Xuri growth factors

CELL EXPANSION

Growth factors regulate many aspects of cellular function, including proliferation and differentiation. They are widely used for the expansion of T lymphocytes and other primary cell types in cell therapy and research applications. Xuri™ IL-2, IL-15, and IL-21 growth factors are developed following the recommendations of USP <1043> "ancillary materials for cell, gene, and tissue-engineered products" and manufactured under relevant current good manufacturing process (cGMP) guidelines. Xuri growth factors allow rapid development of a robust and reproducible cell therapy process.

Rapid process development

Each lot of Xuri growth factor includes clear information on specific activity. Activity-based information allows the consistent dosing of cell culture medium, which will help to minimize process variation. With Xuri growth factors you can standardize your process and achieve reproducible cell processing without the need to validate each new lot of growth factor.

High quality

Xuri IL-2 is produced under a certified GMP license that is regularly qualified by the licensing authorities. The manufacturing process is in accordance with relevant International Conference on Harmonization (ICH) guidelines. The product release procedures are executed under a quality management system following ISO13485. Both sterility and endotoxin levels are tested according to USP guidelines. Xuri growth factors are intended to provide a high quality and safe cellular product for *ex vivo* applications.

USP <1043> compliance

A smooth regulatory submission process is key when dealing with cell therapy requirements. To help you assess and document your production processes, each Xuri growth factor has a comprehensive documentation support file. These support files follow USP <1043> guidelines for "ancillary materials for cell, gene, and tissue-engineered products[1]".

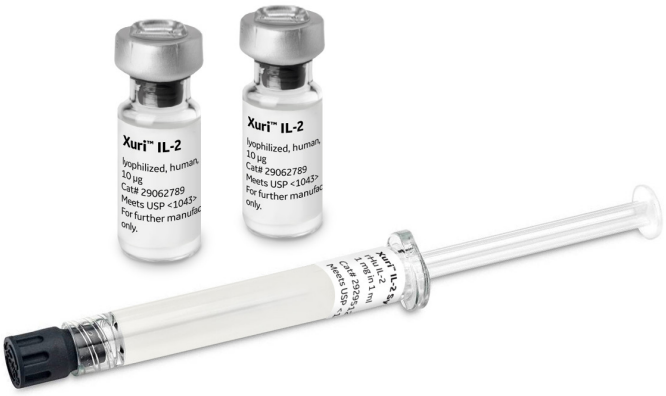

Fig 1. Xuri IL-2 provides reliable and efficient expansion of T lymphocytes. Two formats are available – lyophilized in vials (left) or solution in syringes (right).

Xuri IL-2

Xuri IL-2 (Fig 1) is a reliable reagent for the expansion of T lymphocytes (Fig 2). It is available as:

- lyophilized product in vials
- Stabilized liquid solution (1 mg/mL) supplied in syringe format. The syringe format helps to reduce contamination risk without impacting productivity or performance.

Xuri IL-2 is a proven technology that performs well when used in combination with the Xuri Cell Expansion System. To further accelerate cell expansion process development, we provide a detailed protocol for T cell expansion. This protocol delivers reproducible cell proliferation results in static and perfusion cultures (Fig 2). Even after 14 days of culture, the majority of cells were in the early/intermediate stages of differentiation based on CD27 and CD28 expression patterns (Fig 3), without detectable senescence of the cells.

[1] Xuri Growth Factor products meet USP <1043> "ancillary materials for cell, gene, and tissue-engineered products", within the responsibilities applicable to a supplier. Other aspects of USP <1043> will be the responsibility of the end-user to assess. Cytiva cannot fulfil USP <1043> in regards to application and therapy specific aspects (e.g., use in finished therapeutic, assessment of removal from a finished therapeutic and possibly biocompatibility, cytotoxicity or adventitious agent testing).

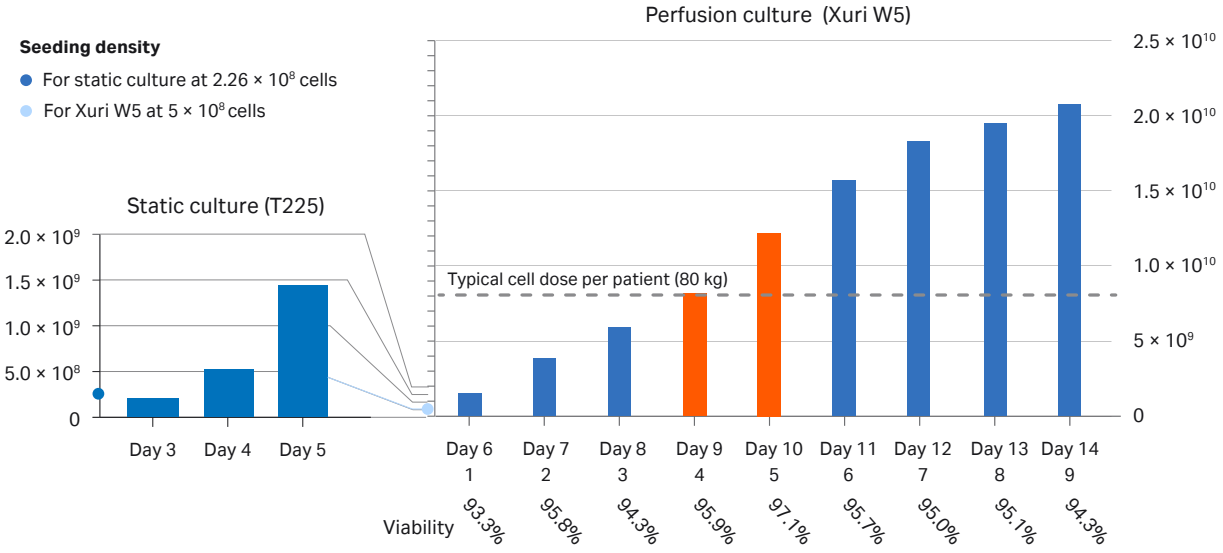

Fig 2. T lymphocytes isolated from peripheral blood were initially cultivated in static cell culture (T225 flasks) with 5% human serum plus 200 IU/mL Xuri IL-2 (left graph). Cells were activated using antibody-coated beads. Cells were counted from day three onwards, and expanded until they passed the number needed for seeding in a 2 L Cellbag™ bioreactor (5 × 10^8). Cells were transferred and cultivated on a Xuri Cell Expansion System W5 for an additional 9 d under perfusion. Media composition and Xuri IL-2 concentrations were kept as in static culture (see application note 28965052). Cell count and viability analyses were performed each day.

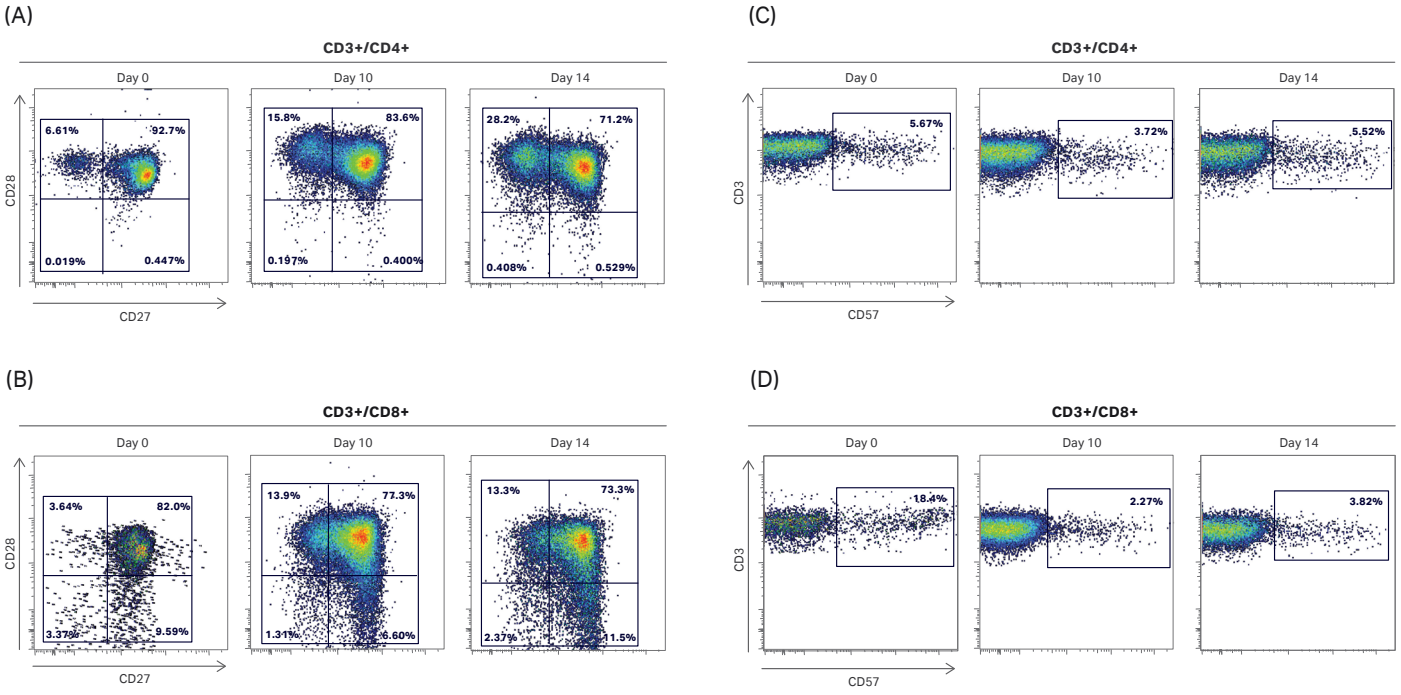

Fig 3. Expression of co-stimulatory molecules CD28 and CD27 as well as CD57 in CD3+/CD4+ T (helper) cells and CD3+/CD8+ (cytotoxic) T cells in Xuri Cell Expansion System W5 at day 0, day 10 and day 14 of culture (lymphocytes were gated based on their forward- and side-scatter profile). (A) Percentage CD28+ and CD27+ within CD3+/CD4+ T cells; (B) Percentage CD28+ and CD27+ within CD3+/CD8+ T cells. (C) Percentage CD57+ within CD3+/CD4+ T cells; (D) Percentage CD57+ within CD3+/CD8+ T cells. The expression patterns of the cell surface markers CD27 and CD28 give an indication of the differentiation state of T cells. Naïve or early differentiated T cells are CD27+/CD28+, effector T cells are CD27+/CD28- or CD27-/CD28+ and late effectors or 'aged' T cells are CD27-/CD28-. Expression was high and after 14 d of culture the majority of cells were in the early/intermediate stages of differentiation. CD57 expression represents senescence of cells. Flow cytometric analysis showed no accumulation of CD57+ T cells.

Xuri IL-2 availability in formulated expansion medium

Consistent availability of growth factors throughout culture is a key requirement for cell manufacturing. The concentration of Xuri IL-2 and Human IL-2 IS, premium grade (Miltenyi Biotec) was evaluated over 8 days at two concentrations and temperatures. Enzyme-linked immunosorbent assay (ELISA) data showed that Xuri IL-2 maintained its concentration profile until day 8 (Fig 4).

Performance of Xuri IL-2 Syringe

Performance of Xuri IL-2 Syringe (three lots) and lyophilized Xuri IL-2 were compared. Performance was determined by measuring ability to support cell growth, cell viability, phenotype (CD3/CD4/CD8); and activation level (CD25). Proliferation (Fig 5A) and cell viability (Fig 5B) were similar for all three lots of Xuri IL-2 Syringe. The results were comparable with those of the lyophilized product. Phenotype and activation levels (Fig 5C) were comparable across all three lots of Xuri IL-2 Syringe and the lyophilized product.

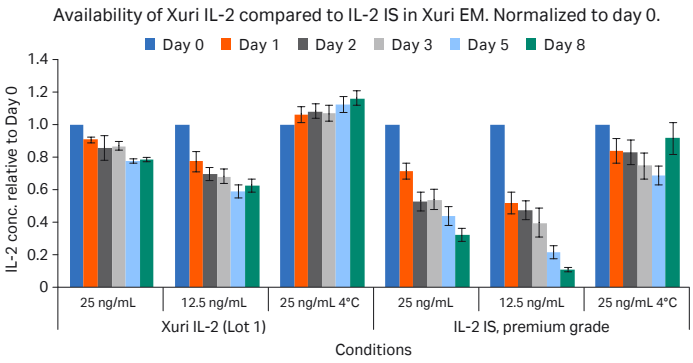

Fig 4. IL-2 stability profile over 8 d in Xuri Expansion Medium (EM), normalized to day 0. GMP compliant Xuri IL-2 and IL-2 IS, premium grade were added to Xuri basal medium containing 5% human AB serum at two different concentrations (25 and 12.5 mg/mL), and kept in a humidified tissue culture incubator at 5% CO_2 and 37°C. A control sample of Xuri EM with 5% human AB serum containing either Xuri IL-2 or IL-2 IS was left at 4°C. Media were collected at days 0, 1, 2, 3, 5, and 8, protease inhibitors were added, and samples were frozen in a -80°C freezer until analysis. After samples from all time points were collected, samples were thawed and IL-2 concentration was determined using an OptEIA™ IL-2 ELISA kit (BD Biosciences). Results are mean ± standard deviation, n = 3.

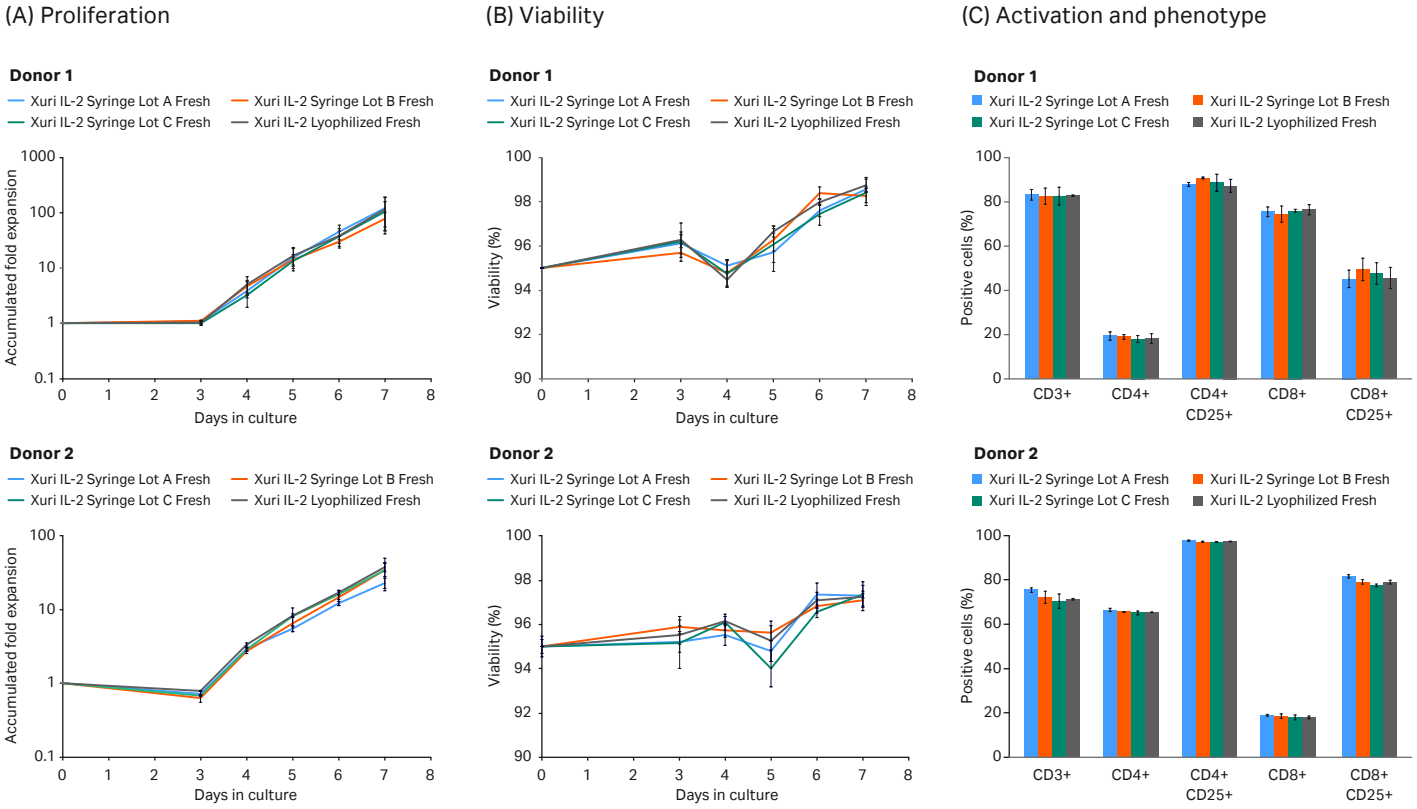

Fig 5. Performance of three lots of Xuri IL-2 Syringe (solution) in T cell expansion. Lyophilized Xuri IL-2 served as a control. (A) Proliferation, (B) Viability, and (C) Phenotype. Human peripheral blood mononuclear cells (PBMCs) were obtained from 2 donors. IL-2 was added to 350 IU/mL in ImmunoCult™ XF T Cell Expansion Medium (STEMCELL Technologies) supplemented with 5% heat-inactivated human serum (Gemini Bio-Products). On day 0, T lymphocytes were activated using CD3/CD28/CD2 soluble activators (STEMCELL Technologies) and cultivated in T-25 flasks for 8 d at 37°C and 5% CO_2. Cells counts were performed daily to determine the daily fold increase and viability. Cells were stained with dye-labeled antibodies (PerCP Cy™ 5.5-CD3, V500-CD4, Alexa Fluor™ 488-CD8, and PE-CD25) and subjected to flow cytometry using a BD LSRFortessa™ system (antibodies and flow cytometer from Becton Dickinson). Results are mean ± standard deviation, n = 3.

Xuri IL-15

Interleukin 15 (IL-15) is known to regulate the activation, proliferation, and differentiation of human lymphocytes. In cultivation procedures it is used in the expansion of T lymphocytes and natural killer (NK) cells.

Xuri IL-21

Interleukin 21 (IL-21) is known to regulate the proliferation and differentiation of human lymphocytes. In cultivation procedures it can be used to influence the phenotype of the final expanded T cell population.

Ordering information

Product	Pack size	Product code
Xuri IL-2 Syringe, 1 mg/mL (USP<1043> for further manufacturing only)	1 mg	29295127
Xuri IL-2 (USP<1043> for further manufacturing only)	10 µg	29062789
Xuri IL-2 (USP<1043> for further manufacturing only)	1 mg	29062790
Xuri IL-15 (USP<1043> for further manufacturing only)	40 µg	29112116
Xuri IL-21 (USP<1043> for further manufacturing only)	40 µg	29112119

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