

# CTS Immune Cell SR for Serum Free Culture and Expansion of Human T Cells

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## INTRODUCTION

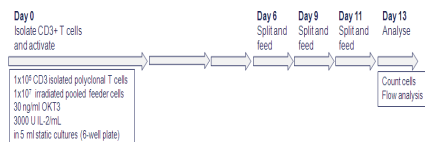
The manufacture of a majority of clinical T cell products for immunotherapy applications requires *in vitro* T cell culture and expansion. Commercialization of T cell manufacturing processes requires reagents that meet regulatory guidelines and ultimately help reduce manufacturing cost of goods. A key component in many T cell culture protocols, in addition to cell culture media and growth factors, is human serum. Human serum is expensive and requires extensive testing prior to use for manufacturing of a cGMP-compliant T cell product. To this end, we have tested a xeno-free serum replacement, CTS<sup>™</sup> Immune Cell SR. CTS Immune Cell SR contains only defined components and can be used in combination with several different cell culture media to support *in vitro* culture and expansion of T cells.

## EXPANSION OF OKT3-ACTIVATED T CELLS

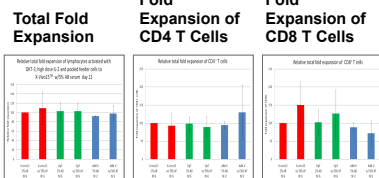
### METHODS

Polyclonal T cells were negatively isolated from fresh PBMC, activated *in vitro* with OKT3 mAb, irradiated pooled feeder cells and high dose IL-2 and expanded for two weeks.

Cell culture media tested were CTS<sup>™</sup> Optmizer<sup>™</sup> T Cell Expansion SFM, Lonza X-VIVO<sup>™</sup> 15 and CTS AIM-V Media.



### RESULTS



T cells expanded in cultures supplemented with pooled human serum or CTS Immune Cell SR show similar growth kinetics and total fold expansion after 2 weeks in culture. Both CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets are expanded.

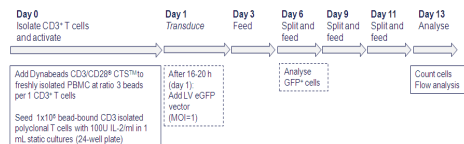
## EXPANSION OF CTS<sup>™</sup> DYNABEADS<sup>™</sup> CD3/CD28 ISOLATED AND ACTIVATED T CELLS

### METHODS

Polyclonal T cells from fresh PBMC were isolated and activated with Dynabeads CD3/CD28 CTS and expanded for two weeks.

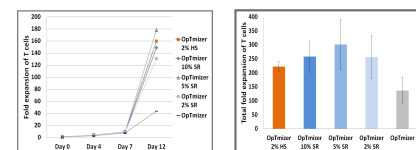
Cell culture media tested were CTS Optmizer T Cell Expansion SFM and Lonza X-VIVO<sup>™</sup> 15 (not shown).

Cell culture media were supplemented with either pooled AB human serum or 10% CTS Immune Cell SR.



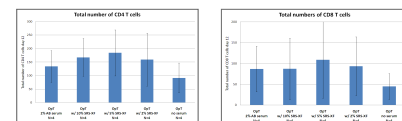
### RESULTS

#### Total Fold Expansion

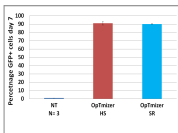


T cells expanded in CTS Optmizer supplemented with human serum or CTS Immune Cell SR show similar growth kinetics and total fold expansion after two weeks in culture. Left panel: Growth kinetics from 1 representative donor. Right panel: Fold expansion of total T cells at the end of culture (day 12). Bars represent averages of 4 normal donors.

#### CTS Immune Cell SR supports expansion of CD4 and CD8 T cells



#### Lentiviral transduction



CTS Immune Cell SR supports T cell lentiviral vector transduction when compared to human sera.

## CTS<sup>™</sup> IMMUNE CELL SR EXPANDED CART-19 T CELLS ARE EFFECTIVE *IN VIVO*

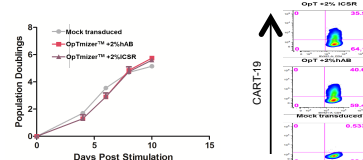
### METHODS

Human CD4<sup>+</sup> and CD8<sup>+</sup> T cells were procured from the Human Immunology Core at the University of Pennsylvania and stimulated with Dynabeads CD3/CD28 CTS in CTS Optmizer SFM supplemented with 2% pooled human AB serum or 2% CTS Immune Cell SR and transduced with CART-19 lentiviral vectors. 6 —10 week ddNOD-SCID-Yc<sup>-/-</sup> (NSG) mice were bred in-house under an approved IACUC protocol and maintained in pathogen-free conditions. Animals were injected I.V. via tail vein with 10<sup>6</sup> Nalm-6 leukemia followed by 10<sup>6</sup> T cells injected 7 days after.

Animals were monitored for signs of high tumor burden by luciferase bioluminescence.

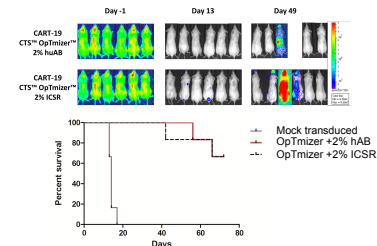
### RESULTS

#### Comparable expansion and transduction of CART-19 cells in human AB serum and CTS Immune Cell SR



CART-19 cells grown with CTS Immune Cell SR supplementation expanded to comparable yields (left panel) and transduction efficiencies (right panel) when compared to cells grown in human serum. Mock transduced cells shown as negative control.

#### *In vivo* efficacy of CART-19 cells expanded in CTS Immune Cell SR



CART-19 cells grown in CTS Optmizer with CTS Immune Cell SR supplementation showed similar potency and efficacy as control CART-19 grown in CTS Optmizer with human serum as measured by tumor burden bioluminescence (top) and animal survival (bottom panel).

## CONCLUSIONS

- CTS Immune Cell SR supports expansion of Dynabeads CD3/CD28 CTS-activated polyclonal T cells and REM – expanded T cells when supplemented into commonly used T cell culture media.
- CTS Immune Cell SR supports lentiviral transduction and expansion of gene-modified T cells, and maintains favourable immune function and *in vivo* efficacy of CAR-T cells.

## REFERENCES

- Ex vivo expansion of human T cells for adoptive immunotherapy using the novel Xeno-free CTS Immune Cell Serum Replacement. Smith C et al. *Clin Transl Immunology* 2015 Jan 15; 4

## TRADEMARKS/LICENSING

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