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Reliable Proliferation and Differentiation of Desired T cell Subpopulations with CellGenix® Recombinant Human Interleukin-2 (IL-2)

Authors and affiliations

Gilli Galore Haskel[1], Efrat Merhavi-Shoham[1], Sivan Ofir[1], Naama Dror[1], Mika Shapiro[1], Roni Bareli[1], Andres Castillo[2], Shanya Jiang[2], Gaurav Kaushik[2], Rohit Saklecha[2], Remmer Janssen[3], Jalil Hakimi[4], Adva Maimon[4], Kat Kozyrytska[2], Maya Fuerstenau-Sharp[5]

1. 4C Biomed Services Ltd. 5, Hamelacha St., Netanya 4250540, Israel
2. Sartorius Stedim North America Inc., 565 Johnson Avenue, Bohemia, NY 11716, USA
3. Sartorius CellGenix GmbH Am Flughafen 16 | 79108 Freiburg | Germany
4. Biological Industries Israel Beit-Haemek, Kibbutz Beit-Haemek, 25115, Israel
5. Sartorius Stedim Biotech GmbH, August-Spindler-Straße 11, 37079 Göettingen, Germany

Abstract

The selection of optimal growth factors and cytokines for reproducible process conditions is of critical importance to the generation of high-yield, high-quality cell and gene therapies. The current industry standard is to produce these drugs using research use only (RUO) grade materials in development then transitioning into GMP qualified materials later in clinical production. This approach carries added risk and costly comparability studies to validate performance. CellGenix® Preclinical cytokines allow for a seamless transition from process development into GMP production with lot-to-lot consistency and excellent performance. To demonstrate this performance in the expansion of T cells, CellGenix® Preclinical Recombinant Human (rh) IL-2 was evaluated alongside FDA-approved Proleukin® (aldesleukin). In this study we compared both the viable cell growth and the subpopulation ratios of CD4+ and CD8+ T cells isolated from peripheral blood mononuclear cells (PBMCs). The results suggest that cells expanded with CellGenix® Preclinical Recombinant Human (rh) IL-2 maintain similar cell growth and subpopulation ratios when compared to Proleukin®.

Find out more: www.sartorius.com/en/applications/cell-and-gene-therapy

Introduction

T cells play a vital role in pathogen elimination and tumor immunosurveillance. The human body can produce an array of specialized T cells that provide unique responses to the diverse spectrum of tumor cells and pathogens (viruses, bacteria, and parasites). The modulation of T cells is an exciting prospect for the development of novel therapies for immuno-oncology and autoimmune applications, such as adoptive cell therapy and vaccine development. This approach has been validated by the approval of multiple autologous chimeric antigen receptor T cell (CAR-T) therapies in the United States and Europe, and hundreds of clinical trials evaluating adoptive cell-based therapies for the treatment of liquid or solid tumors are ongoing.[1]

The production of CAR-T and other autologous T cell-based therapies requires robust expansion of specific subpopulations of patient-derived immune cells. Interleukin-2 (IL-2) is a cytokine that plays a key role in promoting T cell and B cell expansion, and recombinant IL-2 is commonly used in the production of CAR-T therapies.[2] Several derivatives of recombinant IL-2 have been developed for research use only. These IL-2 formulations are usually produced using animal components, and their quality and consistency may be highly variable. These IL-2 formulations cannot be used to manufacture investigational or approved cell-based therapies because they do not comply with Current Good Manufacturing Practice (CGMP) regulations, which are designed to ensure the identity, strength, quality, and purity of drug products, and prohibit the use of any animal-derived components.

To comport with CGMP regulations, manufacturing processes for CAR-T and other cell-based therapies must utilize GMP-grade IL-2. However, the shift from the use of research-grade IL-2 to GMP-grade IL-2 may require revision and/or requalification of manufacturing processes, potentially increasing manufacturing costs and product development timelines.

CellGenix® Preclinical Recombinant Human (rh) IL-2 was designed specifically as a cost-effective, GMP alternative IL-2 formulation that enables a seamless transition from research and process development to manufacturing for clinical applications. CellGenix® rh IL-2 is an animal-derived component-free cytokine available in Preclinical grade for translational applications and in GMP grade for clinical applications. CellGenix® Preclinical rh IL-2 has proven with high activity levels — comparable to CellGenix® GMP-grade IL-2 — enabling cost-efficient production of cellular therapies while reducing process variabilities due to eliminating animal-derived components and minimizing process changes when transitioning to CGMP manufacturing.

Proleukin® (aldesleukin) is a recombinant IL-2 product indicated for the treatment of adults with metastatic renal cell carcinoma and metastatic melanoma. Because it is manufactured in accordance with CGMP regulations it offers high quality and consistency, which makes it attractive as an IL-2 reagent in quality control assays for cell therapy manufacturing processes. In this study, we demonstrate that CellGenix® Preclinical grade IL-2 performs as well as or better than Proleukin® with respect to cell expansion, CD4+/CD8+ ratio consistency, and T cell subpopulations when used to stimulate cultured peripheral blood mononuclear cells (PBMCs) obtained from two human donors. These results support the use of CellGenix® Preclinical grade IL-2 as a cost-effective component to support the development of cell-based therapy manufacturing.

Methods

Cell collection and processing

PBMCs were originated from two healthy donors that were obtained from the central Blood Bank (Israeli National Blood Services, Magen David Adom). PBMCs were separated by centrifugation on a density gradient medium (Lymphoprep) according to standard protocols, frozen with serum-free cell freezing medium (NutriFreez D10 Cryopreservation Medium) and stored in liquid nitrogen (~50x10^6 cells/tube).

On day 0, frozen vials were removed from liquid nitrogen (one vial per donor) and thawed in serum-free media, according to standard protocols. The number of viable cells was determined using Trypan Blue on an automated cell counter (Countess™ II). 1x10^5 cells were stained for the expression of CD4 and CD8 according to standard protocols. Briefly, cells were washed and incubated with control or specific fluorophore-conjugated antibodies (anti-CD8-APC and anti-CD4-FITC) for 30 minutes on ice. Cells were washed twice and re-suspended in FACS buffer (0.5% BSA, 2mM EDTA, 0.02% Sodium Azide). Cells were analyzed on a CytoFLEX instrument and the data were analyzed using FCS Express software (De Novo™ Software).

Cell stimulation

1.875x10^6 PBMCs from each donor were stimulated with 50 ng/mL OKT3 and 300 IU/mL hIL-2 (CellGenix® or Proleukin®), using two different media (Medium 1 and Medium 2). Each cell group was seeded into 3 replicate wells of a G-Rex® 24-well plate at 0.5x10^6 cells/well at a final volume of 8 mL of the appropriate medium and incubated in a 37°C, 5% CO_2 incubator.

On days four and seven, 6 mL of media were removed from all wells. Cell aliquots were obtained from each well and the number of viable cells was determined using Trypan Blue on an automated cell counter and fold expansion was calculated. Based on cell counts, appropriate fresh media was added to the wells to reach 8 mL/well. On day 10, cells were separately collected from all wells. The number of viable cells was determined using Trypan Blue on an automated cell counter and the final fold expansion was calculated.

After counting, replicates were combined and 5x10⁶ cells from each treatment group were processed for T cell marker characterization by spectral flow cytometry. 1x10⁶ cells were stained with a viability dye using Zombie NIR™ Fixable Viability Kit and incubated covered for 20 minutes at room temperature followed by a washing step. Tandem signal enhancer solution was added to the wells and incubated covered for 5 minutes at 4°C. A panel of 10 markers (CD3, CD4, CD8, CD62L, CCR7, CD45RA, CD45RO, CD25, CD127, and CD95) or matching isotype controls were added and incubated covered for 10 minutes at 4°C followed by a washing step. Cells were fixed, washed, and re-suspended in running buffer and acquired on a 4 laser Cytek® Aurora instrument.

Results

Expansion of IL-2 stimulated cells is comparable between CellGenix® Preclinical rh IL-2 and Proleukin®

Cells from each donor were cultured in Medium 1 or Medium 2 and stimulated with CellGenix® Preclinical rh IL-2 or Proleukin®. Viable cell count (Table 1) and fold expansion (Figure 1) were assessed at days 4, 7 and 10. Cell viability and fold expansion were similar for CellGenix® Preclinical rh IL-2 and Proleukin®-stimulated cells at each time point and did not differ between the two media tested.

Table 1. *Viable cell counts for cultured PBMCs stimulated with IL-2. A represents Donor 1, B represents Donor 2*

A) Donor 1

		Viable Cell Count (x10⁶)		
		Day 4	Day 7	Day 10
Medium 1	CellGenix® IL-2	0.15	0.85	3.84
	Proleukin®	0.09	0.44	4.22
Medium 2	CellGenix® IL-2	0.24	2.13	6.42
	Proleukin®	0.23	1.45	7.98

B) Donor 2

		Viable Cell Count (x10⁶)		
		Day 4	Day 7	Day 10
Medium 1	CellGenix® IL-2	0.17	0.95	6.71
	Proleukin®	0.14	0.71	5.43
Medium 2	CellGenix® IL-2	0.33	3.57	20.38
	Proleukin®	0.33	1.87	19.58

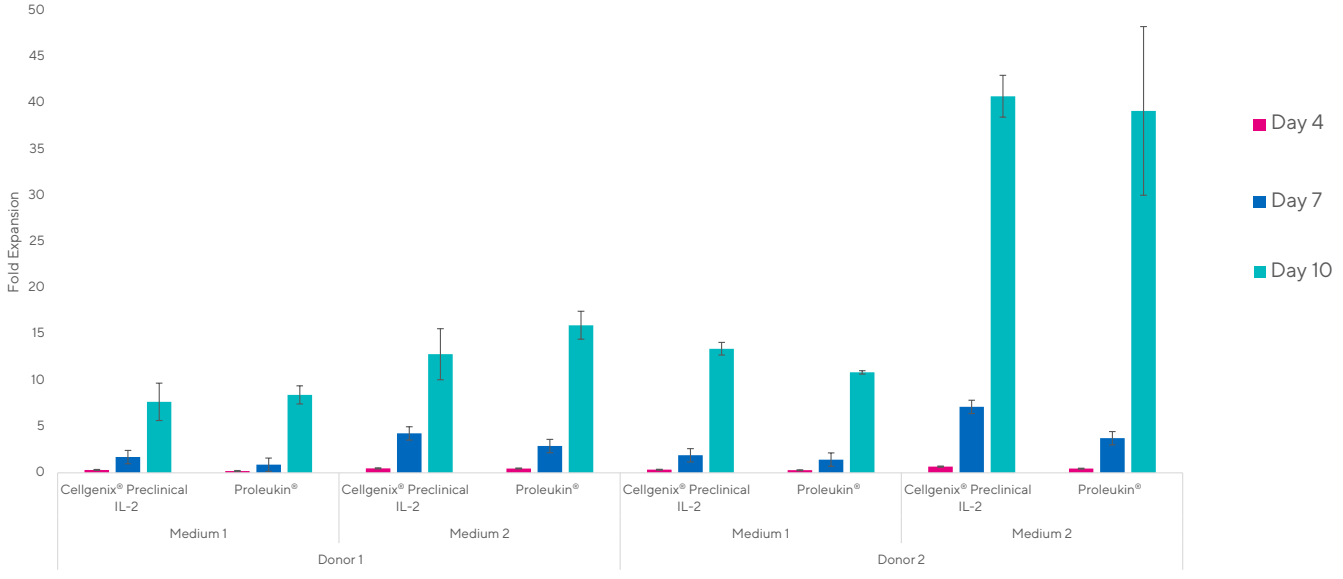

Figure 1. *Fold expansion of donor PBMCs was similar with CellGenix® Preclinical rh IL-2 and Proleukin®.* Frozen donor PBMCs were thawed and stimulated with CellGenix® Preclinical rh IL-2 or Proleukin®. Fold expansion was assessed on day 4, 7 and 10. Numbers represent average fold ± SE of triplicates per treatment group.

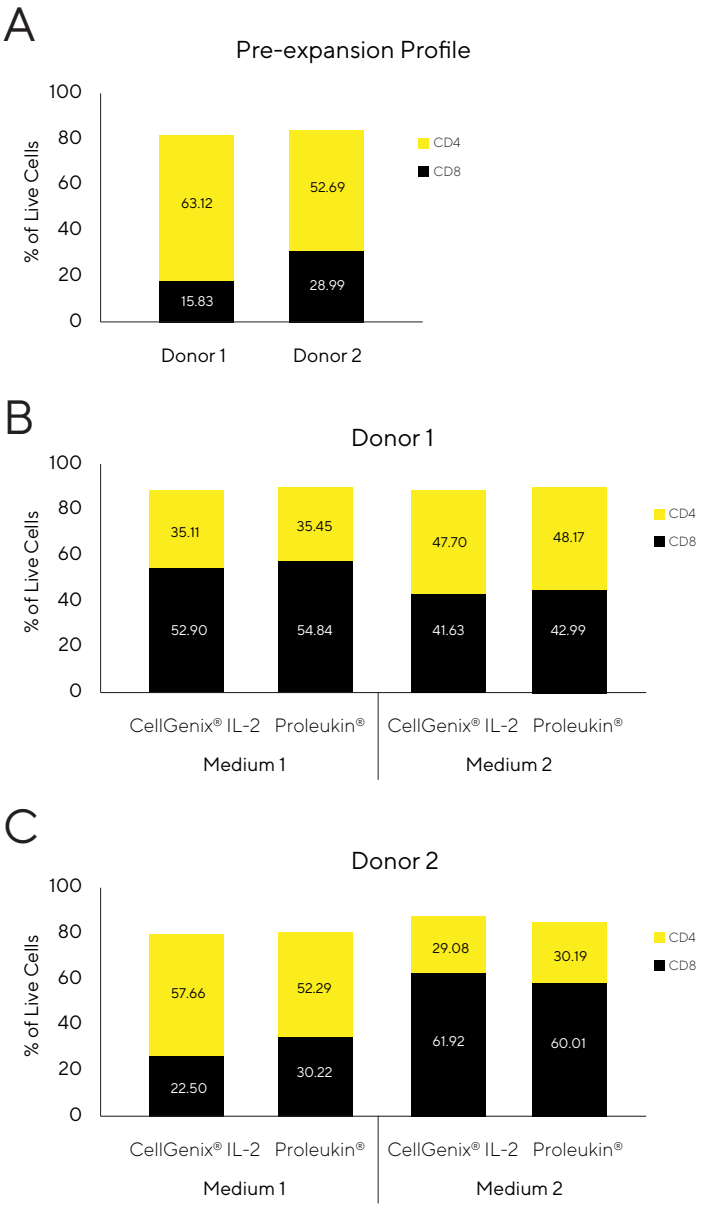

Figure 2. *Expression of CD4 and CD8 before and after IL-2 stimulation of PBMCs from two healthy donors was similar between CellGenix® Preclinical rh IL-2 and Proleukin®.*
A: The CD4+/CD8+ ratio was similar between the two donors on Day 0.
B and C: CD4 and CD8 expression were assessed after 10 days following IL-2 stimulation and reported as a percentage of total live cell counts for each donor, medium used and IL-2.

Table 2. *T cell subpopulations and selected markers for cultured PBMCs stimulated with IL-2.*

Cell Type	Abbreviation	Marker Expression
Naïve T cells	$T_{Naïve}$	CD3+, CD8+/CD4+, CD45RA+, CD45RO-, CD62L+, CD197+, CD95-
Central memory T cells	T_{CM}	CD3+, CD8+/CD4+, CD45RA-, CD45RO+, CD62L+, CD197+
Effector memory T cells	T_{EM}	CD3+, CD8+/CD4+, CD45RA-, CD45RO+, CD62L-, CD197-
Effector memory RA T cells	T_{EMRA}	CD3+, CD8+/CD4+, CD45RA+, CD45RO-, CD62L-, CD197-
Memory regulatory T cells	$_{m}T_{reg}$	CD3+,CD4+, CD25+, CD45RO+, CD127-
Stem memory T cells	T_{SCM}	CD3+, CD8+/CD4+, CD45RA+, CD45RO-, CD62L+, CD197+, CD95+

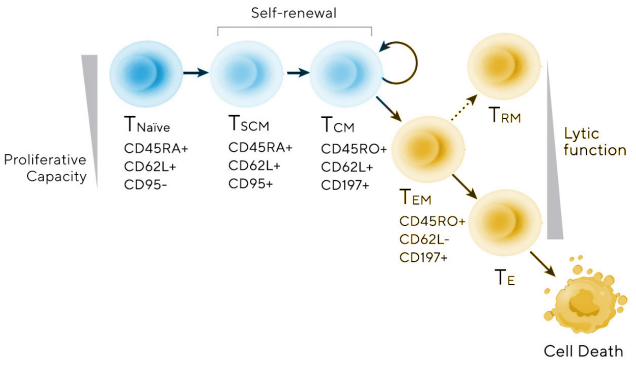

Figure 3. *Representation of memory T-cell differentiation post antigen stimulation*
After stimulation a portion of naïve T-cells are differentiated into memory T-cells. The memory T-cells are further differentiated into stem cell memory T-cells (T_{SCM}), central memory T-cells (T_{CM}), effector memory T-cells (T_{EM}) and resident memory T-cells (T_{RM}). T_{SCM} and T_{CM} subpopulations have the highest potential of self-renewal while effector T-cells (T_{E}) have the highest killing activity.

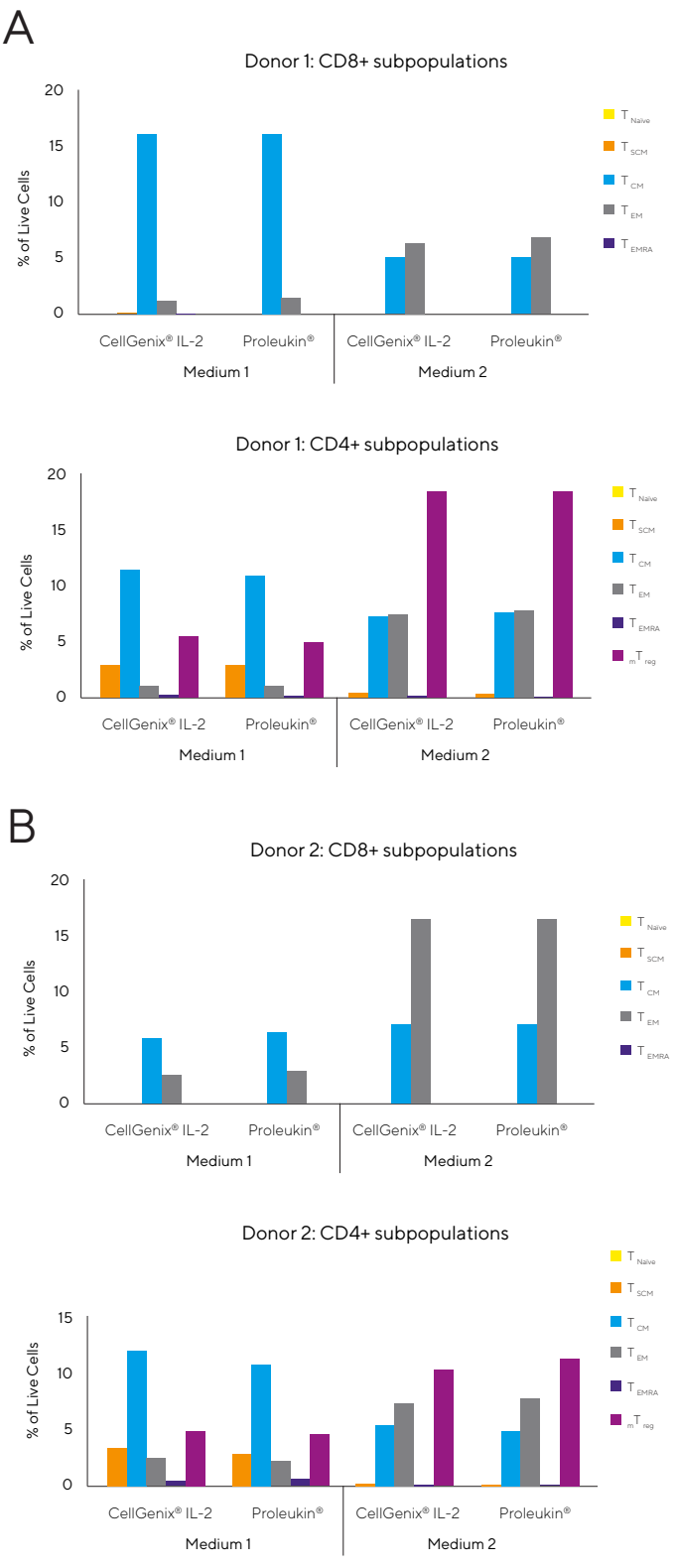

Figure 4. *Percent of CD4+ and CD8+ T cell subpopulation ratio was similar on day 10 in the different treatment groups.* The proportion of CD4+ and CD8+ T cell subpopulations following stimulation with CellGenix® Preclinical rh IL-2 or Proleukin® were similar in Donor 1 (A) and Donor 2 (B).

CD4/CD8 expression ratios were similar between CellGenix® Preclinical rh IL-2 and Proleukin®-stimulated PBMCs

As shown in Figure 2A, the CD4+/CD8+ expression ratio was similar between the two donors on Day 0. Cells from each treatment group were processed for T cell marker characterization by spectral flow cytometry using a panel of 10 markers (CD3, CD4, CD8, CD62L, CCR7, CD45RA, CD45RO, CD25, CD127, CD95) or matching isotype controls (Table 2 and Figure 3). Analysis of total live cells on day 10 (Figure 2B and 2C) revealed that the proportion of cells expressing CD4 or CD8 was similar between CellGenix® Preclinical rh IL-2 and Proleukin®-stimulated PBMCs and was independent of the medium used. Further analysis into the specific T cell subpopulations higlights similarties in profiles between Cellgenix® Preclinical rh IL-2 and Proleukin conditions (Figure 4).

Discussion

IL-2 is an essential reagent for cell therapy manufacturing particularly because of its ability to promote expansion of T cell populations. Proleukin® is considered a standard-bearer in quality and consistency because of the strict regulatory compliance production must adhere to as compared with research use only IL-2. However, its use in the development of cell-based therapies is cost-prohibiting.

CellGenix® Preclinical rh IL-2 was developed to provide a cost-effective alternative to GMP grade cytokines (e.g., CellGenix® GMP grade IL-2) in the development stage, that offers comparable quality and consistency, enabling effective expansion and limiting the quality control assays necessary to transition the development of robust cellular therapies into clinical production.

In this application note, we demonstrated that CellGenix® Preclinical rh IL-2 performs as well as or better than Proleukin® with respect to total cell counts, fold expansion, CD4/CD8 expression ratio, and T cell subpopulations. As growing amounts of clinical data becomes available there is a growing trend that more naïve T cell subpopulations improve critical quality attributes contributing to efficacy.[3] Therefore, it is important to have raw materials that provide consistent and predictable results, especially when transitioning into clinical production. CellGenix® Preclinical rh IL-2 provides this seamless transition when switching to manufacturing with CellGenix® GMP rh IL-2. These studies also show that the ability of CellGenix® Preclinical rh IL-2 to stimulate PBMCs is independent of the media or donor. The results from this investigation reveal that CellGenix® Preclincial IL-2 is comparable to Proleukin®, which is highly beneficial to those engaged in the development of cell-based thera-

pies. These results also support the use of CellGenix® Preclincial IL-2 as a high quality raw material in the development of immune-cell therapies including CAR-T.

Acknowledgement

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Ordering Information

Product Description	Product Name	Item Number
Animal-Derived Component-Free Recombinant Human IL-2	CellGenix® rh IL-2 Preclinical grade	1420-050
Animal-Derived Component-Free Recombinant Human IL-2	CellGenix® rh IL-2 GMP grade	1020-050 1020-100

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

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www.sartorius.com