Highly Stable Liquid IL-7 and IL-15 Closed System Solutions Formulation for Sterility Assurance and Reproducible Biological Activity in Cell Therapy Applications

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ABSTRACT

Cytokines are important factors for T cell development and homeostasis. In addition to the T cell receptor (TCR) and costimulatory receptors, cytokines provide stimulatory signals for full T cell activation, and have pleiotropic effects on T cell proliferation, differentiation and function. Several γ-chain (γc) cytokines, including Interleukin-2, Interleukin-7 and Interleukin-15 (IL-2, IL-7, and IL-15) have been found to stimulate the growth of T cells. Akron Bio has developed and distributed recombinant human (rHu) IL-2, IL-7, and IL-15 in their lyophilized forms for more than a decade, enabling the manufacture of clinical and commercial T cell-based immunotherapies. Recently, Akron Bio launched its Closed System Solutions (CSS)™ line of cGMP products to functionally close critical unit operations in cell and gene therapy process workflows. Autologous cell therapy manufacturing processes require small amounts of these cytokines, which users have historically accommodated by reconstituting and aliquoting larger vials of lyophilized material. However, industry trends toward the closure of key unit operations, greater process automation, and the decentralization of production all necessitate reagents that can be delivered in low-dose, single use, ready-to-use formats that can accommodate speed and consistency without compromising on safety. Cytokines are known to be susceptible to chemical and physical degradation, especially in liquid format and at low concentrations. They are also susceptible to aggregation, surface adsorption, and oxidation. The advancement of cell therapy manufacturing requires the delivery of these cytokines in liquid format, at low concentrations, and in small bioprocessing bags that enable sterile integration into the workflow. This must be achieved without compromising their chemical, physical or biological stability. Leveraging our decades-long expertise in liquid cytokine development, we previously developed and launched a cGMP CSS rHu IL-2 that is currently available on the market. Here, we report the formulation development of our CSS rHu IL-7 and rHu IL-15, showing comparative results from three formulations designed based on their theoretical ability to stabilize cytokine formulation in small bags. We also report results comparing our formulated CSS format rHu IL-7 and rHu IL-15 to the traditional lyophilized format. The formulation development approach adopted for rHu IL-7 and rHu IL-15 CSS format will facilitate the development of a wide range of cytokines in a format that meets new and emergent cell therapy processing needs.

BACKGROUND

Suppliers of ancillary materials required for the manufacture of cell and gene therapies have recently focused on ways to de-risk, standardize, and streamline the use of these materials in workflows, enabling modular process design and cost-effective manufacturing while bolstering the safety profile of these materials. For instance, suppliers are partnering via industry associations and standardization bodies to harmonize testing and reporting, are establishing manufacturing-friendly packaging solutions that integrate with leading manufacturing platforms and are driving toward the deployment of chemically-defined media formulations where feasible, ensuring the use of USP/EP-compliant materials where available. Akron Bio has been providing cytokines, growth factors, and human plasma-derived media supplements to the cell and gene therapy industry since 2006. Akron's products and services are now embedded in nearly 150 clinical and commercial programs in the US, Europe and Asia. Leveraging decades of experience in supporting cell therapy development and responding to emerging market needs, Akron launched its CSS line of products, including AB serum, Human Serum Albumin and rHu cytokines, with rHu IL-2 being the first cytokine launched (Figure 1). Given the criticality of rHu IL-7 and IL-15 in T cell-based immunotherapy manufacturing, Akron decided to broaden its CSS portfolio with these two products. The physicochemical stability of the formulations has been assessed for 12 months for rHu IL-15 CSS and 9 months for rHu IL-7 CSS, to date. We compared the performance of rHu IL-7 and IL-15 liquid CSS formulation with the traditional lyophilized format of rHu IL-7 and IL-15 using T-cells isolated from healthy donors.

Figure 1: Pictures of IL-2 CSS format (A), Human AB Serum CSS Converted from Octaplas® (B), and Human Serum Albumin (HSA) 25% CSS (C) developed by Akron and already on the market. Akron's CSS products are used in several processes on different cell therapy platforms in the industry.

RESULTS

INITIAL FORMULATION DESIGN AND SCREENING USING IL-15

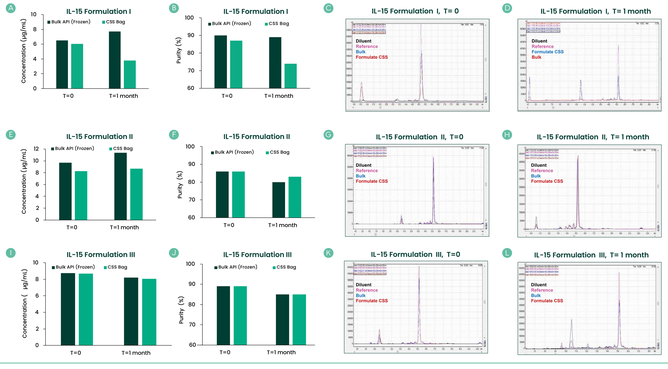

Table 1: Formulations designed for Initial Stability Assessment on IL-15 0.01 mg/mL

Components	Formulation I	Formulation II	Formulation III
rHu IL-15 (mg)	0.01	0.01	0.01
Neutral Buffer System	+	–	+
Acidic Buffer System	–	+	–
Detergent #1	+	–	–
Detergent #2	–	+	+
Formulation Adjuvant	–	+	+

Figure 2: Three formulations (Table 1) were designed and used to formulate IL-15 at 0.01 mg/mL. Two types of detergents were used based on their intrinsic properties and the theoretical prediction that they could prevent aggregation, protein adsorption to the bag container, and IL-15 physicochemical stability. A selected formulation adjuvant was added to Formulation II and Formulation III to assess its ability to prevent oxidation of IL-15. IL-15 concentration and purity were measured by RP-HPLC at time zero (4 hour post-formulation) and 1-month post-formulation for Formulation I (A, B), Formulation II (E, F), Formulation III (I, J). Chromatographic profiles of each IL-15-free formulation (Diluent), an IL-15 standard (Reference), unformulated IL-15 API (Bulk), and IL-15 containing formulations (Formulated CSS) are shown for Formulation I (C,D), Formulation II (G,H) and Formulation III (K,L).

PHYSICOCHEMICAL STABILITY ASSESSMENT FOR IL-15 CSS

IL-15 Formulation II / 12 months stability

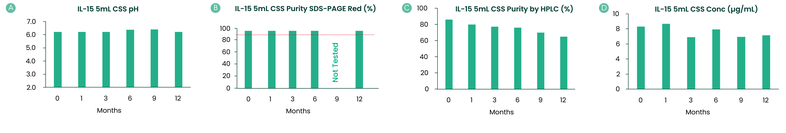

Figure 3: IL-15 was formulated at 0.01 mg/mL in Formulation II and filled at 5 mL in 20mL CSS bags. CSS bags were assessed for changes in physicochemical attributes over 12 months. Bags were stored at 2-8°C. Formulation pH was measured by potentiometry (A). IL-15 concentration and purity was measured by RP-HPLC (C, D). Reduced SDS-PAGE was performed at each time point for further purity assessment. SDS-PAGE graph intend to show if the > 90% purity was met at each time point (B).

IL-15 Formulation III / 12 months stability

Figure 4: IL-15 was formulated at 0.01 mg/mL in Formulation III and filled at 5 mL in 20mL CSS bags. CSS bags were assessed for changes in physicochemical attributes over 12 months. Bags were stored at 2-8°C. Formulation pH was measured by potentiometry (A). IL-15 concentration and purity was measured by RP-HPLC (C, D). Reduced SDS-PAGE was performed at each time point for further purity assessment. SDS-PAGE graph intend to show if the > 90% purity was met at each time point (B).

PHYSICOCHEMICAL STABILITY ASSESSMENT FOR IL-7 CSS

IL-7 Formulation II / 9 months stability

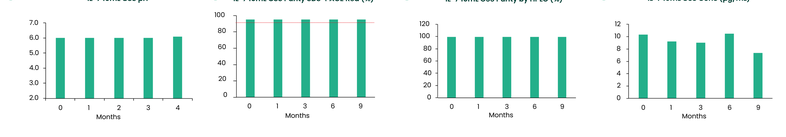

Figure 5: IL-7 was formulated at 0.01 mg/mL in Formulation II and filled at 10 mL in 20mL CSS bags. CSS bags were assessed for changes in physicochemical attributes over 9 months. Bags were stored at 2-8°C. Formulation pH was measured by potentiometry (A). IL-7 concentration and purity was measured by RP-HPLC (C, D). Reduced SDS-PAGE was performed at each time point for further purity assessment. SDS-PAGE graph intend to show if the > 90% purity was met at each time point (B).

IL-7 Formulation III / 9 months stability

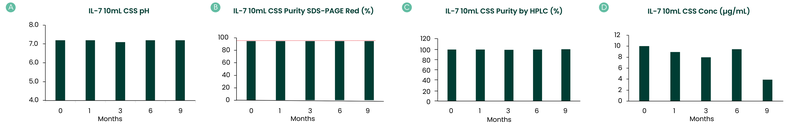

Figure 6: IL-7 was formulated at 0.01 mg/mL in Formulation III and filled at 10 mL in 20mL CSS bags. CSS bags were assessed for changes in physicochemical attributes over 9 months. Bags were stored at 2-8°C. Formulation pH was measured by (A). IL-7 concentration and purity was measured by RP-HPLC (C, D). Reduced SDS-PAGE was performed at each time point for further purity assessment. SDS-PAGE graph intend to show if the > 90% purity was met at each time point (B).

BIOLOGICAL ACTIVITY STABILITY IL-7 AND IL-15 CSS

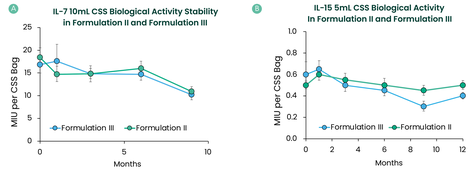

Figure 7: rHu IL-7 and rHu IL-15 were formulated at 0.01 mg/mL in Formulation II or Formulation III and filled at 10 mL in 20mL CSS bag (rHu IL-7) or 5 mL in 20mL CSS bag (rHu IL-15). Each formulation was tested for Biological activity at time zero and various time point over 9 months (rHu IL-7) and 12 months (rHu IL-15). Errors bars represent assay results confidence interval at each timepoint.

FUNCTIONAL COMPARISON WITH LYOPHILIZED IL-7 AND IL-15 USING HEALTHY DONORS DERIVED T-CELLS

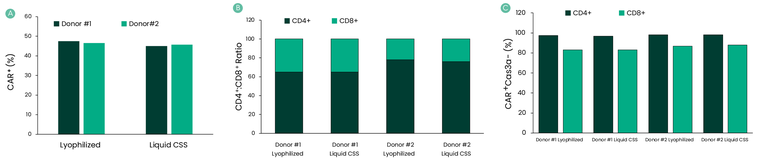

Figure 8: IL-7 and IL-15 CSS were compared with their lyophilized forms with cells isolated from healthy donors. Cryo-preserved apheresis from two healthy donors were thawed, selected, and activated. Culture media were supplemented with reconstituted lyophilized powders of IL-7 and IL-15 or liquid formulation of CSS. Final concentration of IL-7 and IL-15, was 600 IU/mL and 100 IU/mL, respectively. Media was also supplemented with 100 IU/mL of IL-2. Prepared media were stored at 2-8°C and used 12 days post- cytokine addition. Cells were assessed for the percentage of CAR+ (A) and CD4+ CD8+ ratio (B) at harvest. Harvested cells were cryopreserved to mimic the storage of the drug product. Several days after cryopreservation, cells were thawed, and assessed for viability (C).

MATERIALS & METHODS

All formulations were prepared using cGMP compliant chemicals. Recombinant Human IL-7 and IL-15 cytokines were produced and purified using validated processes routinely used to manufacture Akron's commercially available rHu IL-7 and IL-15 lyophilized products. The rHu IL-7 and IL-15 CSS products were filled into Entegris Aramus™ 2D Single-Use Bag Assemblies. Purity was assessed by RP-HPLC and SDS-PAGE. IL-7 and IL-15 concentrations were derived via RP-HPLC by integrating the peak corresponding to non-oxidized rHu IL-7 or IL-15. Biological activity was determined using 2E8 cells for rHu IL-7 and CTLL-2 cells for IL-15. Akron utilizes a parallel line concentration-response model to estimate a relative potency compared to the NIBSC reference standard, applying the principles outlined in USP <1032> Design and Development of Biological Assays & USP <1034> Analysis of Biological Assays.

DISCUSSION

The mechanisms by which cytokines drive T cell activation and expansion have been well studied and their use in cell therapy manufacturing is commonplace. The formulation and presentation of these materials in formats optimized for further manufacturing applications is an area of industry need, especially as manufacturing becomes increasingly modular and automated to meet the needs of larger patient populations. This poster summarized Akron's effort to establish low-dose, liquid cytokines in closed system formats that can retain the products' integrity over time. We demonstrate that rHu IL-7 and rHu IL-15 can be formulated at concentrations as low as 10 mg/mL while maintaining biological activity for 9 months and 12 months, respectively, with long-term stability studies ongoing. The formulation development approach that we designed explored buffer composition that can sustain the stability of a low concentration of these cytokines. The low concentration is likely to promote physicochemical instability and increase the risk of oxidation (1,2,3) which is a known chemical degradation pathway for cytokines. Through formulation development work, these sources of instability were addressed, enabling the team to establish shelf-stable liquid formulations optimized for manufacturing. Next steps include further characterization of these products, an ongoing evaluation of their stability, and the further optimization of these products to enable compatibility with a diverse array of manufacturing protocols.

REFERENCES/ACKNOWLEDGEMENTS

(1) Ruiz L. et al. 2005, J Pharm Pharm Sci 8:207-216. (2) Gitlin G. et al. 1996, Pharm. Res 13:762-769. (3) Torosantucci R. et al. 2013, Mol. Pharm 10:2311-2322. The whole Akron Bio team has been instrumental in the production of this work.