Restoring IL-2 to its cancer immunotherapy glory

Before there was PD1, there was IL-2. Can drug developers channel the effects of this pleiotropic cytokine to take back the lead?

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Checkpoint inhibitors that block PD1 and CTLA4 signalling get all of the credit for setting off the immuno-oncology tsunami. But the cytokine IL-2 put these waves in motion decades before. In 1984, a 33-year-old woman with metastatic melanoma was treated with IL-2, then thought to primarily drive the expansion of effector T cells. Her cancer melted away, and never came back.

The FDA approved a recombinant IL-2 called aldesleukin, at the time owned by Chiron Corporation, for metastatic renal cancer in 1992 and for metastatic melanoma in 1998. Other drug developers chased IL-2-based anti-cancer therapeutics, but the enthusiasm proved temporary. Responses, though deep, were rare. The biologic had to be used at high doses to have an effect. And it was very toxic, triggering potentially fatal vascular leak syndrome and other side effects.

As researchers have studied IL-2, they have found that it has double-edged activity. At high doses, it boosts the proliferation of effector T cells, driving anti-cancer activity. But it also controls the growth of regulatory T cells, immunosuppressive cells that keep the immune system in check.

Now there are new hopes that these activity profiles can be teased apart from one another. IL-2 is back in fashion (TABLE 1). Bristol Myers Squibb (BMS) and Sanofi have each inked billion-dollar-plus deals in recent years to access pegylated IL-2 agents that they can pair with their immuno-oncology offerings. Merck & Co. has partnered on both of those programmes. Roche remains committed to IL-2-based strategies despite some recent setbacks. And a slew of biotech firms have IL-2 candidates in or nearing the clinic.

The explosion of interest in IL-2 is partly a function of the broader hunt for immuno-oncology assets, suspects Abul Abbas, professor emeritus of pathology, who studied IL-2 at the University of California, San Francisco. "The word immuno-oncology seems to generate breathless excitement among pharma executives and investors," he says. But better understanding of the biology of IL-2, the structure of its receptors and the roles it plays in the immune system may be opening up opportunities to overcome the challenges of the past, both in cancer and auto-immune indications (BOX 1).

New technological tools are also contributing factors. "Sometimes it takes new technologies to be able to really unleash otherwise robust biology. I think that's what we're seeing now," says Michael Ehlers, CSO at the venture capital firm ATP, which recently backed Aulos Bioscience's computational approach to anti-IL-2 antibody design.

Sanofi and Synthorx's pegylated biologic SAR444245 uses an unnatural amino acid to improve IL-2's therapeutic profile. Roche is focused on cytokine–antibody fusion candidates. And smaller firms are using IL-2 to de-risk everything from conditionally activated agents that are unmasked only in the tumour environment to mRNA technologies.

For Chris Garcia, a molecular biologist at Stanford University whose group reported the structure of the complete IL-2 receptor complex in *Science* in 2005, the interest in IL-2 highlights growing enthusiasm for protein engineering. "There is a whole renaissance," says Garcia. Natural cytokines do not seem to make good drugs, he says, but old protein engineering concepts combined with better understanding of biologic design may be opening the door to better behaved, less immunogenic engineered cytokines.

"IL-2 is kind of the sentinel. I think we're going to get a lot of answers out of this," says Garcia, who founded Synthekine in 2019 to develop a suite of selective IL-2 therapeutics.

Table 1 | **Select list of IL-2 immuno-oncology candidates**

Drug name	Company	Properties	Lead indications	Phase
Bempegaldesleukin (NKTR-214)	Nektar Therapeutics, Bristol Myers Squibb	IL-2, with six cleavable PEG groups	Melanoma, RCC, bladder cancer	III
Nemvaleukin alfa	Alkermes	Circularly permuted IL-2v–IL2Rα fusion protein	Solid tumours	II
SAR444245	Sanofi/Synthorx	IL-2, with one non-cleavable PEG group	Solid tumours	I/II
RG6279	Roche	IL-2v–anti-PD1 mAb fusion protein	Solid tumours	I
CUE-101	Cue Biopharma	IL-2–HLA complex–HPV16 E7 peptide fusion protein	Head and neck cancer	I
NL-201	Neoleukin Therapeutics	IL-2 protein mimetic, computationally designed		IND
AU-007	Aulos Bioscience	Anti-IL-2 mAb, computationally designed		IND in 2021
STK-012	Synthekine	IL-2 partial agonist, targeting activated T cells		IND in 2021
KY1043	Kymab	IL-2v–anti-PDL1 mAb fusion protein		IND in 2021
BNT151	BioNTech	IL-2v, mRNA encoded		IND in 2021
MDNA11	Medicenna Therapeutics	IL-2 'superkine', albuminated		IND in 2021
WTX-124	Werewolf Therapeutics	Conditionally activated IL-2		Preclinical

HPV-E7, human papilloma virus E7; IL-2v, IL-2 variant; IND, investigational new drug; mAb, monoclonal antibody; RCC, renal cell carcinoma.

Box 1 | **IL-2 in autoimmune indications**

While IL-2-based drugs that can boost effector T cell function are an appealing immuno-oncology prospect, agents that can increase the proliferation of immuno-suppressive regulatory T cells offer auto-immunity applications. Many companies are trying to make the most of this.

Nektar and Eli Lilly's NKTR-358 — a pegylated IL-2 that is designed to preferentially bind the trimeric IL-2 receptor — is in phase II trials in systemic lupus erythematosus and ulcerative colitis, for example. Sanofi's Synthorx has an IL-2 based regulatory T cell-boosting programme that it plans to advance into phase I later this year. And Amgen's AMG-592, an IL-2 mutein with increased regulatory T cell selectivity, is in phase I/II trials in systemic lupus erythematosus and graft versus host disease.

The University of California, San Francisco's Abul Abbas is optimistic that the field is moving in the right direction. "Honestly, there's been more action in stimulating regulatory T cells than in the anti-tumour aspects of IL-2," he says.

But whereas cancer clinical trials tend to be relatively short and well-defined ways to validate a target, auto-immunity trials are often larger, longer affairs, with complex end points and heterogeneous patient populations. "Autoimmune diseases are a tough haul, so they don't attract the same kind of unfettered interest as immuno-oncology," says Abbas.

But for Chris Garcia, a molecular biologist at Stanford University, the jury is still out on the prospects for regulatory T cell potentiators. "So many big drug companies have had IL-2 regulatory T cell potentiators for autoimmunity indications in their pipeline. But where are the data?" he asks. "Some of these companies have shut down those trials, so obviously they're not working very well."

"There is this notion that if you expand regulatory T cells you can treat autoimmunity and if you expand βγ-IL-2 receptor cells you can treat cancer. But this is way oversimplified," he adds.

Parsing pleiotropy

The central challenge in turning IL-2 into an anti-cancer therapeutic is one of cell specificity: if IL-2 boosts the proliferation of both effector T cells and regulatory T cells, is it possible to preferentially activate one population of T cells over another?

For more than a decade now, researchers have looked to the structure of the IL-2 receptor as one means of achieving this control. Regulatory T cells, it turns out, express a trimeric IL-2 receptor that comprises an α-, a β- and a γ-chain. Naive T cells and natural killer (NK) cells, by contrast, carry dimeric receptors that only comprise the β- and γ-chains. Notably, the trimeric receptors have around 100-fold higher affinity for IL-2 than the dimeric receptors, which is why IL-2 has to be used at such high doses to activate dimeric receptors on T cells in cancer applications. So, drug developers have wondered, what happens if you engineer IL-2 so that it can't bind the α-chain, instead preferentially binding the dimeric receptor?

Garcia showed that such an approach was possible, reporting in 2012 in *Nature* on the in vitro evolution of an IL-2 'superkine' variant that has increased affinity for the β-chain of the IL-2 receptor. He licensed this work to Medicenna, which is now preparing to take a candidate into the clinic.

Meanwhile, Nektar and BMS have already started generating clinical data on βγ-chain biased IL-2, with the candidate bempegaldesleukin.

Bempegaldesleukin consists of aldesleukin fused to, on average, six releasable PEG groups. PEG groups were added to IL-2 in part to increase the cytokine's half-life, generating a reservoir of pro-drug that is released as the PEGs are cleaved from the cytokine. But Nektar has also found that by clustering the PEG groups on lysine residues near where IL-2 binds the α-chain of the IL-2 receptor, it can prevent the interaction of IL-2 with the trimeric form of the receptor. And as bempegaldesleukin is hydrolysed to its most active state — which has just a single PEG group attached — it preferentially drives the proliferation of effector T cells over regulatory T cells, the company reported in 2016.

Nektar advanced this drug into clinical trials in 2015 and started working with BMS in 2016 to test it in combination with PD1 blocker nivolumab. Preliminary data were promising in 2017, in cancers including first-line melanoma and second-line, PDL1-negative non-small-cell lung cancer. In 2018, BMS paid US$1.9 billion in upfront payments, plus up to $1.8 billion in milestones, for many rights to the drug.

Subsequent trial results have dampened enthusiasm for this drug, suggesting that it might struggle to compete.

Pivotal data from ongoing trials might yet turn things around for Nektar and BMS. Phase III trial results in metastatic melanoma and in renal cell carcinoma are due by mid-2022. Nektar and Merck & Co. also recently announced plans to start a phase II/III trial of bempegaldesleukin plus pembrolizumab in squamous cell carcinoma of the head and neck, highlighting continued optimism for this agent.

IL-2 advocates see another silver lining in this dataset. "When people looked at the safety data they said, 'Wow, it's not that bad'," says Garcia. "And then everybody jumped in."

Synthorx, for example, has taken a different approach to pegylation. Whereas aldesleukin and bempegaldesleukin are built with the same 20 canonical amino acids as every other human protein, researchers at Synthorx use engineered bacteria to incorporate unnatural amino acids into the constructs they work with. In the case of IL-2, they realized, they could attach a non-cleavable PEG group to this unnatural amino acid to generate a homogenous — and potentially safer and more potent — 'not-α' IL-2 candidate.

Early data suggest that they are on track, says Marcos Milla, CSO at Synthorx. Last year, the company reported preliminary open-label phase I data showing that its drug elicits the expansion of peripheral naive T cells and NK cells, without expanding regulatory T cells. The same trial also suggests that the drug is safe at doses of up to 24 μg/kg — twice what Nektar achieved before it hit dose-limiting toxicities.

"It's all about the therapeutic index," says Marcos Milla, CSO at Synthorx. "We believe that any drug that is worth its salt must show single-agent activity, and aldesleukin showed that but with remarkable toxicity. We believe that our not-α approach allows us to open up the therapeutic index."

Sanofi is similarly enthused, and acquired Synthorx for $2.5 billion in 2019. A 300-patient phase I/II trial of SAR444245 — as monotherapy, in combination with a PD1 blocker and in combination with an anti-EGFR antibody — is underway. Sanofi no doubt hopes to pair this drug with its anti-PD1 cemiplimab. But Synthorx, now a Sanofi subsidiary, has notably also partnered with Merck & Co. on a phase II trial of SAR444245 in combination with pembrolizumab.

"People are starting to talk about IL-2 as a backbone therapy," says Milla. "We have to keep our options very open."

Others have embraced different engineering strategies to direct IL-2 agents to effector cells. Alkermes's phase II candidate nemvaleukin alfa, for instance, consists of an IL-2 variant fused to the α-chain, blocking the candidate's ability to interact with endogenous α-chain on regulatory T cells.

Computational contenders

Computational biologics developers are also taking on IL-2.

A few years ago, David Baker, at the University of Washington's Institute for Protein Design, teamed up with Garcia to computationally create an IL-2 mimetic that will only bind to the dimeric form of the IL-2 receptor. The resulting de novo protein is 25% shorter than aldesleukin (100 amino acids

instead of 132), and has just a 14% similarity with human IL-2 by amino acid sequence.

A de novo approach offers several theoretical benefits over other engineering approaches, Baker, Garcia and colleagues wrote in the 2019 *Nature* paper describing this candidate. Traditional protein engineering is incremental in nature, they argue, and as a result "most of the shortcomings of the parent molecule are inevitably passed on to the resulting engineered variants". By starting from scratch, they argue, they can select the activities that matter most. Baker's prior work with de novo designed proteins also suggests that they are not very immunogenic, a factor that may be linked to their small size and stability.

Baker co-founded Neoleukin Therapeutics to advance this candidate into the clinic, and the company filed an Investigational New Drug (IND) application for NL-201 in December 2020. The FDA put this programme on hold in January, citing the need for new assays to better characterize NL-201, but the company hopes to be able to develop this assay "within the next several months".

Aulos, by contrast, has focused on the computational design of anti-IL-2 antibodies. Pegylated IL-2s, IL-2 variants and fusion constructs all face stability, immunogenicity and manufacturing challenges on the path to the clinic, argues Ehlers. "All of those are semi-complex, from just a biomanufacturing and protein design standpoint." Antibodies, by contrast, are well understood, well behaved and stable starting points. "I was quite attracted to the notion of this simplicity, if you will," he says.

Rather than using exogenous forms of IL-2 to boost effector cell proliferation, Aulos's approach is instead to redirect the body's endogenous IL-2 to these cells. Its lead antibody candidate AU-007 binds endogenous IL-2 in such a way as to physically block the ability of IL-2 to bind with the α-chain on regulatory T cells.

This approach may also make the most of a complex IL-2 feedback cycle, says Ehlers. When IL-2 binds to effector T cells, it stimulates IL-2 production and secretion, he explains. And this newly produced endogenous IL-2 will in turn bind to regulatory T cells. So, even if engineered IL-2 candidates don't themselves activate regulatory T cells, they may nevertheless boost levels of endogenous IL-2 that can activate regulatory T cells and suppress immune activity. Aulos's antibody subverts that process, mopping up any newly produced IL-2 as it is secreted and ensuring it doesn't reach the regulatory T cells.

"The beauty of our approach is that not only do we have a well-behaved molecule that specifically targets immune stimulation and not the regulatory T cells, but it also does it through endogenous IL-2," says Ehlers.

Screening for antibodies that can provide this activity profile is unlikely to succeed, adds Yanay Ofran, acting CEO of Aulos. But computational re-epitoping — using machine learning to model and optimize antibody–antigen interactions — is up to the task, he says. "The ability to really surgically bind an epitope can be a game changer in therapeutics," says Ofran, who is also CEO of Biolojic Design, the company that developed the anti-IL-2 antibody candidate that Aulos has since licensed. Beyond IL-2, computationally designed antibodies might be particularly useful for stabilizing one conformation of a protein over another, or to agonize rather than antagonize a target.

Aulos is on track to file an IND application for AU-007 later this year. "We may very well be the first computationally designed [biologic] that will enter humans. We're neck and neck with the computationally designed IL-2 mimic," says Ofran.

Energizing activated T cells

But a single-minded focus on avoiding IL-2's interaction with the α-chain may be a misstep, says Garcia. "I think that people have sort of gone off on the wrong track," he says.

After all, although naive effector T cells and NK cells express the dimeric receptor, when activated they present the trimeric receptor.

Synthekine's lead programme, STK-012, is an engineered IL-2 that preferentially targets activated effector T cells. "We have found that activated T cells are actually wired a bit differently than other kinds of cells, and that this can be exploited through partial agonists," says Garcia. Synthekine plans to advance STK-012 into the clinic in 2021.

Roche, too, has ended up focusing on activated effector T cells. "From our experiences, reducing [α-chain] binding can be a step forward. But it's not enough," says Pablo Umaña, head of drug discovery in cancer immunotherapy at Roche.

Umaña's team has been working with IL-2 since the mid-2000s. They too have fine-tuned an IL-2 variant that has specific activity for the dimeric form of the receptor. But, rather than use this as a therapeutic on its own, Roche has taken to fusing this variant to various antibodies to target it even more specifically to different cell types.

In the early iterations of this strategy, Roche wanted to increase the concentration of its IL-2 variant in the tumour mass. With cergutuzumab amunaleukin, Roche achieved this by fusing the IL-2 variant to an antibody that targets carcinoembryonic antigen, a cancer antigen that is overexpressed in various cancers. Roche advanced cergutuzumab amunaleukin into the clinic in 2014. With simlukafusp alfa, Roche fused the same IL-2 variant to an antibody targeting FAP, also expressed on a various cancers.

Roche dropped cergutuzumab amunaleukin from its pipeline in 2019, and simlukafusp alfa in 2021. "The magnitude of the benefit, at least in today's world, was not as transformative as we would have hoped," says Umaña. But these agents still drove systemic and tumoural expansion of effector T cells, were reasonably well tolerated and showed hints of potential activity, he points out, and the company remains committed to cytokine–antibody fusion candidates.

"We definitely see these as a confirmation of the hypothesis that removing α-chain binding reduces both the severity of side effects and the preferential expansion of regulatory T cells," says Umaña.

But this still leaves the challenge of preferentially targeting activated effector T cells. Incorporating a non-biased IL-2 variant into a cytokine–antibody fusion is not ideal, he says, because of the toxicity that causes. But if the core role of the α-chain is really just to stabilize the IL-2–IL-2 receptor complex, there might be other ways to promote this interaction, his team theorized. PD1 is also overexpressed on the surface of activated effector T cells. So, they wondered, what about fusing the IL-2 variant to an antibody that binds PD1? As an added benefit, the PD1-blocking component of this candidate takes the brakes off of T cell activity.

Preclinical results are promising, adds Umaña. "If we use our IL-2 variant at a very high dose, and combine it with a very high dose of anti-PD1, we can never match the efficacy of the PD1-targeted IL-2 variant in preclinical models. It's an enormous difference," says Umaña. "It's not about just combining the things; it is really the targeting to those specific cells that makes the difference."

Emerging insights into the biology of PD1 may explain these synergistic effects, he adds. Rafi Ahmed, at Emory University, and colleagues have shown that, whereas PD1 blockade promotes the proliferation of short-lived 'transient' effector cells, combined PD1 blockade and IL-2 activation drives the differentiation of stem cell-like $CD8^+$ T cells into a unique type of 'better effectors'. "These are much more proliferative, they're much more cytotoxic and they're much more efficacious," says Umaña.

Roche advanced its IL-2v–anti-PD1 fusion candidate, RG6279, into a phase I trial in 2020, as both a monotherapy and in combination with the PDL1 blocker atezolizumab. "We will see in the clinic how this works out. But I think it's an exciting time," he adds.