Quality Assurance of Infused CTLs

Test for:	Release Criteria:	Testing Method:
Viability of Clinical Preparation	>75%	Trypan blue exclusion
Cell-Surface Phenotype	Uniformly TCRα/β^+, $CD4^-$, $CD8^+$, $IL\text{-}13^+$	Flow cytometric evaluation with isotype controls.
Vector Rearrangement	Single band	Southern Blot with HyTK-Specific Probe
IL-13 zetakine Expression	Presence of chimeric zeta and endogenous zeta bands	Western Blot with Human Zeta-Specific Primary Antibody
IL-13Rα2 Specific Cytolytic Activity	>25% Specific Lysis at E:T Ratio of 5:1 Against $IL13R\alpha2^+$ Daudi.Lysis of Daudi parental <2X % lysis of parental Daudi at same E:T.	4hr-Chromium Release Assay
Sensitivity to Ganciclovir	<15% Cell viability After 14-days of co-culture in 5 μM ganciclovir.	Trypan blue-exclusion cell enumeration.
Sterility	All screening bacterial/ fungal cultures neg for >14 days. Mycoplasma negative at time of cyropreservation and within 72hrs of each treatment cycle Endotoxin level <5E.I./kg in washed cell preparation. Gram stain of cell culture media negative on day of re-infusion.	Bacterial/fungal by routine clinical specimen culture. Mycoplasma by Gene-Probe RIA. Endotoxin by ELISA. Gram stain by clinical microbiology lab.