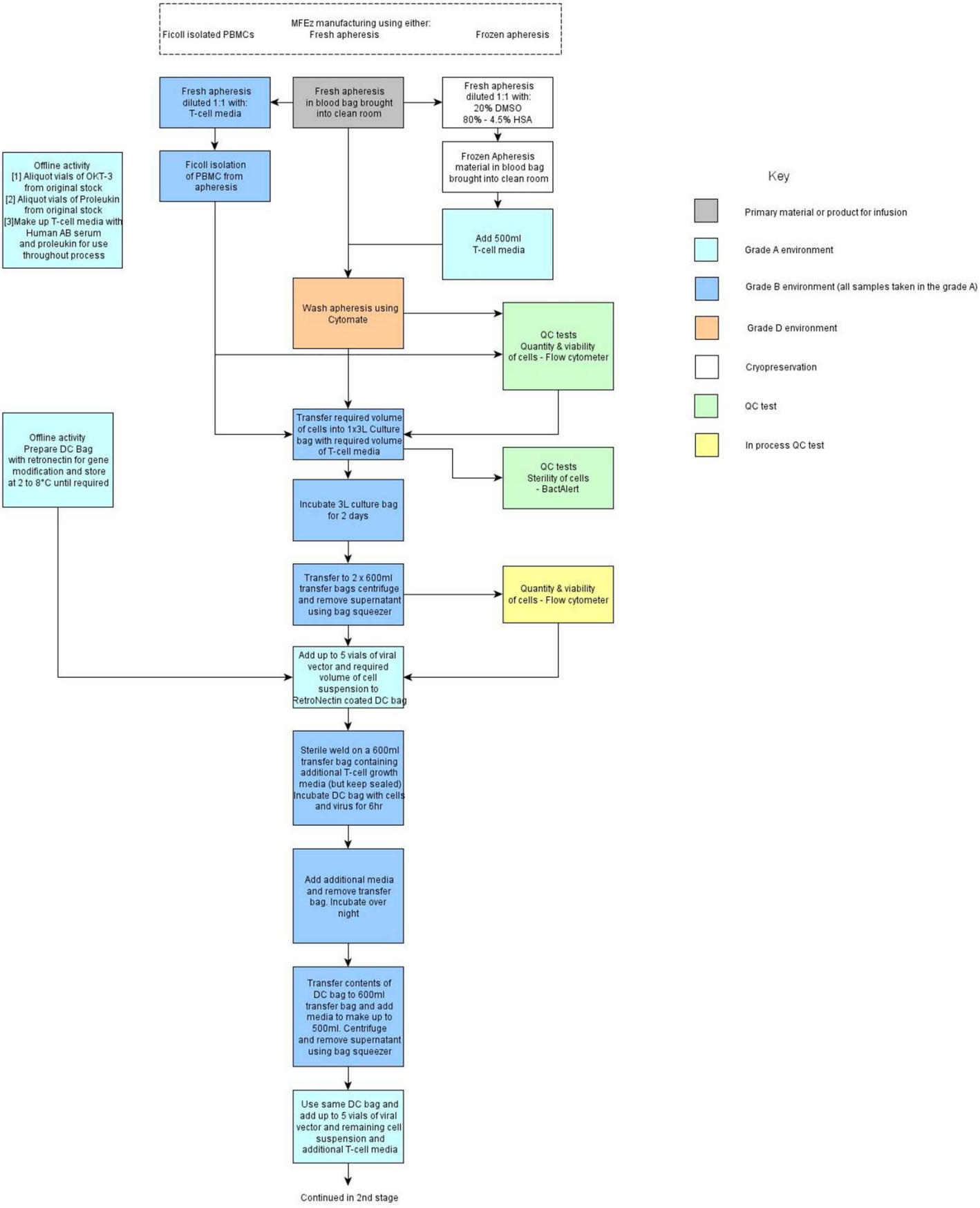
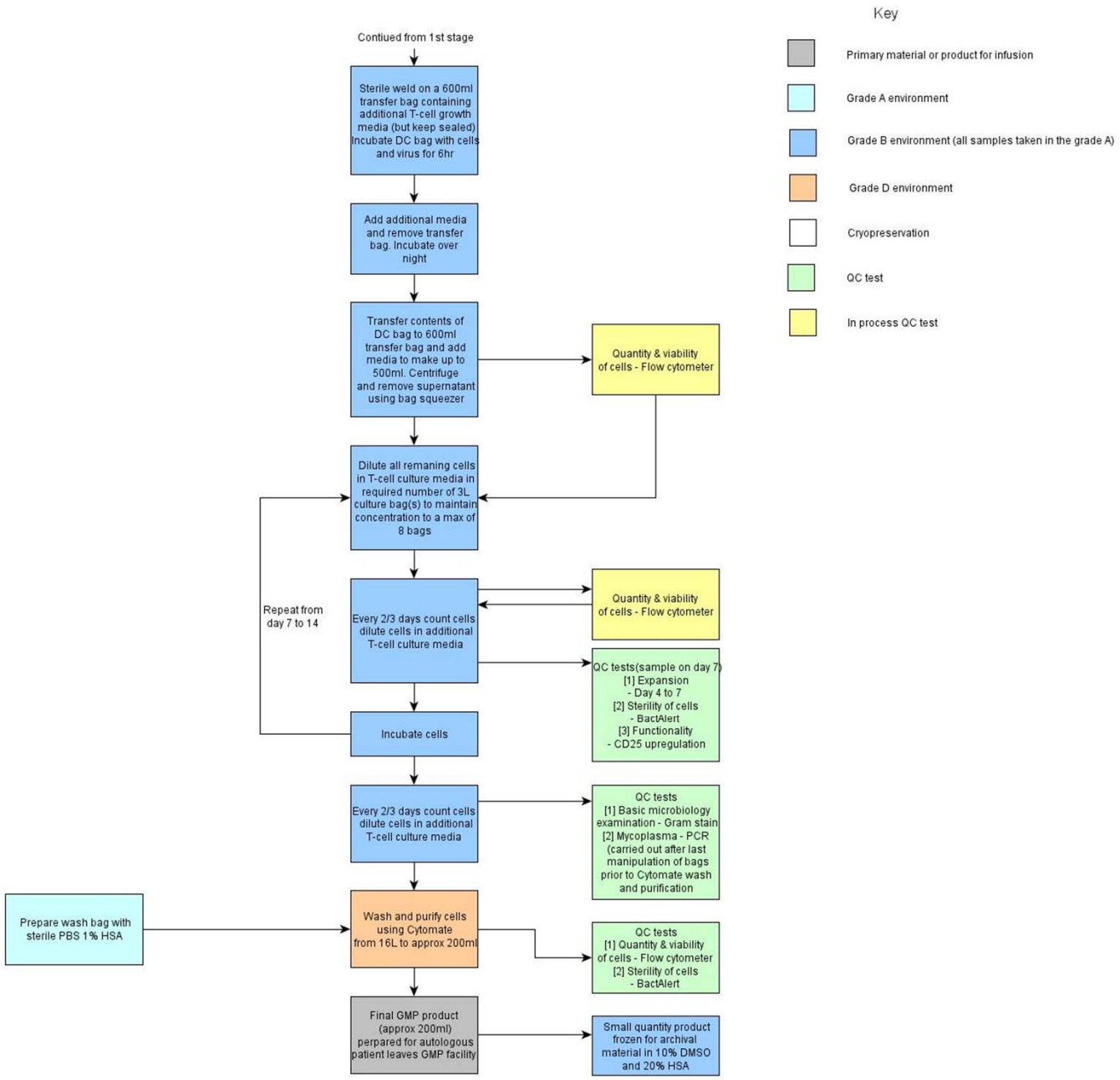


Supplementary Figure 1

(A) Three normal healthy donor buffy coats were used as a source of T-cells after a two day activation using anti-CD3ε and IL-2. The activated T-cells were harvested and transduced with MFEζ retroviral vector before further culture. At time points, CD4/CD8 subset frequency was determined by flow cytometry. (B) Plot of MFEζ CAR expression on fresh and frozen / thawed samples (r^2=0.7109). Relative expression of CD45RAhi, CD27, CD28, CD62L and CCR7 in (C, E) CD4+ and (D, F) CD8+ MFEζ patient T-cells.



Supplementary Figure 2 – Part 1



Supplementary Figure 2 – Part 2
Overview of the process used to generate MFEζ T-cells

Supplemental Table 1
Results from Quality Control testing of Master Cell Bank & End of Production Cells, PGMP15.

Quality Control Tests Master Cell Bank, PGMP15		
Parameter	**Acceptance Criteria**	**Result**
Recovery of cells after thawing	10% confluence is reached after 2 days	45% confluence was reached after 2 days
Titre of the produced supernatant	> 5×10^5 infectious particles per ml supernatant	A titer of 1.3 x 10^6 infectious particles / ml was calculated
Presence of the MFEζ transgene	MFEζ detectable by flow cytometry	The MFEζ transgene could be detected
Safety Tests		
Sterility of the cells (Bactec, 14 day culture)	No growth	No microbial growth was observed
Identity of the cells via *env*-specific PCR	Negative for amphotropic and ecotropic and positive for GALV *envelope* sequences	Pass
Identity of the cells via species PCR analysis	Positive for murine and negative for primate, rat or hamster beta globin sequences	Pass
Detection of mouse virus via mouse antibody production (MAP) test	Following mouse viruses are undetectable: • Ectromelia Virus • Hantaan Virus • K Virus • Lymphocytic Choriomeningitis Virus • Minute Virus • Adenovirus • Cytomegalovirus, • Encephalomyelitis Virus (Theiler's, GDVII) • Hepatitis Virus • Rotavirus (EDIM), • Pneumonia Virus • Polyoma Virus • Reovirus Type 3 • Sendai Virus • Thymic Virus, • Mycoplasma Pulmonis, • Lactic Dehydrogenase Virus	Pass
Test for bovine virus via coculture with KL-2 cells	CPE, hemadsorbtion and hemaglutination undetectable using cells from the MCB. Positive and negative control to	Pass
Test for porcine virus via coculture with PK-13 cells		Pass
Test for adventitious virus		Pass

via coculture with Vero cells	demonstrate the validity of the assay	
Test for adventitious virus via coculture with MRC-5 cells		Pass
Test for replication competent retroviruses (RCR) via extended S+L- assay		Pass
Test for bovine Polyomavirus via PCR	Bovine polyomaviruses sequences are undetectable	No virus detectable
Test for mycoplasma via indicator cell culture and culture method	No detectable mycoplasma by co-culture in solid and liquid media as well as by fluoressence staining of indicator cells.	No mycoplasma detectable

Quality Control Tests for End of Production Cells

Parameter	**Acceptance Criteria**	**Result**
Functionality (Cellular Assay / FACS analysis)	MFEζ detectable	The MFEζ transgene was detected
Safety Tests		
Sterility (BacTec system)	No microbial growth	No microbial growth was observed
RCR (PG4 S+L-)	No RCR detectable	No Cytopathic effect (CPE) was detected when the EPCs were used. A distinct CPE was detected when at least 0.37 $TCID_{50}$ where incubated with the test article and the detector cell line. No CPE was detected when the EPCs were used. A distinct CPE was detected when at least 1.9 $TCID_{50}$ where incubated with the test article and the 293 cells.

Supplemental Table 2 Results for MFEζ Retroviral Vector

Test	Acceptance Criteria	Results
Titre (Cellular Assay / FACS analysis)	Titre > 10^6 cells / ml	A titer of 1.0 x 10^7 infectious particles / ml was calculated
Functionality (Cellular Assay / FACS analysis)	MFEζ detectable	The MFEζ transgene could be detected
Sterility (BacTec system)	No microbial growth	No microbial growth was observed
Endotoxin (Culture assay according to EP)	Endotoxin concentration < 5 EU / ml	The level of endotoxin potency in the supernatant was 0.24 EU/mL
Mycoplasma (Semiquantitative assay according to EP)	No mycoplasma detectable	No mycoplasma could be detected by co-culture in solid and liquid media as well as by fluorescence staining of indicator cells
RCR (PG4 S+L-)	No RCR detectable	No CPE was detected when the retroviral vector containing supernatant PGMP15 was used. A distinct CPE was detected when at least 31 $TCID_{50}$ where incubated with the test article and the detector cell line No CPE was detected when the retroviral vector containing supernatant PGMP15 was used. A distinct CPE was detected when at least 31 $TCID_{50}$ where incubated with the test article and 292 cells

Supplemental Table 3 Justification of Specification for MFEz T cells

Test	Specification	Justification
Retroviral titre	> 10^5 infectious MFEζ retroviral vector particles/ml	A minimum of infectious vector particles/ml is necessary to achieve an effective transduction rate leading to the anticipated functionality of the cells.
Functionality of MFEζ-transgene	MFEζ-protein is detectable on cell surface by FACS	The expression of the transgene product MFEζ on the surface of the cells is a prerequisite for the detection of cancer-related antigens. Absence of expression or inability to bind the bCEA antigen would indicate mutations in the vector which would abolish the anticipated function.
Sterility	No detectable microbial growth in Bactec cultures	Drug substance should be without bacterial contaminants.
Bovine, porcine, murine, adventitious viruses	No virus detectable	Drug substance should be without viral contamination.
RCR	No RCR detectable	Drug substance should be without RCR contamination.
Mycoplasm	No mycoplasm detectable	Drug substance should be without mycoplasma contamination.
Endotoxin	< 5 EU/ml	High Endotoxin levels would indicate bacterial contamination. Because drug product will not be tested individually for Endotoxin levels, all raw materials for manufacturing of drug product should be tested for Endotoxin.

Supplemental Table 4.
Summary of Pre-release and Final product release criteria for MFEζ T cells

Day 7 Pre-Release Certification	**Threshold values**	**Notes**
Fold expansion	≥ 2.5 fold expansion in cell number by day 7	Fold expansion determined by the comparison of day 7 cell counts with day 4 counts (post transduction). Studies with normal donor T-cells suggested that an expansion of < 2.5 fold would result in less than 10^9 total cells after culture.
Transgene Expression	≥ 20% expression of the MFEζ CAR on $CD3^+7\text{-}AAD^-$ cells.	The level of 20% was arbitrarily set based upon in vitro studies of MFEζ function (Gilham et al. J. Immunotherapy 2002).
Sterility	No microbiological growth	48 hour culture in BactAlert aerobic and anaerobic culture bottles.
Final Product Release Criteria	**Threshold values**	**Notes**
T-cell dose	$1x10^9 - 5\ x10^{10}$ viable T-cells	Dose required dependent upon cohort.
T-cell viability	≥ 50% viable cells ($CD3^+$, $Annexin\text{-}V^-$, $7\text{-}AAD^-$).	
Transgene Expression	≥ 20% expression of the MFEζ CAR on $CD3^+7\text{-}AAD^-$ cells.	
Functionality	≥ 2 fold increase in CD25 up-regulation by MFEζ T-cells induced by CEA over un-stimulated MFEζ T-cells	
Sterility	- Microbial cultures negative day 0 and day 7 - No visible bacteria by Gram stain of samples from all culture bags. - Mycoplasma negative by PCR	BactAlert cultures PCR methods used rather than culture for speed of results.