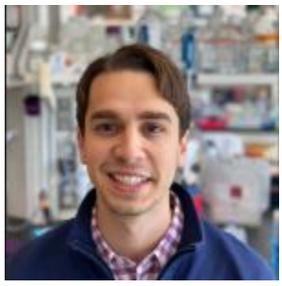
Timmerman Report

The New Frontier of Cytokine Therapies: Engineered Selectivity

By Luciano Santollani, July 26, 2022



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We're in the middle of a cytokine renaissance.

One of the initial cancer immunotherapies, cytokines made their clinical debut over 30 years ago, with Interleukin-2 (IL-2) leading the charge. In the body, this small protein is known to activate and proliferate T cells, making it a naturally promising candidate for immunotherapy.

IL-2 cemented its "clinically validated" status when it achieved an impressive 16% Objective Response Rate in patients with metastatic melanoma treated with a high dose of the cytokine (source). This, along with other promising data in renal cell carcinoma, led Chiron to secure FDA approval for Proleukin in 1992.

Still, there was a problem.

Toxicity — most notably vascular leak syndrome (VLS) — and a narrow therapeutic window quickly led to IL-2 falling out of style in the clinic. In fact, current annual sales of Proleukin hover around $60M (source), a tiny sum in comparison to some of the immuno-oncology blockbusters -- the PD-1 inhibitor pembrolizumab raked in over $17 billion for Merck in 2021.

For the last two decades, much effort has gone into engineering IL-2 to retain or even improve its efficacy while shedding toxicity, finally unleashing its full potential.

To understand what drives IL-2's behavior, we need to dive into the biology of how it works. Specifically, it interacts with cells through a three-part receptor: the beta and gamma chains, which drive IL-2's biological effects, and an optional alpha chain which, when present, strongly increases the affinity between the cytokine and its receptor. There are three key players that express this receptor: (1) CD8 "killer" T cells which mount the anti-tumor response, (2) Natural Killer cells, which also can kill tumor cells but have been associated with toxicity, and (3) CD4 regulatory T cells, or Tregs, which down-regulate the anti-tumor response and always express the a chain, serving as a sink for IL-2.

After initial engineering to improve the half-life of IL-2, the next clear step was to skew it away from the Tregs that could be hampering its efficacy. This led to the creation of "not-alpha" IL-2s, engineered versions of the cytokine that avoided the a chain and minimized the Treg sink. Some research also associates vascular leak syndrome with alpha-receptor binding, so evading those interactions could also reduce IL-2 toxicity. This wave of engineering was led by Nektar's bempeg, a PEGylated IL-2 meant to minimize a chain binding that was recently terminated because of multiple Phase III failures. Others implementing the "not-alpha" approach to IL-2 engineering include Synthorx, Neoleukin, Anaveon, and Alkermes.

However, some believe that simply avoiding the alpha receptor is not enough. South San Francisco, CA-based Asher Bio is doubling down on selectivity by delivering cytokines to specific cell types. For IL-2, they are targeting it to CD8 T cells.

The company, which has raised over $160 million from Third Rock Ventures and other blue-chip biotech investors since inception, was started when co-founders Ivana Djuretic and Andy Yeung saw "an opportunity to solve the problem of cytokines." The problem Yeung is referring to is

pleiotropy, the potential for cytokines to stimulate multiple cell types broadly. The challenge is to stimulate certain cell types, but not so many.

Andy Yeung, co-founder and chief technology officer, Asher Bio

Jeff Tong, partner at Third Rock and a board member of Asher, agrees that for cytokines, this behavior "is part of their power, but also part of their challenge." Djuretic and Yeung, who left their jobs at Pfizer to pursue the opportunity at Asher, were initially backed by Y Combinator before joining forces with Third Rock in 2021. At that time, the Third Rock team were "looking for ways to harness that cell-type specificity" of cytokines, so backing Asher "made natural sense," says Tong — "we had a very shared alignment toward what we were trying to accomplish."

At the center of Asher's story lies their *cis*-targeting technology, a platform for ensuring that cytokines only signal on their target cell — in the IL-2 case, CD8 T cells. The motivation for this comes from data suggesting that the original not-alpha approach may not be delivering on its full promise. Even though these molecules avoid the a chain, they still bind to Tregs via the beta gamma receptor. Not only that, by also activating NK cells, which highly express the beta gamma chains, not-alpha IL-2s may not be addressing the problem of toxicity. Yeung believes this has led to the "sub-optimal" profiles of traditional not-alpha molecules. With a *cis*-targeting approach, Asher believe they can solve these problems.

To do so, the team at Asher has attenuated the cytokine's affinity to its receptor and then fused it to a CD8-targeting antibody. This engineering is done through a combination of structure-guided, library, and computational tools. The attenuation, which Yeung acknowledges seems "counterintuitive," is the key in making all of this work.

At physiological concentrations, the weakened cytokine is "invisible" to other cell types, Yeung says — but, when the antibody binds to its target, the local concentration of the cytokine is now high enough to signal effectively. This isolates the effects of the cytokine to only the cell type of choice, controlling the pleiotropy that has plagued many cytokines in the clinic, he says.

Asher is able to generate a 100-1,000-fold selectivity increase for the target cell, something "not achievable by simply mutating the cytokine" like some of the not-alpha approaches, says Yeung. The team has presented compelling preclinical data for AB248, their CD8-targeted IL-2 lead, at recent conferences, including the American Association for Cancer Research in April (https://asherbio.com/pipeline/presentations-publications/).

Asher is now planning the next big step in development -- a Phase 1a/1b trial is scheduled to begin before the end of the year.

The company envisions this approach being highly modular. Asher is using lessons from CD8-targeting and applying them to IL-21. Yeung says they "could easily generate the next product by simply swapping individual components," unlocking the possibility of multiple differentiated products.

The IL-2 field is very crowded, with many competing approaches having recently entered, or about to enter, the clinic.

Roche is using a similar approach as Asher to target IL-2 to PD-1 expressing cells, generally thought to be more antigen-experienced. Pfizer is similarly using PD-1 as an anchor for IL-15, a naturally "not-alpha" cytokine that shares the beta gamma receptor with IL-2. Synthekine is developing an IL-2 mutein that also selectively targets antigen-experienced cells.

There are companies regulating cytokine activity spatially rather than cellularly through tumor-specific proteases. These "masked" cytokine companies include Xilio and Werewolf, with the former having dosed first patients this year with their lead IL-2. Others are using local retention after intratumoral injection of cytokines to minimize any systemic exposure. This includes

Cullinan Oncology's Amber program, a collagen-binding IL-2/IL-12 fusion, as well as Ankyra Therapeutics.

Because of its initial clinical data from decades ago, IL-2 has always been the testbed for cytokine engineering, but there are many other promising alternatives, such as IL-12 and interferon, that could benefit from the same engineering principles. Once we begin to see how these approaches — *cis*-targeting, partial agonism, protease-unlocking, local retention — shake out for IL-2 in the clinic, we will quickly see them being applied to other cytokines. These recombinant proteins can be manufactured at large scales, meaning that many patients should have the opportunity to get access here and around the world.

The coming years will be an exciting time for cytokines.