Efficacy and Utility of New EXP-Pak™ (Charter Medical) Closed-System Disposable Cell Expansion Bags

Clinical cellular therapy applications often times require a cell expansion or maturation step prior to use. Traditionally, cell expansion or cell culture is performed in "open" systems including multi-well culture dishes or tissue culture flasks. These "open" steps present risks and are not ideal for larger-scale manufacturing. The new EXP-Pak™ cell expansion container is a closed-system, gas permeable bag intended for expansion and culture of non-adherent cells.

The EXP-Pak™ bags are made from a unique polyolefin film that permits gas permeability required for cell expansion and maintenance of cell viability in addition to excellent clarity (when filled with liquid) for viewing of cultures. The bags also feature a tubing harness that allows for filling, sampling, and manipulation steps to take place in a completely closed, sterile manner.

The aim of this study was to investigate the ability to culture and expand cells in the EXP-Pak™ (Charter Medical, Ltd. Winston-Salem, NC) and to also compare expansion rates and cell recovery to alternatively available cell expansion bags (different manufacturers). The results herein demonstrate that the EXP-Pak™ can effectively promote cell culture and expansion.

Methods

Tumor Infiltrating Lymphocytes (TIL)

Briefly, TIL cells for the REP (Rapid Expansion Protocol) were thawed and placed into culture plates. On day 3, culture flasks were set up to initiate the expansion using ≥ 1.0E+6 cells in 150mL media. On day 10 of the expansion, ≥ 1.5E+8 total viable cells on average were transferred in 500mL media to either the EXP-Pak™ (3L) expansion bag or previously validated FEP (3L) bag for final cell expansion and comparison. On days 12, 14, and 17, cell counts and viability were performed and cell culture media was added accordingly.

Human Th2 Rapa Cells

Briefly, Th2 cell cultures are initiated with CD4+ selected lymphocytes and placed into culture in X-Vivo media (Lonza) containing 5% HI autologous or HI AB plasma, 20 IU/mL IL-2 (Chiron), 1000 IU/mL IL-4 (Cell Genix) and 1uM rapamycin (Sirolimus) in either the EXP-Pak™ bag or the PL732 Lifecell® (Baxter) bag. Cells are stimulated on Day 0 with 3:1 (beads per cell) anti-CD3 and anti-CD28 coated beads (4.5uM Dynal tosylactivated). For both 6 day and 12 day, 10% 10x (IL-2 and IL-4) media containing 5% plasma and 1uM rapa is added on days 2 and 4.

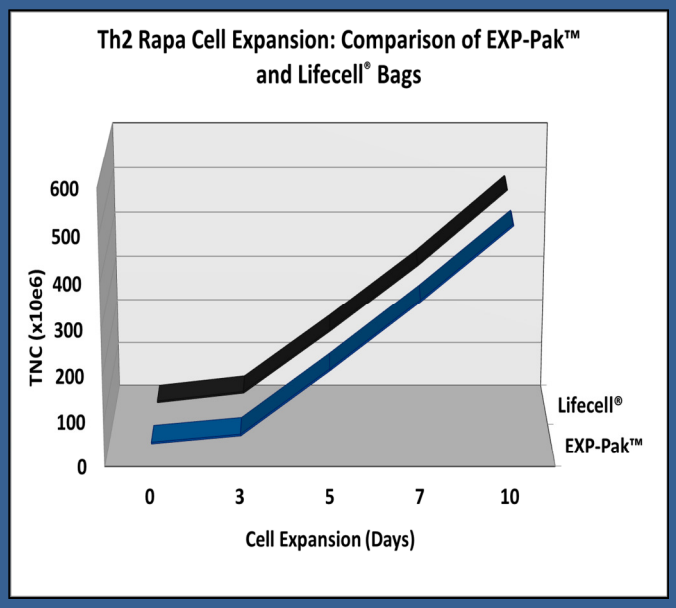
Table 1: Tumor Infiltrating Lymphocyte Expansion

	EXP-Pak™ (3L)			FEP Bag (3L)		
Days	Cells/mL	Bag Volume	Total Viable Cells	Cells/mL	Bag Volume	Total Viable Cells
Day 12	1.79E+6	500mL	8.95E+8	1.5E+6	500mL	7.5E+8
Day 14	2.66E+6	900mL	2.39E+9	2.05E+6	750mL	1.54E+9
Day 17	2.54E+6	2400mL	6.10E+9	1.82E+6	1550mL	2.82E+9

Data Summary

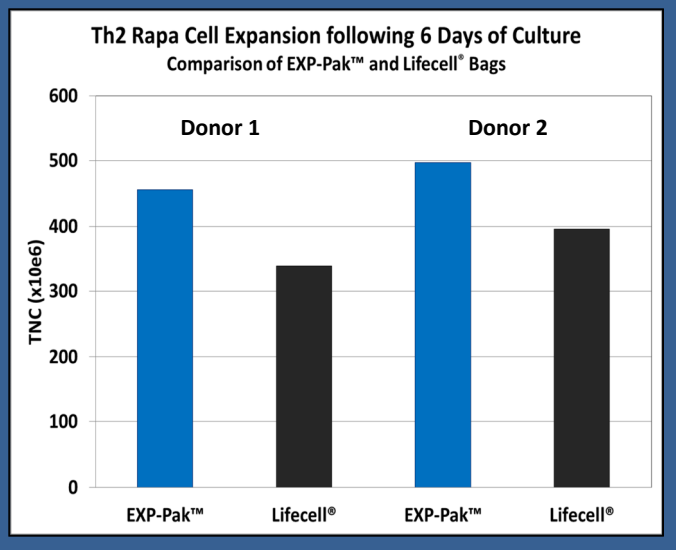
Expansion of Tumor Infiltrating Lymphocytes was performed using the EXP-Pak™ cell expansion bags and compared to a previously validated FEP bag (different manufacturer). Overall cell expansion (cells/mL) and total viable cells in the EXP-Pak™ increased at each of the time points tested in the study (Table 1). Furthermore, when compared to the currently accepted FEP bag used, cell/ml and total viable cells in the EXP-Pak were equivalent or better at each of the days measured. Data from three EXP-Pak™ bags were averaged and compared to the average of six FEP bags. This study demonstrates that effective TIL cell expansion can be performed using the new EXP-Pak™ bags.

Figure 1: Th2 Rapa Expansion



In a separate study, EXP-Pak™ bags were investigated for the expansion of Th2 lymphocytes and compared to cell expansion values achieved using the Lifecell® culture bag (Figures 1 and 2). In the initial experiment, Th2 Rapa cells were cultured and expanded for 10 days in both bags. TNC counts were performed on days 3, 5, 7, and 10 as shown in Figure 1. Similar expansion rates were noted for both bags.

Figure 2: Th2 Rapa Expansion – Donor Comparison



In the second experiment, Th2 Rapa cells from two separate donors were expanded in EXP-Pak™ bags and compared to Lifecell® containers (Figure 2). Following 6 days of cell expansion, TNC counts were performed for each donor pair. The results demonstrate similar to slightly improved overall expansion using the EXP-Pak™ bags for both donor cell comparisons. The results of the studies indicate that EXP-Pak™ bags can be used for Th2 lymphocyte expansion. While additional studies are warranted, the data indicate that comparable values to the Lifecell® container can be achieved using the new EXP-Pak™ closed-system expansion bags.

Conclusion

The data demonstrate that the EXP-Pak™ cell expansion bags provide an ideal environment for culture and expansion of cells. Average cell expansion values (TNC) and total cell viability were demonstrated to be comparable to alternative bags used. Furthermore, the bags provide a completely closed system for sterile filling, sampling, and cell manipulation. In conclusion, the EXP-Pak™ cell expansion containers from Charter Medical, Ltd. offer an optimal, scalable cell expansion environment for a variety of therapeutically important cell types.

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EXP-Pak™ products are intended for expansion and culture of cells