Activity Peak of *in vitro* Expanded Immunocytes

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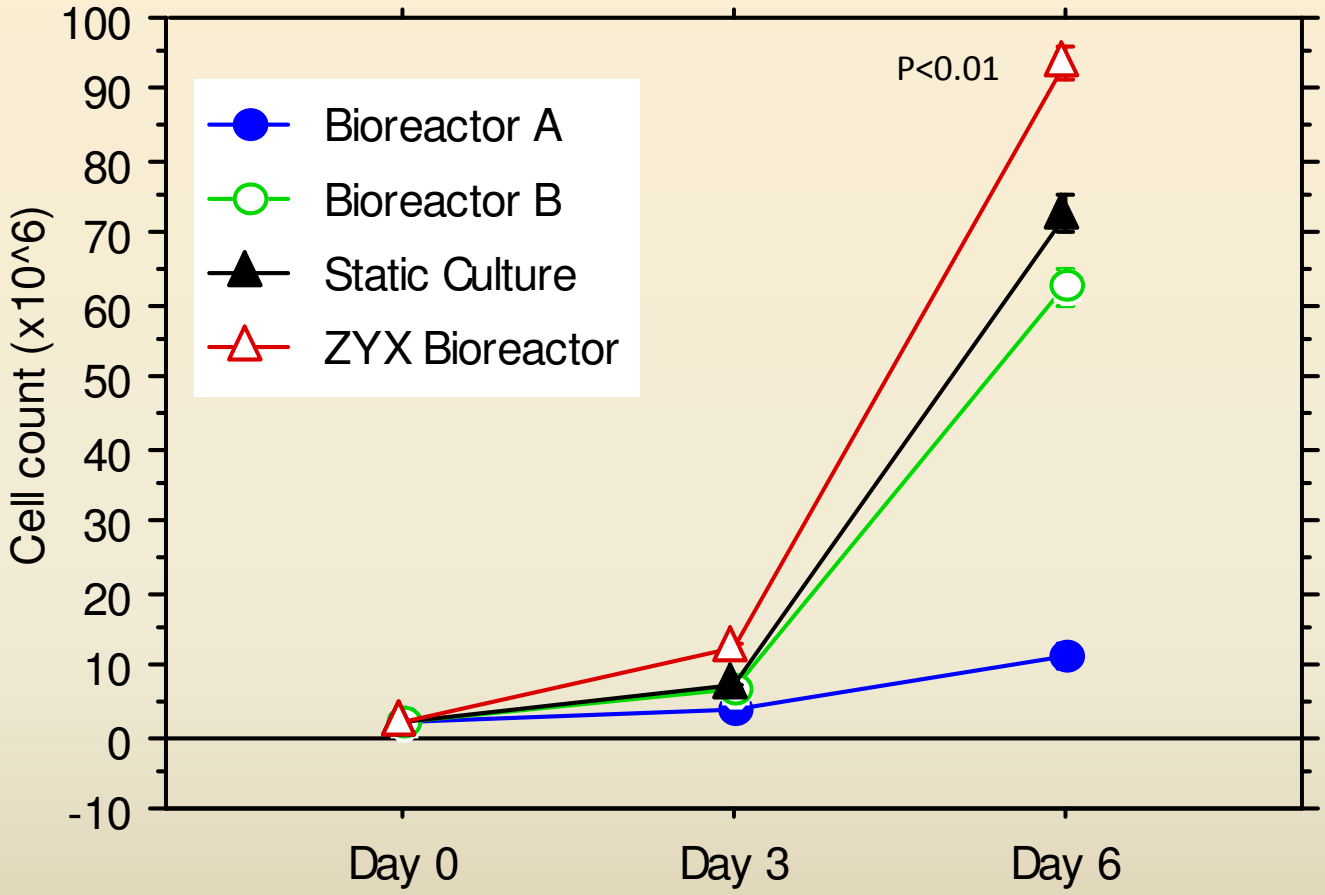
Expanding cell therapy

Antigen-specific Immunocyte in vitro Expansion

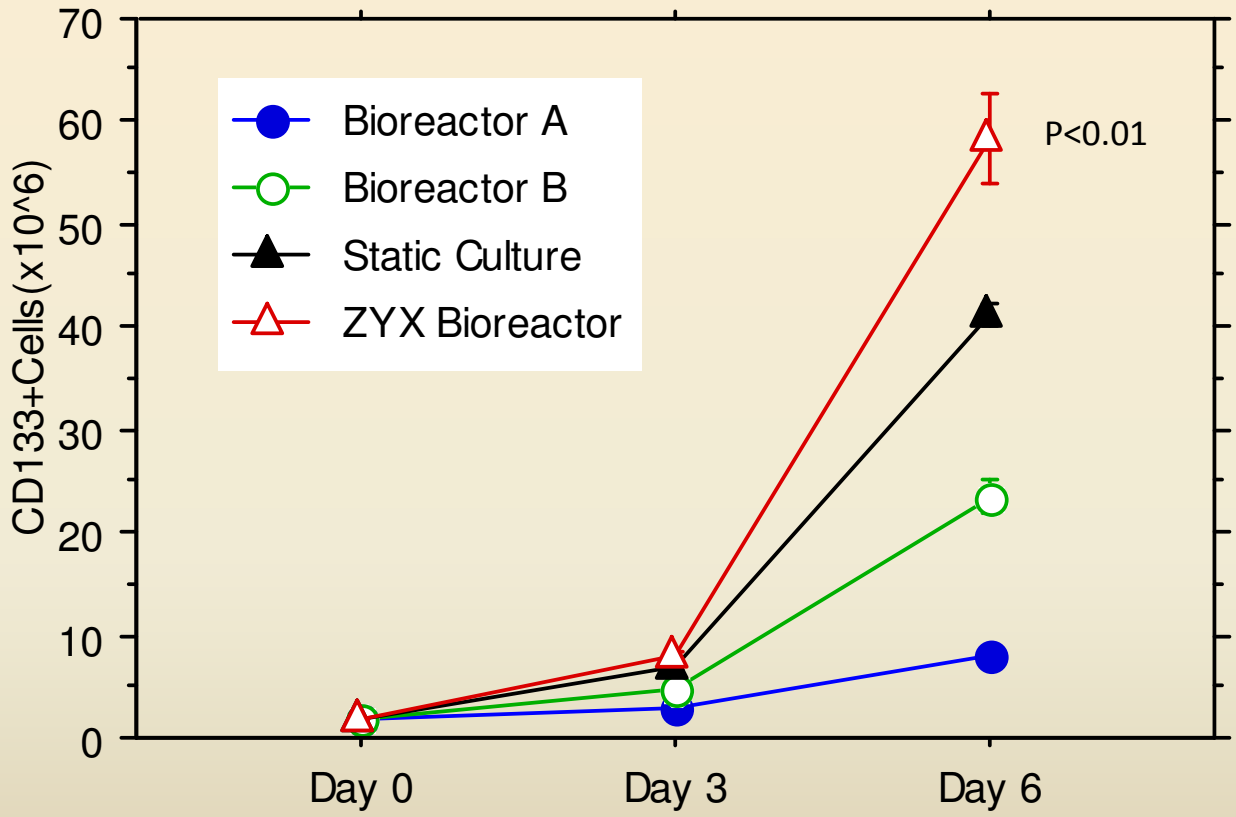
- For cancer and chronic virus infection
- To Reach effective cell number
- Free from the inhibition to the immunocyte growth, which caused by in vivo cancer cells and infected cells
- Selective expansion and isolation of antigen-specific immunocytes

Increased CD34+ HSC Expansion in PBSC Culture Using ZYX Bioreactor



10 ml purified CD34+ peripheral blood hematopoietic stem cells (PBSC) were cultured in different conditions. The initial cell density was 0.2×10^6/ml. ZYX standard Stem cell culture medium II was used in all cultures.

Increased CD133+ HSC Expansion in PBSC Culture Using ZYX Bioreactor



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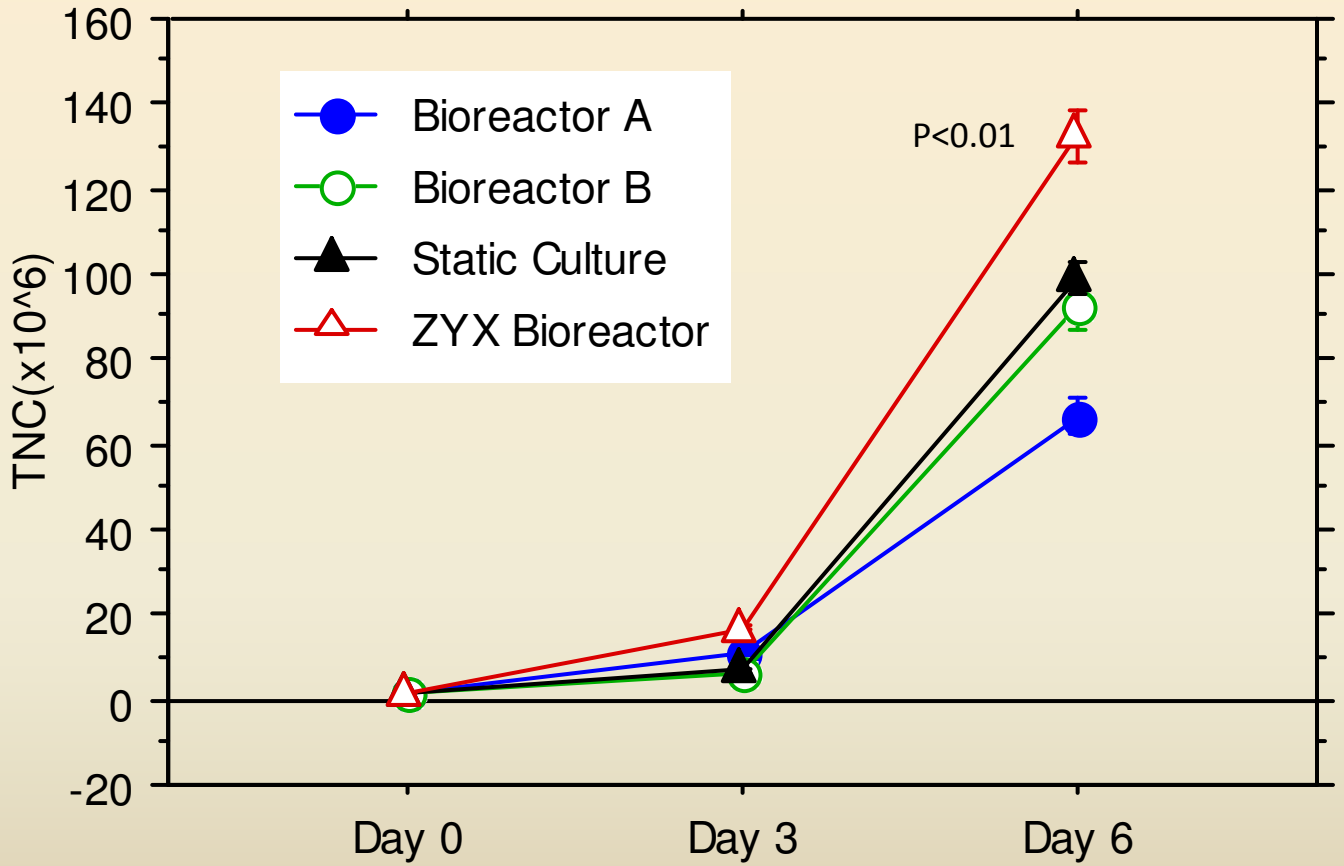
Increased TNC Expansion in PBSC Culture

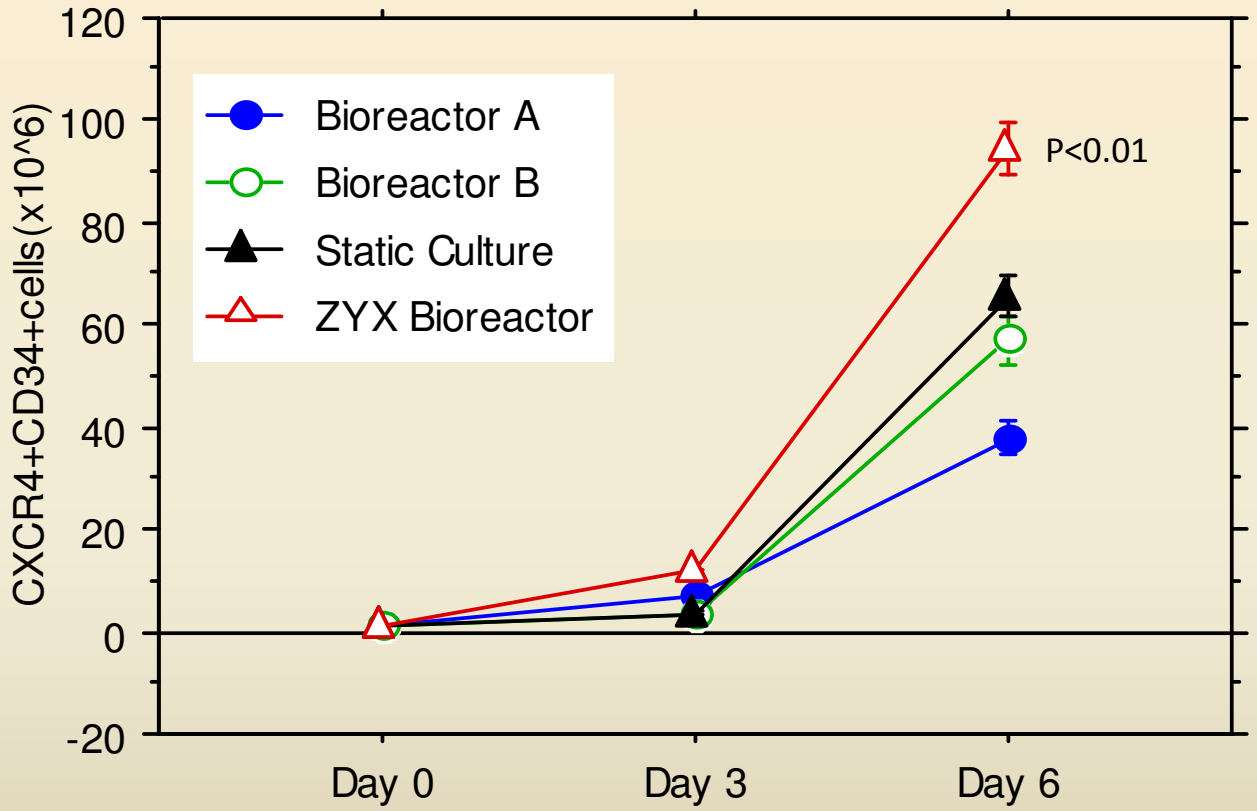
Using ZYX Bioreactor



10 ml purified CD34+ peripheral blood hematopoietic stem cells (PBSC) were cultured in different conditions. The initial cell density was 0.2×10^6/ml. ZYX standard Stem cell culture medium II was used in all cultures. Expansion of Total Nucleated Cells (TNC) was plotted in this chart.

Increased CD34 and CXCR4 Double Positive Cell Expansion in PBSC Culture

Using ZYX Bioreactor



CXCR4 expression is positively correlated with engraftmnt. 10 ml purified CD34+ peripheral blood hematopoietic stem cellc (PBSC) were cultured in different conditions. The initial cell density was $0.2x10^6$/ml. ZYX standard Stem cell culture medium II was used in all cultures. Expansion of Total Nucleated Cells (TNC) was plotted in this chart.

ISHAGE Guidline for CD34+ Cell Enumeration with Flow Cytometry

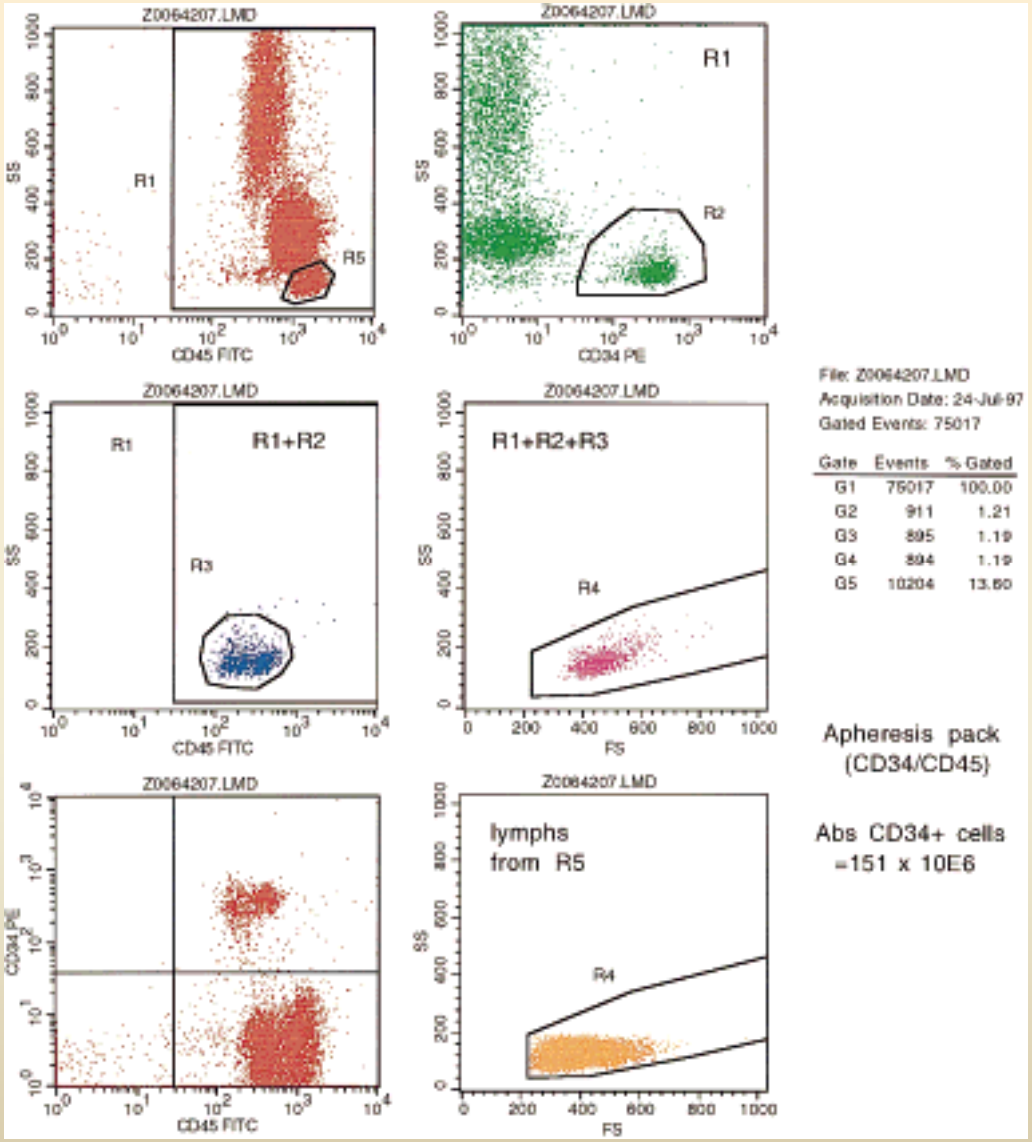
1.CD45 Dim: not differentiated into mature myeloid cells

2.Low side scatter: cells are round with single nuclei

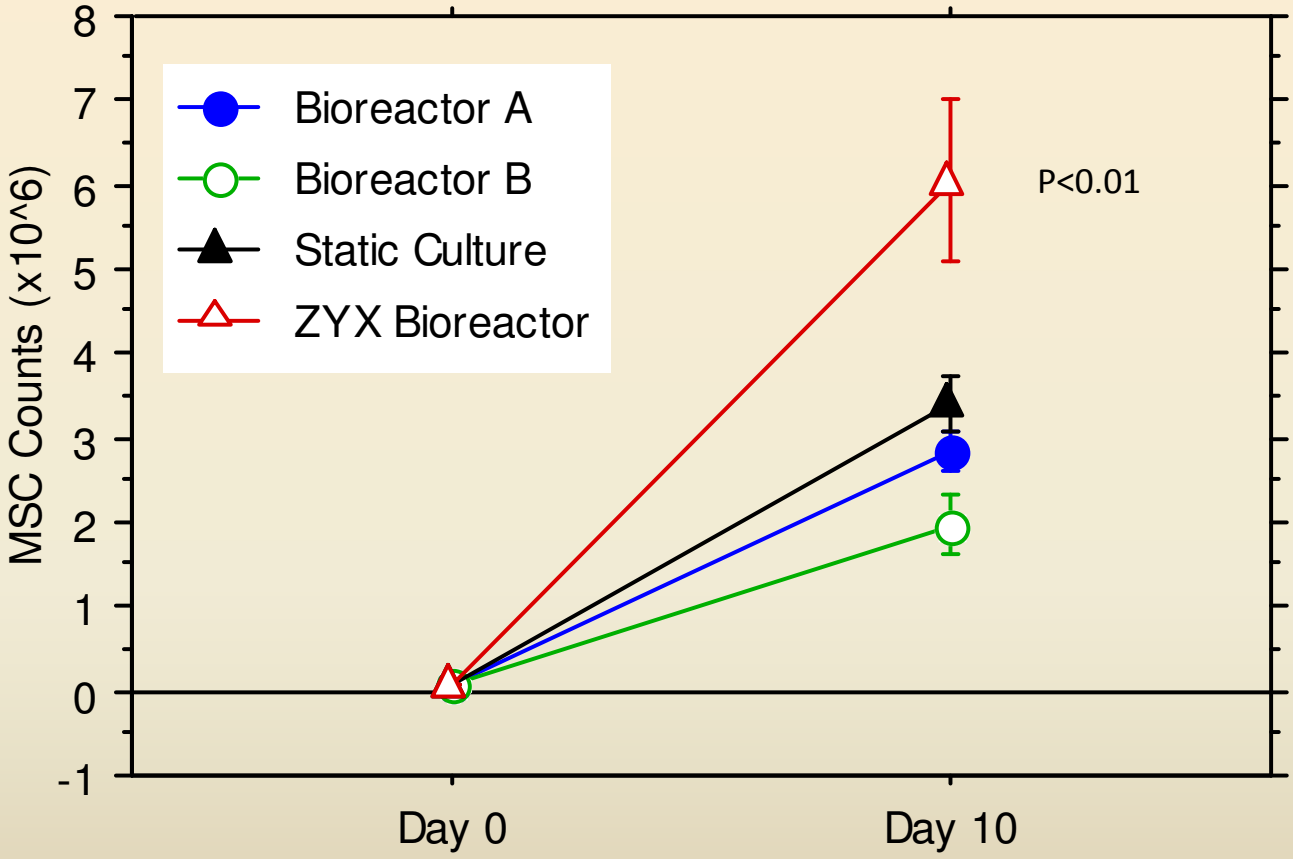
Our Modification:

1.Cell counting beads addition

2. One more color addition for CD38, CD133 or CD90

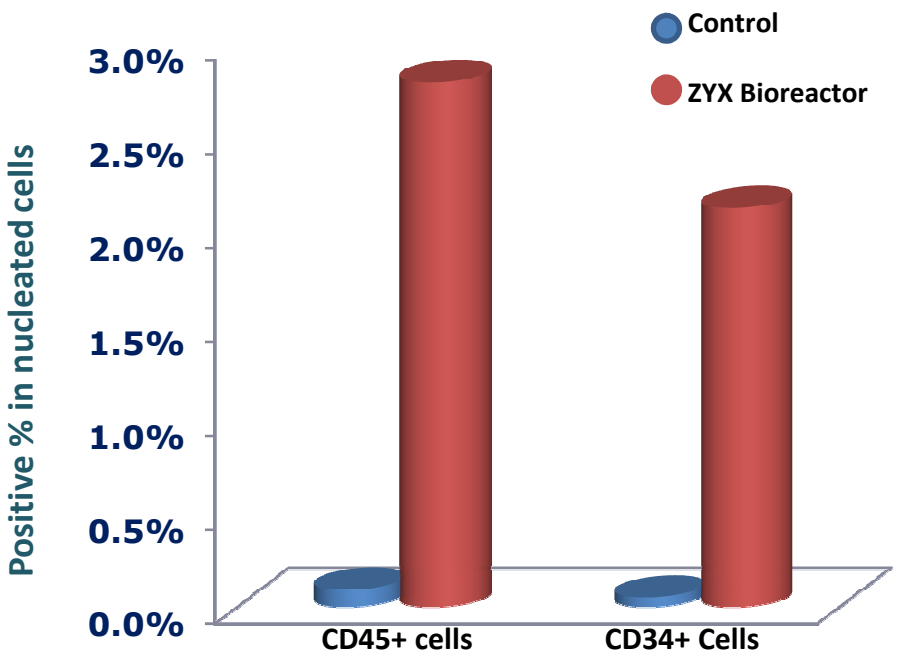


Increased Cord Mesenchymal Stem Cell Expansion in Culture with ZYX Bioreactor



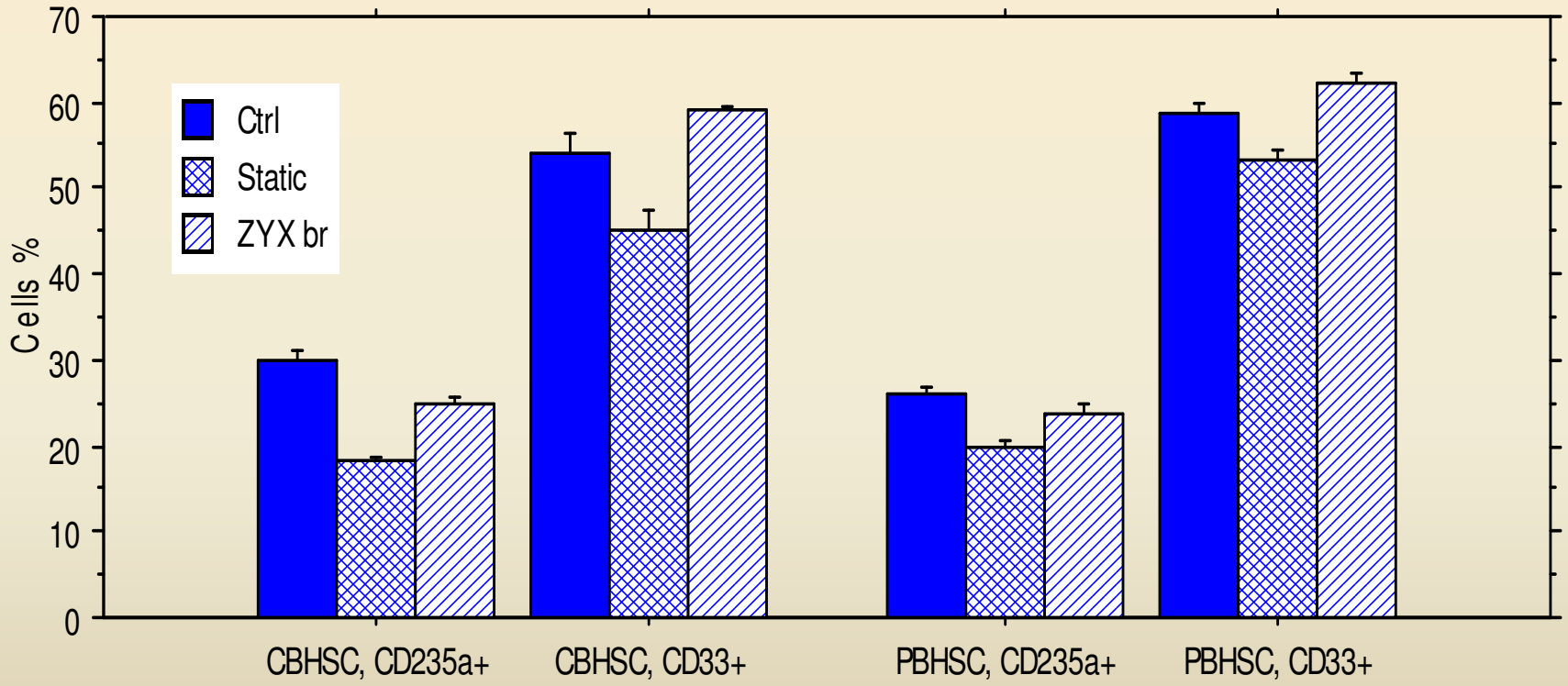
Cord mesenchymal stem cells were cultured in different condition for 10 days. 10^5 cells were seeded in each culture. Cells were collected by the automatic procedure in ZYX Bioreactor, for other culture condition, the standard tripsin cell lifting procedure was used. MSC cell markers were checked before or after expansion

Engraftment Assay with Expanded HSC in NOD/SCID Mice



The expanded human peripheral blood CD34+cells were injected into NOD/SKID mice. Bone marrow cells were harvested 6 weeks following the transplantation and examined for human CD45+ and CD 34+ cells.

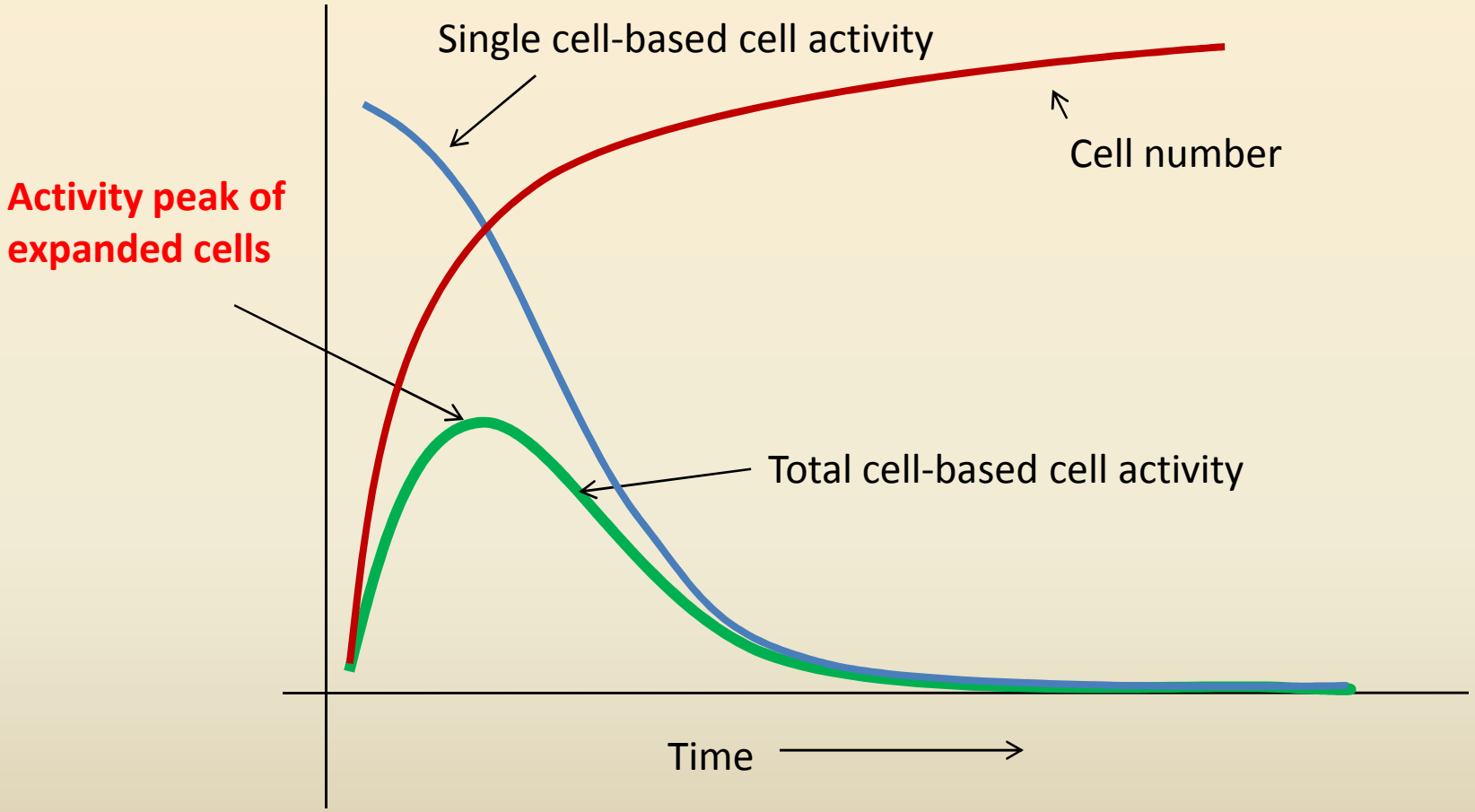
Evaluation of differentiation potency of expanded cord blood HSC (CBHSC) and peripheral blood HSC (PBHSC) using Colony Forming Unit Assay.



Fold increase in total cells, $CD34^+$ cells and total engraftment potency following culture and the ratios of total cells/$CD34^+$ cells and total cells/engraftment potency

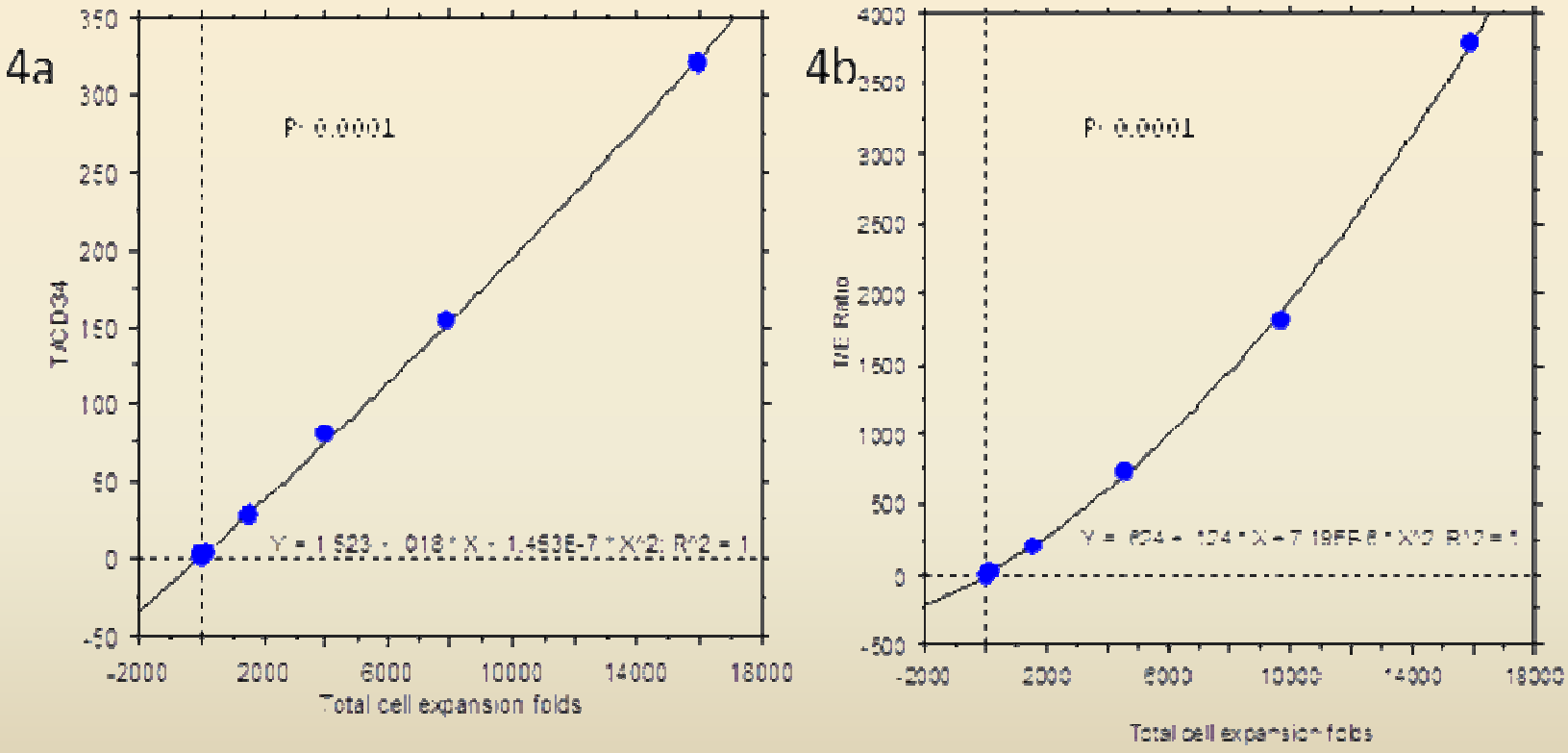
Time points (days)	0	2	4	6	8	10
Total cells	1	3.1	20.5	163	1214	9894
$CD34^+$ cells	1	2.1	8.5	35.6	52.5	49.4
Engraftment potency	1	1.8	5.4	8.5	7.4	4.2
Total cells/$CD34^+$ cells	1	1.476	2.412	4.579	23.124	200.283
Total cells/Engraftment potency	1	1.722	3.796	19.176	204.595	2355.714

Model of Cell Activity in Expanding Cells



Total cell activity=Single cell-based cell activity*Cell number

Regression functions for TNC fold-expansion and ratio of TNC fold-expansion/CD34^{+} cell fold-expansion (4a) and ratio of TNC fold-expansion/engraftment potency fold-increase (4b).



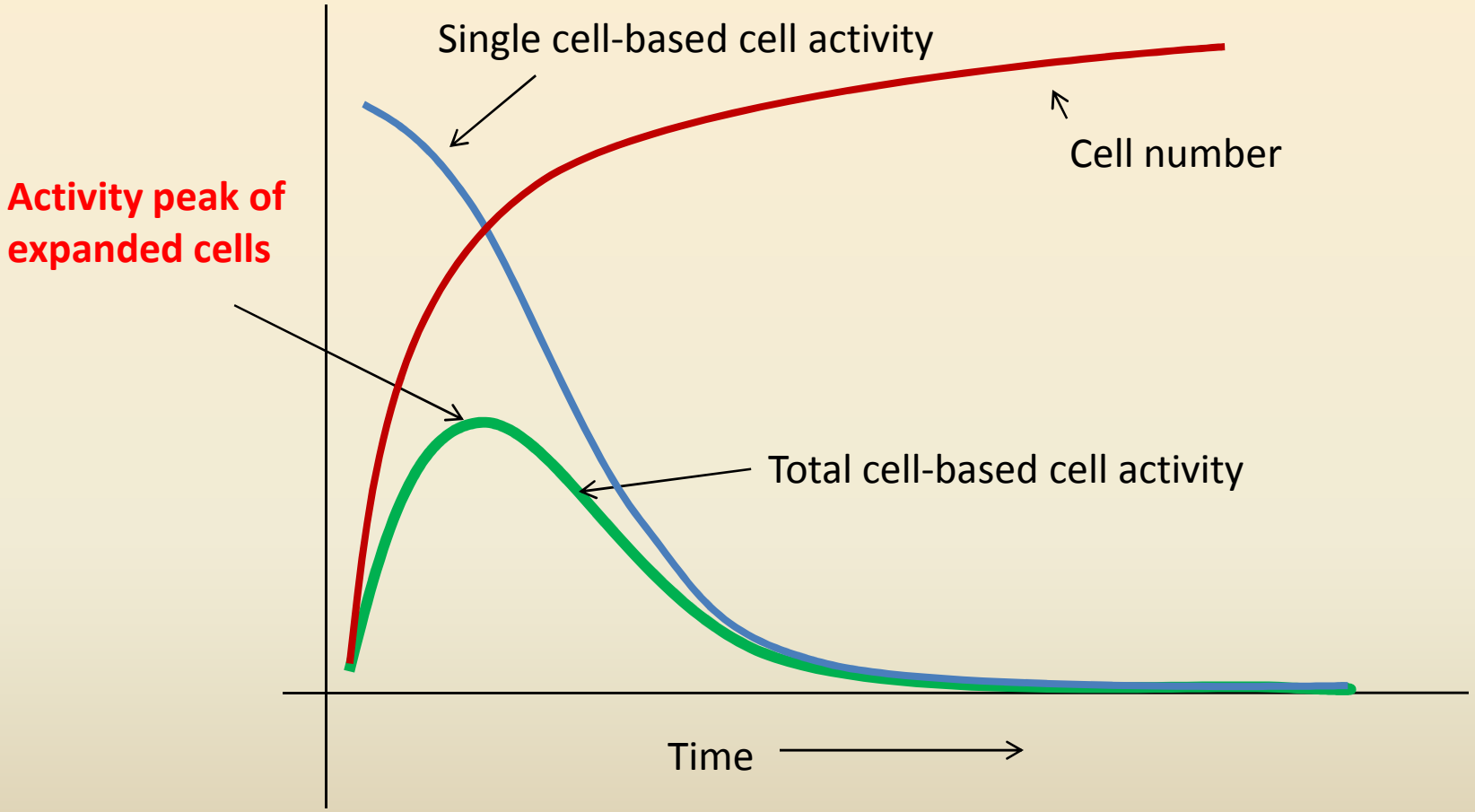
Do the individual cell-based functions of immunocytes decline when the cells are expanded in vitro similar to HSC?

If so, do the activities of the total immunocytes also have a peak point during the cell expansion?

The answer for both questions are **Yes** by following tests:

- B cells (CD19+, CD 20+, CD22+): Immunoglobulin Production
- T cells (CD3+): Cytokine productions: IL-2, IL-12 and IL-18
- **Antigen-specific cytotoxic T cells: virus-infected cell and cancer cell lysis or apoptosis.**

Model of Cell Activity in Expanding Cells



Total cell activity=Single cell-based cell activity*Cell number

How to determine the activity peak of the cells in cell expansion?

For the productions immunoglobulins and cytokines, only one test, such as ELISA, would be enough for the required information.

However, for the antigen specific CTL activity for different antigens from different patients with different culture medium, it is not that easy. Therefore, the development of a simple method to monitor the CTL toxicity to specific cancer cells is very important.

Cell density detection is a optimal method for monitoring CTL toxicity

Methods	Correlation coefficient (R^2)	P Value	Complexicity
Cell density detection	0.98	<0.01	-
Cytokine detection			
ELISA	0.82	<0.05	++
Facs Intracellular stain	0.79	<0.05	++
ELIspots Assay	0.85	<0.05	++
CTL assay	1.0	<0.01	++++

How to determine the activity peak of expanded cancer-specific CTL by monitoring the cell density change and real time reporting the results?

Prerequisites:

1.A cell density detector

2. A device for the alternation of proper static and kinetic culture

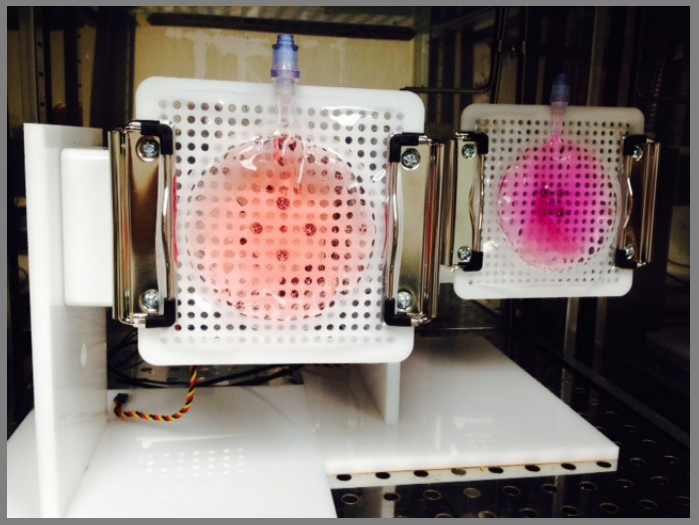
Static culture—provide the adequate contact between antigen particles (cancer cells) and effector cells (T cells), and minimize the shear-stress on the cells.

Kinetic culture---ensure effector cells can receive adequate metabolic support and provide a condition for cell density detection.

3.Cells can be evenly distributed in the culture container in the static culture following kinetic culture

4.A suitable program can be used to automatically control and adjust the frequency and interval between static and kinetic culture as well as the speed and changes of the speed of the cell culture container moving in kinetic culture

Among many Bioreactors, only ZYX Bioreactor can meet these requirements

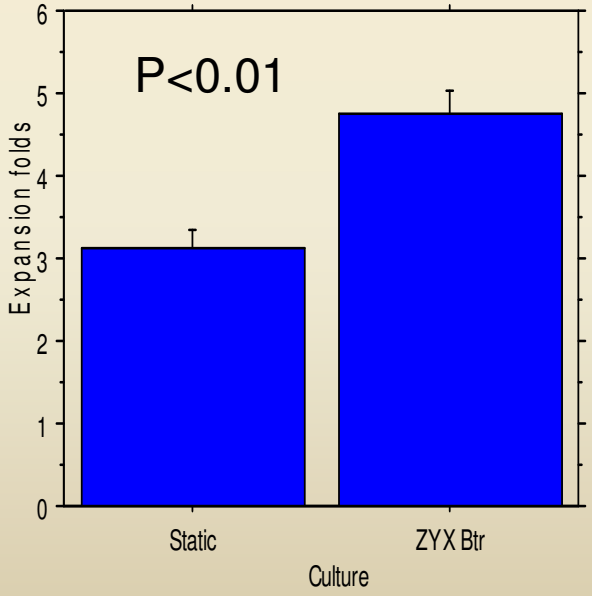


Comparison of CMV-CTL number and cytotoxicity between the ZYX btr and the static culture

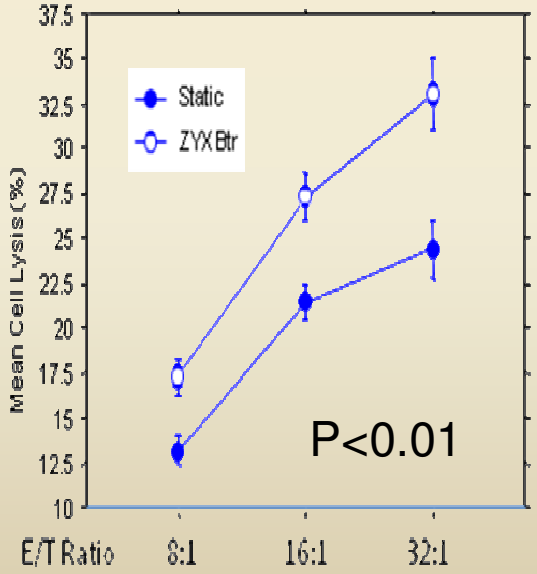
CMV pp65-specific CD8+ CTL expansion	Fold increase (6th day)	Activities (lysis%) at different E:T ratios		
		4:1	8:1	16:1
ZYX btr (n=6, Means±SD)	4.76±0.62	17.24±2.31	27.36±3.11	33.85±3.84
Static culture (n=6, Means±SD)	3.12±0.55	13.18±1.95	21.50±2.46	28.26±3.77

Splenocytes from cancer cell line-primed BALB/c mice → Cancer cell stimulation and CD8+ selection and expanded in ZYX Btr or Static → CTL activity tested in vitro or in vivo (BALB/c mice)

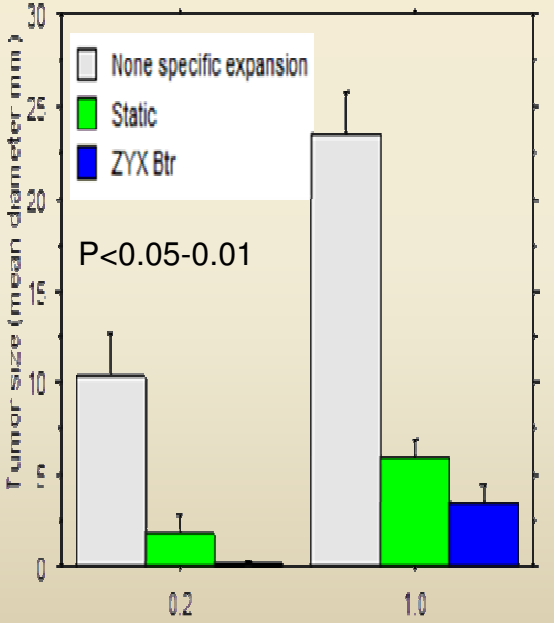
Comparison of Total Cancer-specific CTL Expansion between ZYX Bioreactor and Static Culture in Mice



Comparison of Expanded Mice Cancer-specific CTL Cytotoxicity to Cancer Cells between ZYX Bioreactor and Static Culture



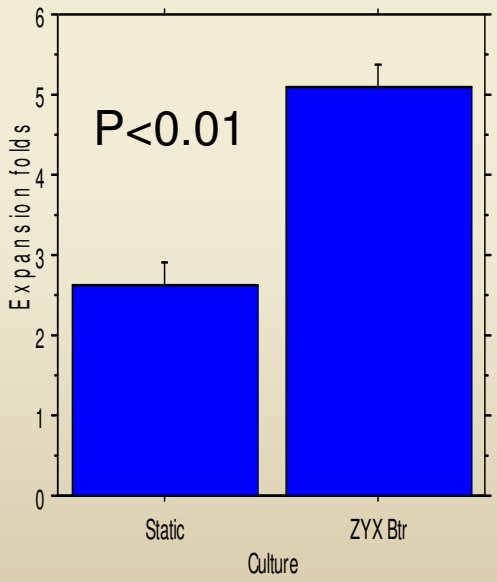
Enhanced Mouse Cancer Cell inhibition in vivo by Cancer-specific CTL Expanded in ZYX Bioreactor



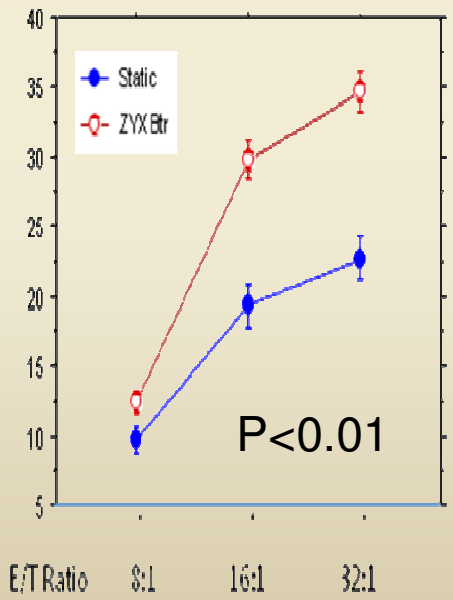
Inoculated cancer cell number($x10^6$)

Blood mononuclear cells from patient with lung cancer → Cancer cell stimulation and CD8+ selection and expanded in ZYX Btr or Static → CTL activity tested in vitro or in vivo (NOD/SCID mice)

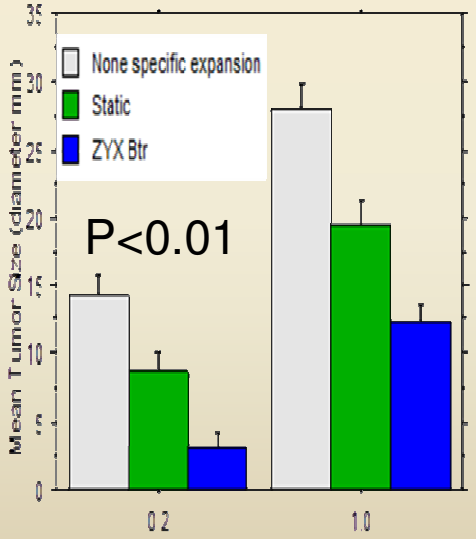
Comparison of Total Human Cancer-specific CTL Expansion between ZYX Bioreactor and Static Culture



Comparison of Expanded Human Cancer-specific CTL Cytotoxicity to Cancer Cells between ZYX Bioreactor and Static Culture



Enhanced Human Cancer Cell inhibition in NOD/SCID Mice by Cancer-specific CTL Expanded in ZYX Bioreactor



Inoculated cancer cell number($x10^6$)

Conclusion

1.When the activity of individual cell-based antigen-specific immunocytes declines in the cell expansion, the total cell activity increases at early expansion stage until it reaches a peak.

2. The activity of antigen-specific immunocytes correlates the changes of cell density, cytokine production and many other factors, but the cell density detection would be the be the best option for monitoring the CTL activity.

3. ZYX Bioreactor can determine the optimal cell harvest time by monitor the change of cell density for the highest antigen-specific CTL cytotoxicity.

Acknowledgement

Why not Car-T???

1. Car-T is a GMO (Genetically Modified Organism,Letenvirus)
   E-asCTL is Non-GMO
2. Car-T procedure is very complicated
   E-asCTL is much simpler
3. Car-T procedure is time consuming, takes several months
   E-asCTL takes a couple of weeks
4. Car-T procedure is very expansive
   E-asCTL is much less expansive

Now we need to know which one is more effective?