Stability and Dependability in Cell Manufacturing: The Development of an AI-Modified IL-2 Heat Stable Agonist Protein

In this study, we have utilized artificial intelligence (AI)/predictive analytics to develop a thermal stable IL-2 protein that contains a stabilized synthetic core and engages only the IL-2 dimeric βγ receptor complex. This IL-2 Heat Stable Agonist Protein has enhanced stability, enabling it to withstand high temperatures and extended culture durations, making it an ideal choice for cell therapy manufacturers.

Introduction

Interleukin-2 (IL-2) is an essential reagent when manufacturing immune-based therapies, including T cells and natural killer (NK) cells. Cell manufacturing relies on stable and dependable reagents for consistency in culture conditions and standardization of developmental procedures, and IL-2 is no exception. Adding cytokines to media reservoirs a few days before experiments is optimal, but practical concerns such as cytokine degradation, availability of refrigeration equipment, and general shipping and storage issues pose challenges. To address these issues, we utilized artificial intelligence (AI)/ predictive analytics to develop a heat stable IL-2 protein. Structurally, this protein improves upon the disordered and unstable regions found in the standard IL-2 protein, while maintaining efficient engagement and activation of the IL-2βγ receptors. The IL-2 Heat Stable Agonist Protein outperforms the standard IL-2 protein in heat stability, IL-2βγ affinity, and duration in media, while delivering equivalent results for T cell outgrowth. Incorporating the IL-2 Heat Stable Agonist Protein into cell therapy manufacturing workflows can help address some of the increasingly demanding operational and process development requirements that the field is facing.

Key Takeaways

- The IL-2 Heat Stable Agonist Protein was engineered to engage only the IL-2 dimeric βγ receptor complex, making it an excellent choice for culturing T cells or tumor-infiltrating lymphocytes (TILs).
- The melting temperature (Tm) of the Recombinant Human IL-2 Heat Stable Agonist Protein is much higher than that of the standard IL-2 protein, and it retains activity at both 37 ˚C and 95 ˚C.
- The IL-2 Heat Stable Agonist Protein has enhanced stability in media over extended culture durations, allowing greater consistency throughout the production process.
- The IL-2 Heat Stable Agonist Protein and the standard IL-2 protein promote comparable $CD8^+/CD4^+$ T cell outgrowth, with similar T cell phenotypes.

Experimental Workflow

Isolated frozen T cells were thawed, plated, and activated with anti-CD3, anti-CD28 antibodies conjugated to magnetic beads. Cells were maintained at a cell density of 0.25 x 10^6 or 0.5 x 10^6 cells/cm^2 after day 12 with 10ng/mL Recombinant Human IL-2 Heat Stable Agonist Protein (R&D Systems, Catalog # BT-002HS) or standard IL-2. IL-2 was refreshed every 2-3 days. *Note: The standard IL-2 protein used in this study is R&D Systems™ Animal-free Recombinant Human IL-2 (Catalog # BT-002-AFL), which is the commonly used Aldesleukin version of IL-2.*

Results

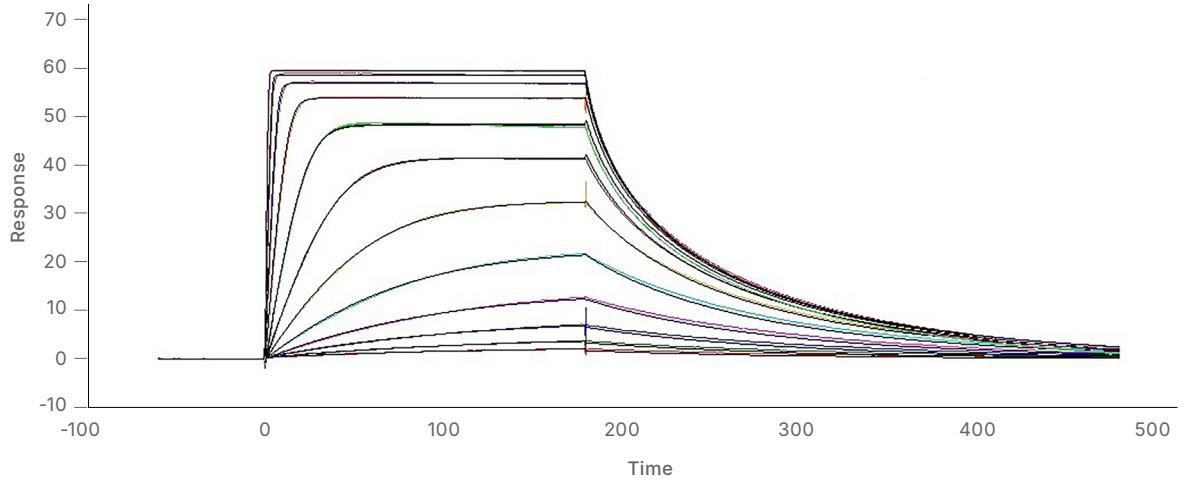

Figure 1. Affinity Measurements and Binding Kinetics of the Interaction between IL-2 Rβ and the IL-2 Heat Stable Agonist Protein by SPR. Recombinant Human IL-2 Rβ Fc Protein (R&D Systems, Catalog # 10919-2B) was captured on a Sensor Chip Protein AGL, and binding to Recombinant Human IL-2 Heat Stable Agonist Protein (R&D Systems, Catalog # BT-002HS) was measured at a concentration range between 0.097nM and 200 nM. The double-referenced sensorgram was fit to a 1:1 binding model to determine the binding kinetics and affinity, with an affinity constant of KD=2.55 nM. *Note: The IL-2 Heat Stable Agonist was modeled to engage only the IL-2 dimeric βγ receptor complex.*

	Standard IL-2	IL-2 Heat Stable Agonist Protein
Tm	56 °C	72 °C

Table 1. R&D Systems IL-2 Heat Stable Agonist Protein has a Significantly Higher Melting Temperature than the Standard IL-2 Protein. The melting temperatures (Tm) of the standard Animal-free Recombinant Human IL-2 (R&D Systems, Catalog # BT-002-AFL) and Recombinant Human IL-2 Heat Stable Agonist Protein (R&D Systems, Catalog # BT-002HS) were determined through a thermal shift assay using SYPRO® Orange.

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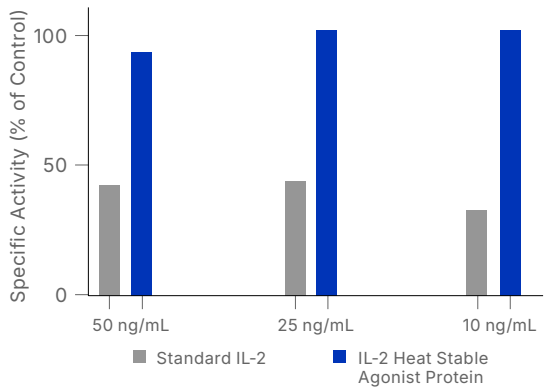

Figure 2. The IL-2 Heat Stable Agonist Protein is More Stable in Media than the Standard IL-2 Protein. The stability of the Recombinant Human IL-2 Heat Stable Agonist Protein (R&D Systems, Catalog # BT-002HS) was evaluated in media at three different concentrations (50, 25, and 10 ng/mL) and compared to the stability of the standard Animal-free Recombinant Human IL-2 Protein (R&D Systems, Catalog # BT-002-AFL) at the same concentrations. Specifically, the standard IL-2 protein and the IL-2 Heat Stable Agonist Protein were stored in media at the indicated concentrations in a humidified tissue culture incubator at 5% CO_2 and 37 ˚C for 10 days. The specific activity was measured using a 96-hour NK92 cell proliferation assay. The data demonstrates that the IL-2 Heat Stable Agonist Protein maintained specific activity at all concentrations after 10 days in media at 37 ˚C, while the activity of the standard IL-2 protein was reduced.

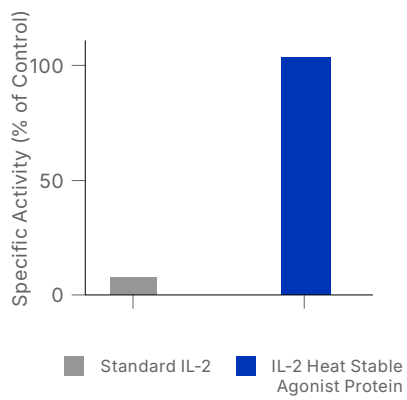

Figure 3. The IL-2 Heat Stable Agonist Protein Maintains Stability at Extreme Temperatures. To analyze the stability of the IL-2 Heat Stable Agonist Protein (R&D Systems, Catalog # BT-002HS) at extreme temperatures, this protein and the standard Animal-free Recombinant Human IL-2 Protein (R&D Systems, Catalog # BT-002-AFL) were subjected to 95 ˚C for 10 min., allowed to cool and then added to a culture of NK92 cells to assess their abilities to support cell proliferation. The data demonstrates that only the IL-2 Heat Stable Agonist Protein retains activity following exposure to 95 ˚C.

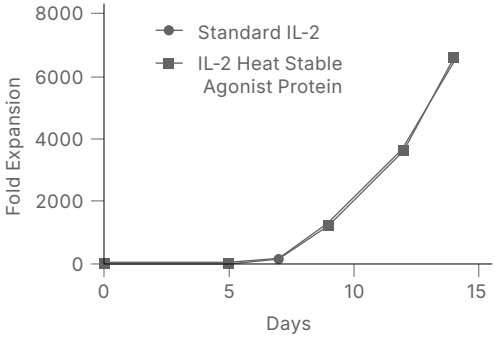

Figure 4. The IL-2 Heat Stable Agonist Protein Provides Consistent CD4⁺/CD8⁺ T Cell Proliferation in Side-by-Side Testing with the Standard IL-2 Protein. $CD4^+$ and $CD8^+$ T cells were activated with anti-CD3-, anti-CD28-conjugated beads, and grown for 14 days in media containing 10 ng/mL Animal-free Recombinant Human IL-2 (R&D Systems, Catalog # BT-002-AFL) as the standard IL-2 protein or the IL-2 Heat Stable Agonist Protein (R&D Systems, Catalog # BT-002HS). IL-2 conditions were refreshed every 2-3 days. The average fold expansion of the cells was determined every 2-3 days and showed that the IL-2 Heat Stable Agonist Protein supports similar levels of T cell expansion as the standard IL-2 protein.

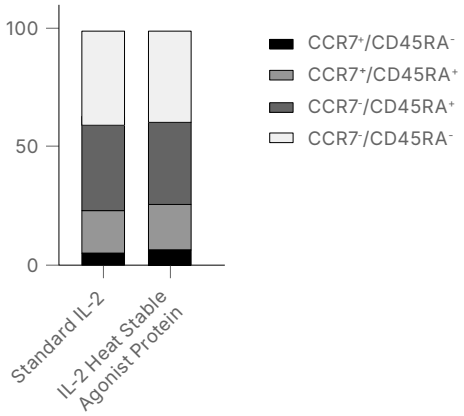

Figure 5. T Cells Expanded with Standard IL-2 or IL-2 Heat Stable Agonist Protein Display Similar Phenotypes. $CD4^+$ and $CD8^+$ T cells were activated with anti-CD3-, anti-CD28-conjugated beads, and grown for 14 days in media containing 10 ng/mL Animal-free Recombinant Human IL-2 (R&D Systems, Catalog # BT-002-AFL) as the standard IL-2 protein or the IL-2 Heat Stable Agonist Protein (R&D Systems, Catalog # BT-002HS). IL-2 conditions were refreshed every 2-3 days. After 21 days, the phenotypes of the cells were compared by flow cytometry using a PE-Cy7-conjugated mouse anti-human CD45RA monoclonal antibody and a Brilliant Violet 711-conjugated mouse anti-human CCR7 monoclonal antibody to determine the number of $CD4^+$ and $CD8^+$ naive ($CD45RA^+$ $CCR7^+$), central memory ($CD45RA^-$ $CCR7^+$), terminal effector memory ($CD45RA^+$ $CCR7^-$), and effector memory ($CD45RA^-$ $CCR7^-$) T cells. The percentages of each of these cell populations were found to be similar whether the cells were expanded with the standard IL-2 or the IL-2 Heat Stable Agonist Protein.

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Conclusion

To help address some of the practical challenges of using cytokines in cell therapy manufacturing processes, we have developed an IL-2 Heat Stable Agonist Protein using artificial intelligence/predictive analytics. By evaluating the performance of this protein relative to the standard IL-2 protein under various conditions including time, temperature, medium, concentrations, and T cell outgrowth, we have found that the IL-2 Heat Stable Agonist Protein has a higher melting temperature and maintains its activity profile at all tested concentrations at both 37 ˚C and 95 ˚C. As a result, this breakthrough provides the opportunity for improved dependability in the manufacturing of immune-based therapies. It can ultimately offer researchers confidence in approaching the operational and process development requirements necessary for cell therapy manufacturing.