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Cytokine engineering for targeted cancer immunotherapy

Lucia Bonati[1] and Li Tang[1,2]

Abstract

Cytokines are key modulators of the immune responses and represent promising therapeutics for a variety of cancers. However, successful translation of cytokine-based therapy to the clinic is limited by, among others, severe toxicities and lack of efficacy due to cytokine pleiotropy and off-target activation of cells. Engineering cytokines with enhanced therapeutic properties has emerged as a promising strategy to overcome these challenges. Advances in protein engineering and protein-polymer conjugate technologies have fostered the generation of cytokines with enhanced target cell specificity and longer half-life than the native ones. These novel cytokines exhibit reduced systemic toxicities while focusing the activities at the tumor site, thus, enhancing antitumor immunity. The growing toolbox of cytokine engineering strategies will further stimulate the development of smart cytokine-based immunotherapies with enhanced efficacy and safety profiles.

Addresses

[1] Institute of Bioengineering, Lausanne, CH-1015, Switzerland

[2] Institue of Materials Science and Engineering, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, CH-1015, Switzerland

Corresponding author: Tang, Li (li.tang@epfl.ch)

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Introduction

Cytokines are small proteins (typically < 30 kDa) that are produced by immune cells and play an essential role in maintaining physiological immune homeostasis and regulating pathophysiological processes, such as cancer and autoimmune diseases [1]. Cytokines are divided in different subclasses based on their receptors' conformation, including chemokines, interferons (IFNs), interleukins (ILs), and tumor necrosis factors (TNFs) (reviewed elsewhere [2–5]). They act by binding to specific receptors inducing receptor dimerization or membrane reorganization. Receptor aggregation activates distinct kinases, which phosphorylate serine and tyrosine residues of the receptor's cytoplasmic domains and activate distinct transcription factors for nuclear translocation and gene expression regulation. For example, many IFN and IL receptors selectively associate with Janus kinases (JAKs), which initiate receptor phosphorylation and subsequent recruitment of one or more Signal Transducer and Activator of Transcription (STAT) proteins. In contrast, TNF binding to its cognate receptor induces the recruitment of specific complexes and kinases, which lead to activation of downstream transcription factors including AP-1 and the nuclear factor κB (NF–κB) [5–9].

Given their essential role in modulating the immune responses, many cytokines have been identified as therapeutic candidates for the treatment of a variety of cancers [10]. To date, several cytokines have been approved for cancer immunotherapy in the clinic, including recombinant IL-2, IFNα, and TNF [11]. However, the clinical application of cytokine-based immunotherapies is greatly hampered by toxicities and/or modest efficacies. This effect is mainly due to the pleiotropic nature of cytokines causing undesired binding and activation of off-target cells. Moreover, native cytokines typically have a short serum half-life and narrow therapeutic window, which limits their therapeutic efficacy [12].

Engineering of cytokines with enhanced specificity, localized activity, and longer half-life is a promising approach to unleash their full therapeutic potential. Advances in cytokine structural biology and synthetic biology have boosted the development of engineered cytokines either through structure-based protein modifications or through the fusion of cytokines with antibody-derived fragments (Table 1) [12–14]. On the other hand, a variety of polymer–cytokine conjugates and peptide–cytokine fusions have been developed to provide therapeutic cytokines with prolonged half-life, increased specificity, and low immunogenicity (Table 1) [15–18]. Moreover, beyond directly engineering the cytokine molecule, antibodies can be used to target either the cytokine or the cytokine's receptor, thus altering their functional behavior [19,20]. In this review, we highlight recent progress in engineering cytokine molecules for targeted cancer immunotherapy

Table 1

Summary of recent advances in cytokine engineering strategies.

Strategy	Cytokines	Key results	Representative references
1. Molecular engineering			
Immunocytokines	IL-2, IL-12, IFNγ, TNFα	↑ Tumor localization, ↑ Half-life	[24]
Site-directed mutagenesis	IL-2	↑ Selective activation of cytotoxic immune cells	[33,34,79]
Combinatorial mutagenesis	IL-2, IL-10, IL-18	↑ Selective activation of cytotoxic immune cells, ↑ Bioactivity	[37,38,40,42,43]
De novo protein design	IL-2, IL-15	↑ Selective activation of cytotoxic immune cells	[41]
2. Chemical engineering			
PEGylation	IFNα, TNF	↑ Half-life, ↑ Aqueous solubility	[15]
Conjugation to synthetic polypeptides	IFNα	↑ Half-life, ↑ Stability, ↑ Tissue permeability, ↓ Immunogenicity	[51,53,54]
[a]ECM-targeting cytokines	IL-2, IL12, CCL4	↑ Tumor localization, ↓ Systemic toxicities	[62–65,68]

[a] ECM, extracellular matrix; TNF, tumor necrosis factor; IFN, interferon; PEG, polyethylene glycol.

using molecular and structural variations as well as chemical modifications (Figure 1). We also share our vision on the current limitations of cytokine engineering and technological advances needed for the successful translation of these strategies into the clinic.

Molecular engineering of therapeutic cytokines

Molecular engineering strategies aim at enhancing the therapeutic properties of cytokines by fusing them with other protein domains, such as antibody fragments, or by altering the protein's molecular structure using codon mutagenesis. These techniques have proven successful in overcoming some shortcomings of native cytokines, including dose-limiting toxicities, off-target effects, short half-life, and insufficient activity. In this subsection, we first review recent efforts to increase cytokine tissue specificity by using cytokine–antibody fragment fusions. Next, we illustrate current advances in the modulation of cytokine activities employing mutagenesis-based approaches. For the purpose of this review, modification with peptides, although very similar to proteins in composition, is discussed in the chemical engineering subsection due to their small size and similarity to synthetic polymers.

Immunocytokines

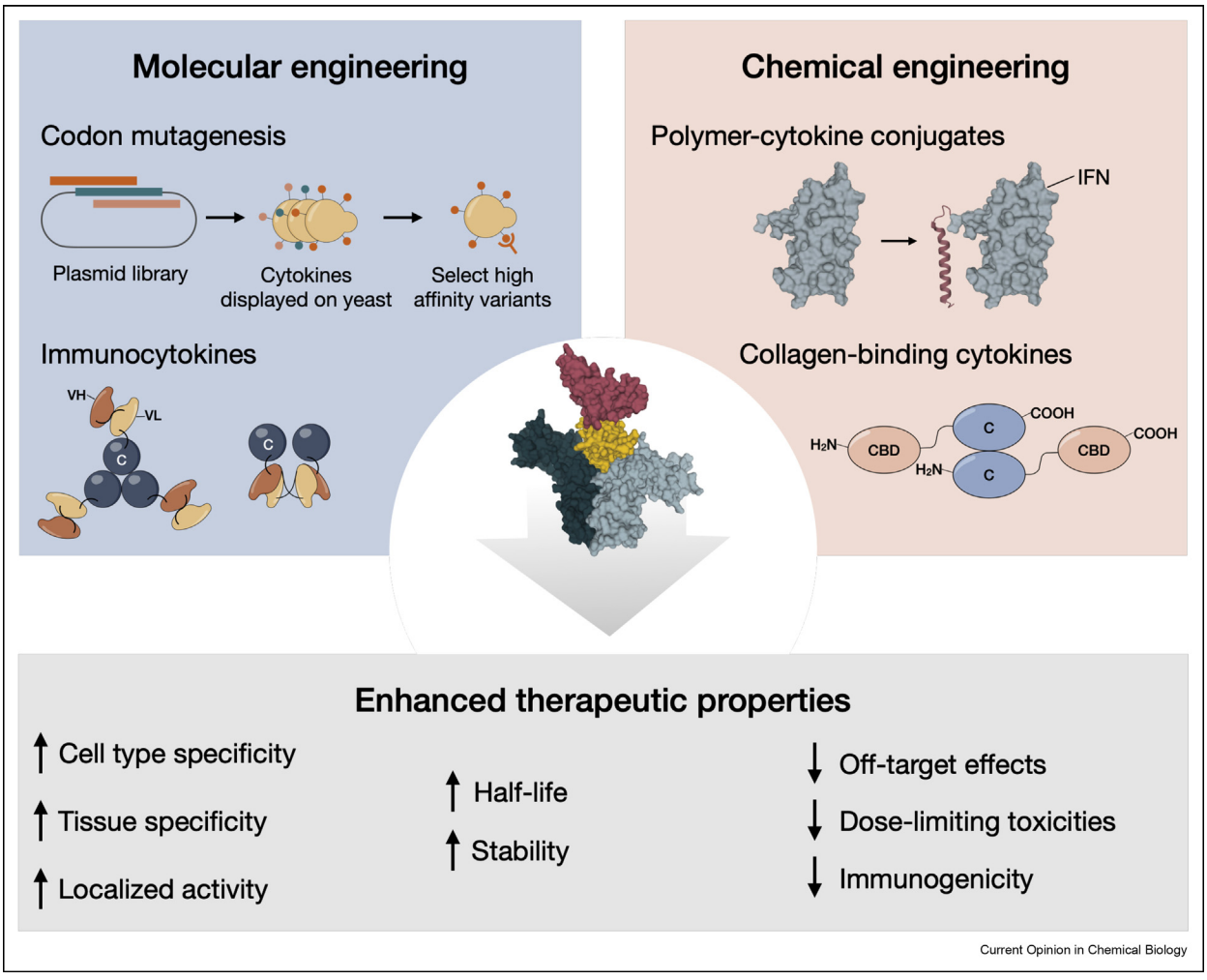
Targeted delivery of cytokines to the tumor microenvironment (TME) can trigger strong antitumor immunity and turn so-called immunologically "cold" tumors into "hot" tumors with high immune cell infiltration [11,21]. However, systemic administration of cytokine-based therapies is limited by their pleiotropic nature, their short half-life, and substantial side effects [15,16]. Antibody–cytokine fusion proteins, called immunocytokines, consist of the fusion of cytokines to full-sized antibodies or antibody fragments, which provide the molecule with the capability to target tumor-associated antigens. This led to current clinical testing of multiple immunocytokines, including IL-2, IL-12, and TNF-based fusions for the treatment of different malignancies (extensively reviewed elsewhere [11,22–26]).

The architecture of the antibody fragment greatly impacts the targeting performance of immunocytokines. In general, the size and the spatial arrangements are considered key optimization parameters as they can influence binding affinity, pharmacokinetics, extravasation and tumor uptake, and effective doses [24]. Recently, sophisticated immunocytokine formats were developed for the treatment of so far refractory tumors. As an elegant example, Dougan et al. developed an alpaca-derived heavy-chain only fragment (VHH) targeting programmed death-ligand 1 (PD-L1) fused to IL-2 or IFNγ to treat pancreatic ductal adenocarcinoma, which was unresponsive to native immune checkpoint blockade antibodies [27]. They showed that immunocytokines consisting of a small antibody fragment favored tumor penetration and conferred enhanced antitumor activity compared to their full-sized counterparts.

In addition, in a study by Weiss et al., several immunocytokines containing different L19-derived fragments, an antibody that targets a tumor-associated epitope of extracellular fibronectin, were generated for the treatment of glioblastoma [28]. Fusions of L19 fragments in single chain variable fragment (scFv) or diabody (a dimer of scFvs) formats with TNFα and IL-12 could enable effective antitumor activity both in mouse models and patients. Notably, the intravenous route of administration of the immunocytokines represents a significant advantage compared to previously used intratumoral injection route [29] or surgery for local delivery of a cytokine encoding viral vector [21].

Despite the promising results of immunocytokines, work by Tzeng et al. showed that the biodistribution of a

Figure 1



Overview of cytokine engineering strategies in the context of cancer immunotherapy. VH, heavy-chain variable domain; VL, light chain variable domain; C, cytokine; IFN, interferon; CBD, collagen-binding domain.

full size antibody-IL-2 immunocytokine was mainly governed by cytokine–receptor interactions rather than by tumor antigen–antibody fragment interactions [30]. This study pointed out the complexity of *in vivo* fate of immunocytokines, due to multiple potential targeting interactions, and the need for a case-by-case assessment of tumor targeting capacity. In summary, these preclinical and clinical studies highlight the potential of immunocytokines for tumor treatment. Nonetheless, robust optimization is required to find the right cytokine–antibody combination and structure.

Mutagenesis

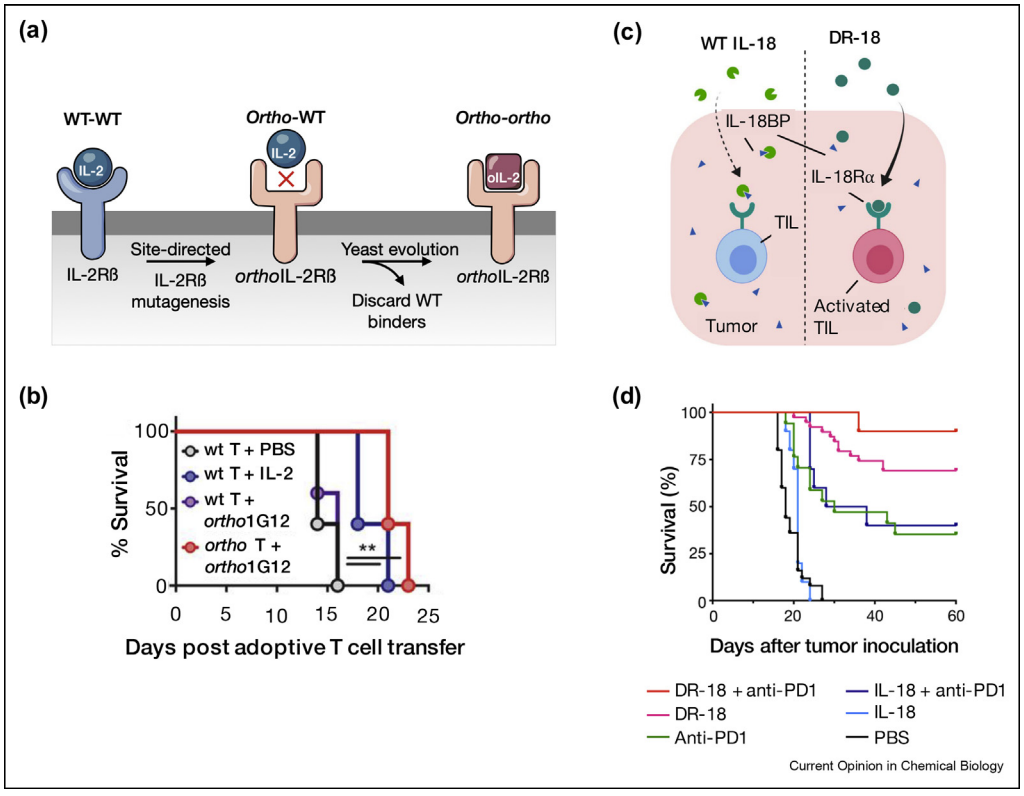
Mutagenesis technologies can be used to directly engineer cytokine's binding sites with altered receptor specificity and affinity. To date, different mutagenesis systems have been applied to cytokines including site-directed mutagenesis and combinatorial library-based platforms, such as phage or yeast display [14].

One cytokine that has been extensively explored for mutagenesis is IL-2, which plays a pivotal role in the immune response by promoting immune cell proliferation, differentiation, and cytolytic activity [31]. In the past decades, wild-type IL-2 has been used in the clinic for the treatment of several cancers, but it has been shown to induce severe toxicities and side effects [32]. These effects are mainly due to the pleiotropic nature of IL-2, which acts on effector cytotoxic immune cells as well as immunosuppressive regulatory T cells (Tregs). Specifically, IL-2 signals through activation of the IL-2 receptor (IL-2R), which exists in a dimeric or trimeric format. The intermediate-affinity dimeric receptor consists of an IL-2Rβ and a γ_c subunit, whereas the

high-affinity trimeric receptor includes an IL-2Rα, an IL-2Rβ, and a γ_c subunit. Dimeric receptors are mainly expressed on cytotoxic T cells and natural killer (NK) cells, while trimeric receptors are predominantly found on Tregs. Importantly, this effect results in preferential stimulation of Tregs leading to immunosuppression in the TME [31].

In order to achieve favored activation of cytotoxic T cells for cancer therapy, many engineering efforts have been focused on altering the binding affinity of IL-2 towards distinct receptor subunits. Site-directed rational mutagenesis is based on the prediction of free energy changes upon amino acid mutation within a protein–protein interface. However, in the case of cytokine–receptor interactions, the underlying energetics are highly unpredictable. For this reason, site-directed mutagenesis approaches have been mostly used to weaken receptor-cytokine interactions [33–36], e.g., the binding between IL-2 and the IL-2Rα subunit. Conversely, yeast and phage display were successfully used to generate IL-2 variants with increased affinity to one or more receptor subunits. The Wittrup and Garcia groups pioneered the development of several IL-2 variants with redirected specificity towards one distinct IL-2R subunit resulting in preferential expansion of cytotoxic T cells and NK cells [37–39]. While engineered cytokines provide a potent tool to target distinct endogenous immune populations, they are still incapable of precisely targeting adoptively transferred cells. Recent work by Sockolosky et al. has provided convincing evidence that IL-2 specificity can be redirected towards adoptively transferred T cells using an orthogonal (*ortho*) cytokine-receptor system in which both cytokine and receptor are engineered to signal exclusively together (Figure 2a,b) [40]. Notably, engineered *ortho*IL-2 exhibited a high degree of specificity *in vivo*, negligible off-target toxicity, and elicited

Figure 2



(a) Schematic overview of orthogonal IL-2/IL-2R pairs consisting of engineered IL-2 and IL-2R that signal exclusively together and do not cross-react with wild-type (WT) cytokine and receptor. **(b)** Survival curves of mice bearing subcutaneous B16F10 tumors treated with adoptive transfer of pmel-1 transgenic $CD8^+$ T cells expressing WT IL-2R (wt T) or orthoIL2-Rβ (ortho T), in combination with WT or orthogonal IL-2 mutant (ortho1G12). ortho, orthogonal. Adapted with permission from a study by Sockolosky et al. [40]. **(c)** Schematic illustration of the DR-18 technology. Engineered decoy-resistant IL-18 (DR-18) does not bind the decoy receptor IL-18 binding protein (IL-18BP) and maintains the capability to bind tumor-infiltrating lymphocytes. **(d)** Survival curves of mice bearing MC-38 tumors after the treatment with WT IL-18 plus anti-PD-1 antibody, or DR-18 alone or in combination with anti-PD-1 antibody. TIL, tumor-infiltrating lymphocytes; PBS, phosphate-buffered saline; anti-PD1, anti-programmed cell death protein 1 antibody. Adapted with permission from a study by Zhou et al. [42].

enhanced anticancer immune responses against B16F10 mouse melanoma tumors. Although engineering mutant receptor-cytokine pairs as highly potent as the wild-type remains challenging, this approach is promising for the specific expansion of genetically engineered T cells, such as CAR T cells. Alternatively, de novo cytokines can be designed and synthesized. Silva et al. used computational methods to elegantly demonstrate this idea [41]. They generated IL-2 and IL-15 mimetics, termed neoleukin-2/15, which specifically bind to the β and γ subunits of IL-2R with no requirement of binding to IL-2Rα for signaling initiation. These synthetic cytokines showed a therapeutic activity superior to wild-type IL-2 in several tumor models with greatly reduced toxicity and immunogenicity.

Besides IL-2, other cytokines have been recently employed for mutagenesis. In a recent attempt to enhance the antitumor activity of IL-18, Zhou et al. used directed evolution to engineer a 'decoy-resistant' IL-18 variant (DR-18), which does not bind to the decoy receptor IL-18BP, thereby maintaining its activity in the circulation (Figure 2c,d) [42]. Strikingly, administering DR-18 to tumor-bearing mice resulted in a selective expansion of stem-like $TCF1^{+}CD8^{+}$ T cells in tumor and biased differentiation towards polyfunctional T cells rather than towards an exhausted phenotype. In addition, DR-18-induced proliferation of NK cells in MHC class I-deficient tumors, providing a new tool for the treatment of immune checkpoint blockade therapy-resistant tumors. Another recent example of cytokine mutagenesis involves an engineered IL-10 mutant with increased affinity towards the low-affinity IL-10Rβ, which acts as a strong gene expression regulator in both $CD8^{+}$ T cells and monocytes [43].

Collectively, these preclinical studies highlight the immense potential that mutagenesis-based engineering strategies hold for cancer immunotherapy. However, this technology provides solutions to only one drawback of wild-type cytokines, i.e., pleiotropy, thus inevitably passing on the engineered variants several other shortcomings coming from the parental molecule, such as short half-life and low stability. Therefore, the combination of mutagenesis and other cytokine engineering technologies (e.g., chemical engineering) may be necessary to obtain an optimal therapeutic cytokine molecule.

Chemical engineering of therapeutic cytokines

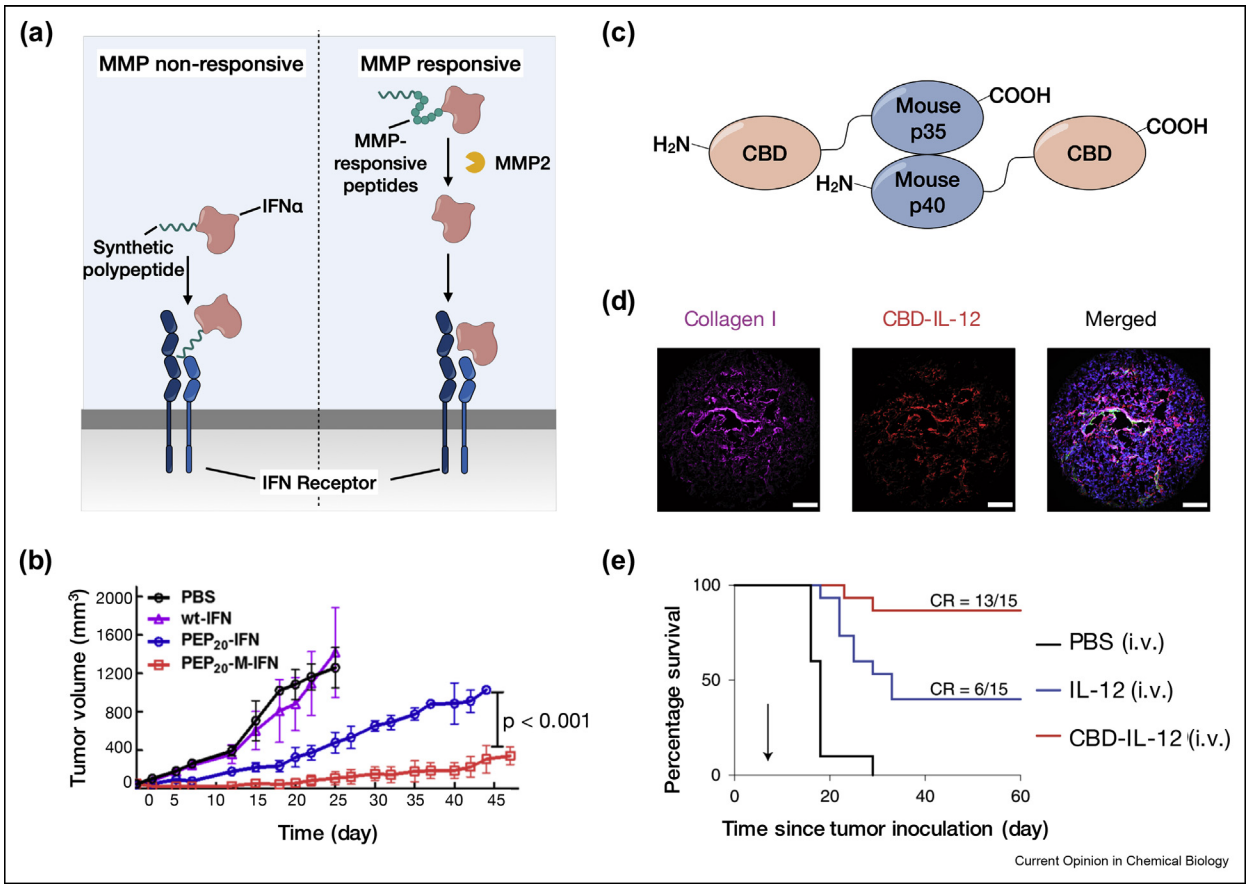
In addition to engineering cytokines by modifying their amino acid sequence, another approach to improve their pharmacological properties (e.g., solubility and pharmacokinetics) is through conjugation with synthetic polymers or peptides. Functional groups on the surface of cytokines are typically used to covalently link polymers. The most well-known strategy of this kind is the conjugation of proteins with polyethylene glycol (PEG), known as PEGylation. PEGylation has gained vast success in prolonging cytokines' half-life, leading to several clinically approved PEGylated cytokines [15]. Despite these successes, the varying protein bioactivity caused by nonspecific conjugation as well as the nondegradable nature of PEG have recently drawn increasing concerns [44,45]. To address these issues, biodegradable and non-immunogenic alternatives to PEG have emerged, such as amino acid-based synthetic polypeptide.

Alternatives to PEG

In recent years, a wide range of PEG alternatives has been investigated [46–49]. Despite the potential success of these methods, none of them yet provided satisfactory biodegradability and pharmacological performance. Chemical conjugation of synthetic polypeptides to therapeutic cytokines, known as PEPylation, represents a valid alternative to PEG because of its excellent chemical modularity and increased biodegradability [16]. In an elegant example, site-selective conjugation of polypeptide with protein was achieved upon the introduction of two orthogonal "chemical handles" at the C- and N-termini of the polypeptide [50]. Importantly, the PEPylated IFNα showed significantly enhanced protease resistance and thermostability. Building on this work, additional efforts were focused on employing cyclic polypeptide structures in the modification of antitumor therapeutic biomolecules. In two distinct studies, Lu and colleagues investigated the effects of a PEPylated IFN with a head-to-tail macrocyclic polypeptide [51] and another PEPylated IFN bearing a fixed helical polypeptide [52], respectively, in mouse models. Strikingly, both strategies resulted in improved protease resistance, thermostability, and cytotoxicity *in vitro*, as well as low immunogenicity *in vivo*. In particular, macrocyclization of the IFN–polypeptide conjugate displayed enhanced pharmacokinetics as well as tumor retention and tissue penetration. These examples provide promising insights into the use of PEPylated cytokines for cancer therapy.

Nevertheless, the engineering of tissue-specific and inducible cytokines remains challenging. Recently, Wang et al. took advantage of the overexpressed matrix metalloproteinases (MMPs) in the TME and designed a peptide–cytokine conjugate linked through a MMP-responsive peptide sequence between the IFNα structure and the synthetic polypeptide (Figure 3a, b) [53]. In the presence of high concentrations of MMP, the peptide substrate is cleaved, and the protein activity can be instantly restored to its full capacity. Moreover, systems that are responsive to multiple stimuli have been developed. For example, a temperature-responsive and MMP-cleavable IFNα have been recently engineered [54]. By fusing a body temperature-responsive elastin-like polypeptide, an MMP substrate, and IFNα, the

Figure 3



(a) Schematic illustration of a matrix metalloproteinase (MMP)-responsive IFNα. An MMP-cleavable peptide sequence was inserted between the synthetic polypeptide and IFNα allowing for peptide cleavage in response to MMP overexpressed in tumor. **(b)** Tumor growth curves of mice bearing OVCAR3 xenografts tumors. Adapted with permission from a study by Wang et al. [53]. **(c)** Schematic illustration of the fusion of a collagen-binding domain (CBD) with the mouse p35 and p40 subunits of IL-12 to afford a collagen-binding IL-12 (CBD-IL-12). CBD was fused to each of the subunits through a $(GGGS)_2$ linker. **(d)** Representative human melanoma cryosections with immunohistochemistry staining showing the binding of CBD-IL-12 to collagens. Scale bar: 100 μm. **(e)** Survival curves of mice bearing EMT6 murine mammary carcinoma receiving CBD-IL-12, wild-type IL-12, or PBS through intravenous (i.v.) injections. CR, complete response. Adapted with permission from a study by Mansurov et al. [65]. IFNs, interferons; PBS, phosphate-buffered saline; wt-IFN, wild-type IFNα; PEP_{20}-IFN, PEPylated IFNα without an MMP-responsive peptide; PEP_{20}-M-IFN, PEPylated IFNα with the MMP-responsive peptide sequence.

authors were able to achieve synergy of the body temperature responsiveness and MMP-cleavability, thus dramatically improving antitumor efficacy compared with the native IFNα. These strategies combine advantages afforded by PEPylation with benefits from unconjugated protein, including high tumor retention, deep tumor penetration, and intact antitumor potency.

Taken together, these studies suggest that the conjugation of synthetic polypeptides to cytokines represents a viable alternative to PEGylation, opening up new opportunities for the development of responsive release systems for cytokine therapeutics. However, the receptor-binding domains of PEPylated cytokines typically did not undergo any modification; thus, the risk of off-target toxicities deriving from the pleiotropic nature of cytokines persists.

Extracellular matrix-targeting cytokines

The TME comprises many different types of cells including stromal cells as well as an evolving extracellular matrix (ECM) [55,56]. Collagen is a major component of the ECM contributing to the molecular architecture and mechanical properties of tissues [57]. Importantly, it has been observed that many tumors contain increased amounts of collagen compared to healthy tissues [58,59]. Early evidence that collagen could act as a promising target was shown by fusing a collagen-binding domain (CBD) to epidermal growth factor receptor (EGFR)-specific antibody fragments

[60,61]. Recently, a variety of ECM-binding cancer therapeutics were developed for enhanced tumor targeting [62,63]. For example, scientists from the Hubbell group elegantly demonstrated the potential of CBD-conjugated cytokines by fusing IL-2 to a peptide, which has high affinity to collagen I and III [64]. Strikingly, CBD-modified cytokines enhanced the tumor infiltration of $CD8^+$ T cells and achieved increased antitumor efficacy compared to their unmodified counterparts. This strategy took advantage of the increased vascular permeability of tumor blood vessels, which allow the CBD-conjugated cytokines to accumulate in the TME. Building on this work, CBD-conjugation was further used for the tumor-targeted delivery of several cytokines and chemokines including CCL4 and IL-12 leading to more effective and safer cancer immunotherapies [65,66].

IL-12 is considered an attractive antitumor cytokine as it can act on both the innate and adaptive immune system [65]. However, administration of IL-12 in clinical trials resulted in high accumulation in healthy tissues and limited penetration in tumor tissues [67]. In a recent report by the Wittrup lab, IL-12 was fused to lumican, a protein that binds collagen I and IV. Intratumoral injection of lumican-conjugated IL-2 and IL-12 induced increased cytokine retention in the tumor matrix [68]. In a following study, Mansurov et al. [65] developed tumor-targeted CBD-IL-12 therapy that could be administered intravenously (Figure 3c-e). In line with previous reports, CBD-IL-12 administration led to cytokine accumulation in both B16F10 and EMT6 tumors, resulting in enhanced intratumoral IFNγ production and $CD8^+$ T cell recruitment. To summarize, conjugation of cytokine to ECM-targeting peptides/proteins represents a promising engineering approach for tumor-targeted delivery of immunostimulatory molecules for the treatment of solid tumors.

Perspective

Cytokine-based immunotherapies have been used to treat various tumors. However, considerable toxicities and modest efficacies of therapeutic cytokines pose major challenges that require further cytokine engineering. In this review, we highlighted some of the most recent reports leveraging molecular and chemical engineering strategies to improve safety and efficacy of cytokine-based cancer therapy. However, significant challenges remain to be addressed.

Immunocytokines as well as chemically modified cytokines maintain their pleiotropic capability to target any cell type that expresses their receptor. This effect remains the leading cause of cytokine-derived toxicities and represents a considerable hurdle for clinical translation. Finding the optimal balance between the activation of desired immune cells versus off-target cells (e.g., $CD8^+$ T cell versus Tregs) remains a key challenge. Future efforts in cytokine engineering will focus on cytokines with improved pharmacokinetics combined with more specific receptor-targeting capabilities. This may be achieved through masking the receptor-binding domains of cytokines using antibodies or synthetic polymer molecules, thus preventing binding to certain receptor subunits expressed only on undesired cells but not the target cells [69,70].

To achieve targeted immunotherapy, it is crucial to provide cytokines with the capability to discriminate between targeted and non-targeted tissues. We envision that engineering stimuli-responsive cytokine-based immunotherapies (e.g., a cytokine responsive to low pH in TME) will provide a promising solution to on-target-off-tumor toxicities. Moreover, regulating the timing of cytokine delivery with disease progression is crucial for optimal therapeutic performance. In recent years, advancements in cell and genetic engineering technologies have enabled researchers to leverage the capability of immune cells to migrate to tumors and recognize cancer cells for targeted delivery of cytokines. T cells have been engineered to secrete cytokines including IL-2, IL-7, IL-12, IL-15, IL-21, CCL19 [71–75], and the p40 subunit of IL-23 [76], or to carry cytokine-loaded nanoparticles [77,78]. Cell-based cytokine delivery provides inducible cytokine production allowing for precise tuning of the amplitude, duration, and localization of cytokines. Such strategies combining cell engineering with cytokine engineering have shown great promise. However, accompanied toxicities, such as autonomous growth of the cytokine-secreting T cells and cytokine storm, have to be assessed for clinical translation.

Combination therapy with multiple cytokines is an attractive option as it offers the possibility to achieve synergistic therapeutic effects. However, the molecular differences between cytokines lead to dramatically different pharmacological properties, making clinical translation challenging. A solution may reside in the generation of bi-/multispecific cytokines that bind two or multiple targets with one molecule. This strategy could lead to the development of one single molecular product, thus accelerating the approval procedures and reducing development costs. Nevertheless, whether different cytokines combined in one molecule still retain their activities needs to be evaluated.

In conclusion, the growing toolbox of cytokine engineering strategies that can be combined and tuned for maximum safety and efficacy will continue to enable the

development of novel therapeutic solutions for the treatment of refractory diseases.

Author contributions

L.B. and L.T. planned and wrote the review.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Wang X, Lupardus P, LaPorte SL, Garcia KC: **Structural biology of shared cytokine receptors**. *Annu Rev Immunol* 2009, **27**: 29–60.

2. Griffith JW, Sokol CL, Luster AD: **Chemokines and chemokine receptors: positioning cells for host defense and immunity**. *Annu Rev Immunol* 2014, **32**:659–702.

3. Lazear HM, Schoggins JW, Diamond MS: **Shared and distinct functions of type I and type III interferons**. *Immunity* 2019, **50**: 907–923.

4. Lin JX, Leonard WJ: **The common cytokine receptor γ chain family of cytokines**. *Cold Spring Harb Perspect Biol* 2018, **10**: 1–38.

5. Kalliolias GD, Ivashkiv LB: **TNF biology, pathogenic mechanisms and emerging therapeutic strategies**. *Nat Rev Rheumatol* 2016, **12**:49–62.

6. Haan C, Kreis S, Margue C, Behrmann I: **Jaks and cytokine receptors-An intimate relationship**. *Biochem Pharmacol* 2006, **72**:1538–1546.

7. Moraga I, Spangler J, Mendoza JL, Garcia KC: **Multifarious determinants of cytokine receptor signaling specificity**. *Adv Immunol* 2014, **121**:1–39.

8. Gorby C, Martinez-Fabregas J, Wilmes S, Moraga I: **Mapping determinants of cytokine signaling via protein engineering**. *Front Immunol* 2018, **9**:2143.

9. Aggarwal BB: **Signalling pathways of the TNF superfamily: a double-edged sword**. *Nat Rev Immunol* 2003, **3**:745–756.

10. Baldo BA: **Side effects of cytokines approved for therapy**. *Drug Saf* 2014, **37**:921–943.

11. Neri D: **Antibody–cytokine fusions: versatile products for the modulation of anticancer immunity**. *Cancer Immunol Res* 2019, **7**:348–354.

12. Vazquez-Lombardi R, Roome B, Christ D: **Molecular engineering of therapeutic cytokines**. *Antibodies* 2013, **2**:426–451.

13. Scheller J, Engelowski E, Moll JM, Floss DM: **Immunoreceptor engineering and synthetic cytokine signaling for therapeutics**. *Trends Immunol* 2019, **40**:258–272.

14. Spangler JB, Moraga I, Mendoza JL, Garcia KC: **Insights into cytokine-receptor interactions from cytokine engineering**. *Annu Rev Immunol* 2015, **33**:139–167.

15. Ekladious I, Colson YL, Grinstaff MW: **Polymer–drug conjugate therapeutics: advances, insights and prospects**. *Nat Rev Drug Discov* 2019, **18**:273–294.

16. Liu X, Wu F, Ji Y, Yin L: **Recent advances in anti-cancer protein/peptide delivery**. *Bioconjugate Chem* 2019, **30**:305–324.

17. Pelegri-Oday EM, Lin EW, Maynard HD: **Therapeutic protein-polymer conjugates: advancing beyond pegylation**. *J Am Chem Soc* 2014, **136**:14323–14332.

18. Mitragotri S, Burke PA, Langer R: **Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies**. *Nat Rev Drug Discov* 2014, **13**:655–672.

19. Spangler JB, Moraga I, Jude KM, Savvides CS, Garcia KC: **A strategy for the selection of monovalent antibodies that span protein dimer interfaces**. *J Biol Chem* 2019, **294**: 13876–13886.

20. Kureshi R, Bahri M, Spangler JB: **Reprogramming immune proteins as therapeutics using molecular engineering**. *Curr Opin Chem Eng* 2018, **19**:27–34.

21. Chiocca EA, Yu JS, Lukas RV, Solomon IH, Ligon KL, Nakashima H, Triggs DA, Reardon DA, Wen P, Stopa BM, *et al.*: **Regulatable interleukin-12 gene therapy in patients with recurrent high-grade glioma: results of a phase 1 trial**. *Sci Transl Med* 2019, **11**:1–13.

22. Young PA, Morrison SL, Timmerman JM: **Antibody-cytokine fusion proteins for treatment of cancer: engineering cytokines for improved efficacy and safety**. *Semin Oncol* 2014, **41**: 623–636.

23. Bootz F, Neri D: **Immunocytokines: a novel class of products for the treatment of chronic inflammation and autoimmune conditions**. *Drug Discov Today* 2016, **21**:180–189.

24. * Hutmacher C, Neri D: **Antibody-cytokine fusion proteins: biopharmaceuticals with immunomodulatory properties for cancer therapy**. *Adv Drug Deliv Rev* 2019, **141**:67–91.
This review provides a thorough overview of the design and engineering of immunocytokines. The authors discuss key features of preclinical and clinical-stage products, as well as their potential limitations and emerging opportunities.

25. Müller D: **Antibody fusions with immunomodulatory proteins for cancer therapy**. *Pharmacol Ther* 2015, **154**:57–66.

26. Neri D, Sondel PM: **Immunocytokines for cancer treatment: past, present and future**. *Curr Opin Immunol* 2016, **40**:96–102.

27. * Dougan M, Ingram JR, Jeong HJ, Mosaheb MM, Bruck PT, Ali L, Pishesha N, Blomberg O, Tyler PM, Servos MM, *et al.*: **Targeting cytokine therapy to the pancreatic tumor microenvironment using PD-L1–specific VHHs**. *Cancer Immunol Res* 2018, **6**: 389–401.
The authors present a simple method to reduce tumor burden of pancreatic tumor using single-domain antibodies (VHHs) against PD-L1 fused to IL-2 or IFNγ. This approach could significantly increase the number of intratumoral $CD8^+$ T cells and influence macrophages differentiation towards a more anti-inflammatory M1-like phenotype.

28. ** Weiss T, Puca E, Silginer M, Hemmerle T, Pazahr S, Bink A, Weller M, Neri D, Roth P: **Immunocytokines are a promising immunotherapeutic approach against glioblastoma**. *Sci Transl Med* 2020, **12**:1–11.
The authors present extracellular fibronectin-targeting immunocytokines for the treatment of glioblastoma. This strategy leads to enhanced lymphocyte tumor infiltration and increased the amounts of proinflammatory cytokines within the tumor microenvironment.

29. vom Berg J, Vrohlings M, Haller S, Haimovici A, Kulig P, Sledzinska A, Weller M, Becher B: **Intratumoral IL-12 combined with CTLA-4 blockade elicits T cell-mediated glioma rejection**. *J Exp Med* 2013, **210**:2803–2811.

30. Tzeng A, Kwan BH, Opel CF, Navaratna T, Wittrup KD: **Antigen specificity can be irrelevant to immunocytokine efficacy and biodistribution**. *Proc Natl Acad Sci Unit States Am* 2015, **112**: 3320–3325.

31. Liao W, Lin JX, Leonard WJ: **Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy**. *Immunity* 2013, **38**:13–25.

32. Rosenberg SA: **IL-2: the first effective immunotherapy for human cancer**. *J Immunol* 2014, **192**:5451–5458.

33. * Vazquez-Lombardi R, Loetsch C, Zinkl D, Jackson J, Schofield P, Deenick EK, King C, Phan TG, Webster KE, Sprent J, *et al.*: **Potent antitumour activity of interleukin-2-Fc fusion proteins requires Fc-mediated depletion of regulatory T-cells**. *Nat Commun* 2017, **8**:1–12.
By engineering an IL-2 variant with abolished CD25 binding, the authors identify the depletion of regulatory T cells as a major determinant for antitumor activity.

34. Shanafelt AB, Lin Y, Shanafelt MC, Forte CP, Dubois-Stringfellow N, Carter C, Gibbons JA, Cheng SL, Delaria KA, Fleischer R, *et al.*: **A T-cell-selective interleukin 2 mutein exhibits potent antitumor activity and is well tolerated in vivo**. *Nat Biotechnol* 2000, **18**:1197–1202.

35. Gillies SD, Lan Y, Hettmann T, Brunkhorst B, Sun Y, Mueller SO, Lo KM: **A low-toxicity IL-2-based immunocytokine retains antitumor activity despite its high degree of IL-2 receptor selectivity**. *Clin Canc Res* 2011, **17**:3673–3685.

36. Carmenate T, Pacios A, Enamorado M, Moreno E, Garcia-Martínez K, Fuente D, León K: **Human IL-2 mutein with higher antitumor efficacy than wild type IL-2**. *J Immunol* 2013, **190**:6230–6238.

37. Liu DV, Maier LM, Hafler DA, Wittrup KD: **Engineered interleukin-2 antagonists for the inhibition of regulatory T cells**. *J Immunother* 2009, **32**:887–894.

38. Levin AM, Bates DL, Ring AM, Krieg C, Lin JT, Su L, Moraga I, Raeber ME, Bowman GR, Novick P, *et al.*: **Exploiting a natural conformational switch to engineer an interleukin-2 "superkine**. *Nature* 2012, **484**:529–533.

39. Rao BM, Driver I, Lauffenburger DA, Wittrup KD: **High-affinity CD25-binding IL-2 mutants potently stimulate persistent T cell growth**. *Biochemistry* 2005, **44**:10696–10701.

40. ** Sockolosky JT, Trotta E, Parisi G, Picton L, Su LL, Le AC, Chhabra A, Silveria SL, George BM, King IC, *et al.*: **Selective targeting of engineered T cells using orthogonal IL-2 cytokine-receptor complexes**. *Science* 2018, **359**:1037–1042.
This paper presents an elegant technology in which T cells are genetically engineered with a receptor that specifically binds to its engineered cytokine counterpart. This is the first report of a method to specifically expand genetically engineered cells.

41. ** Silva DA, Yu S, Ulge UY, Spangler JB, Jude KM, Labão-Almeida C, Ali LR, Quijano-Rubio A, Ruterbusch M, Leung I, *et al.*: **De novo design of potent and selective mimics of IL-2 and IL-15**. *Nature* 2019, **565**:186–191.
Using a computational design approach, the authors generate IL-2 and IL-15 mimetics with enhanced specificity to $CD8^+$ T cells and superior therapeutic activity. These engineered cytokines show high stability and undetectable immunogenicity.

42. ** Zhou T, Damsky W, Weizman O El, McGeary MK, Hartmann KP, Rosen CE, Fischer S, Jackson R, Flavell RA, Wang J, *et al.*: **IL-18BP is a secreted immune checkpoint and barrier to IL-18 immunotherapy**. *Nature* 2020, **583**:609–614.
The authors engineered a variant of the IL-18 cytokine that is resistant to its endogenous negative regulation mechanism and exerts potent antitumor effects in mouse tumor models.

43. * Gorby C, Sotolongo Bellón J, Wilmes S, Warda W, Pohler E, Fyfe PK, Cozzani A, Ferrand C, Walter MR, Mitra S, *et al.*: **Engineered IL-10 variants elicit potent immunomodulatory effects at low ligand doses**. *Sci Signal* 2020, **13**:1–18.
The authors generated a variant of the IL-10 cytokine with high affinity for the beta receptor subunit to enhance IL-10-mediated gene expression.

44. Tong R, Cheng J: **Anticancer polymeric nanomedicines**. *Polym Rev* 2007, **47**:345–381.

45. Mima Y, Hashimoto Y, Shimizu T, Kiwada H, Ishida T: **Anti-PEG IgM is a major contributor to the accelerated blood clearance of polyethylene glycol-conjugated protein**. *Mol Pharm* 2015, **12**:2429–2435.

46. Keefe AJ, Jiang S: **Poly(zwitterionic)protein conjugates offer increased stability without sacrificing binding affinity or bioactivity**. *Nat Chem* 2012, **4**:59–63.

47. Thomas A, Müller SS, Frey H: **Beyond poly(ethylene glycol): linear polyglycerol as a multifunctional polyether for biomedical and pharmaceutical applications**. *Biomacromolecules* 2014, **15**:1935–1954.

48. Mancini RJ, Lee J, Maynard HD: **Trehalose glycopolymers for stabilization of protein conjugates to environmental stressors**. *J Am Chem Soc* 2012, **134**:8474–8479.

49. Hu J, Wang G, Zhao W, Liu X, Zhang L, Gao W: **Site-specific in situ growth of an interferon-polymer conjugate that outperforms PEGASYS in cancer therapy**. *Biomaterials* 2016, **96**:84–92.

50. Hou Y, Yuan J, Zhou Y, Yu J, Lu H: **A concise approach to site-specific topological protein-poly(amino acid) conjugates enabled by in situ-generated functionalities**. *J Am Chem Soc* 2016, **138**:10995–11000.

51. ** Hou Y, Zhou Y, Wang H, Wang R, Yuan J, Hu Y, Sheng K, Feng J, Yang S, Lu H: **Macrocyclization of interferon-poly(α-amino acid) conjugates significantly improves the tumor retention, penetration, and antitumor efficacy**. *J Am Chem Soc* 2018, **140**:1170–1178.
The authors present an ingenious strategy to improve cytokines circulation half-life and antitumor efficacy through cyclization of the polypeptide conjugate. This method achieves increased antitumor activity by increasing cytokines tumor penetration and retention.

52. * Hou Y, Zhou Y, Wang H, Sun J, Wang R, Sheng K, Yuan J, Hu Y, Chao Y, Liu Z, *et al.*: **Therapeutic protein PEPylation: the helix of nonfouling synthetic polypeptides minimizes antidrug antibody generation**. *ACS Cent Sci* 2019, **5**:229–236.
This review discusses recent advances in the field of protein-peptide conjugation as well as future directions in the field.

53. ** Wang H, Hou Y, Hu Y, Dou J, Shen Y, Wang Y, Lu H: **Enzyme-activatable interferon-Poly(α-amino acid) conjugates for tumor microenvironment potentiation**. *Biomacromolecules* 2019, **20**:3000–3008.
The authors report a simple method to generate enzyme-activatable protein–polypeptide conjugates. This approach permits inducible release of cytokines through degradation of a peptide substrate for matrix metalloproteinases.

54. Wang Z, Guo J, Sun J, Liang P, Wei Y, Deng X, Gao W: **Thermoresponsive and protease-cleavable interferon-polypeptide conjugates with spatiotemporallyprogrammed two-step release kinetics for tumor therapy**. *Adv Sci* 2019, **6**:1–8.

55. Whiteside TL: **The tumor microenvironment and its role in promoting tumor growth**. *Oncogene* 2008, **27**:5904–5912.

56. Zhou ZH, Ji CD, Xiao HL, Zhao H Bin, Cui YH, Bian XW: **Reorganized collagen in the tumor microenvironment of gastric cancer and its association with prognosis**. *J Canc* 2017, **8**:1466–1476.

57. Ricard-Blum S: **The collagen family**. *Cold Spring Harb Perspect Biol* 2011, **3**:1–19.

58. Provenzano PP, Inman DR, Eliceiri KW, Knittel JG, Yan L, Rueden CT, White JG, Keely PJ: **Collagen density promotes mammary tumor initiation and progression**. *BMC Med* 2008, **6**:1–15.

59. Butcher DT, Alliston T, Weaver VM: **A tense situation: forcing tumour progression**. *Nat Rev Canc* 2009, **9**:108–122.

60. Liang H, Li X, Chen B, Wang B, Zhao Y, Zhuang Y, Shen H, Zhang Z, Dai J: **A collagen-binding EGFR single-chain Fv antibody fragment for the targeted cancer therapy**. *J Contr Release* 2015, **209**:101–109.

61. Liang H, Li X, Wang B, Chen B, Zhao Y, Sun J, Zhuang Y, Shi J, Shen H, Zhang Z, *et al.*: **A collagen-binding EGFR antibody**

fragment targeting tumors with a collagen-rich extracellular matrix. *Sci Rep* 2016, **6**:1–14.

62. Ishihara J, Fukunaga K, Ishihara A, Larsson HM, Potin L, Hosseinchi P, Galliverti G, Swartz MA, Hubbell JA: **Matrix-binding checkpoint immunotherapies enhance antitumor efficacy and reduce adverse events**. *Sci Transl Med* 2017, **9**:1–14.

63. Sasaki K, Ishihara J, Ishihara A, Miura R, Mansurov A, Fukunaga K, Hubbell JA: **Engineered collagen-binding serum albumin as a drug conjugate carrier for cancer therapy**. *Sci Adv* 2019, **5**:1–12.

64. ** Ishihara J, Ishihara A, Sasaki K, Lee SSY, Williford JM, Yasui M, Abe H, Potin L, Hosseinchi P, Fukunaga K, *et al.*: **Targeted antibody and cytokine cancer immunotherapies through collagen affinity**. *Sci Transl Med* 2019, **11**:1–12.
By fusing a collagen-binding domain to IL-2 and to immune checkpoint blockade antibodies, the authors describe a strategy to decrease systemic toxicity of the cytokine therapy and achieve significant tumor growth suppression in multiple murine cancer models, such as B16F10.

65. ** Mansurov A, Ishihara J, Hosseinchi P, Potin L, Marchell TM, Ishihara A, Williford JM, Alpar AT, Raczy MM, Gray LT, *et al.*: **Collagen-binding IL-12 enhances tumour inflammation and drives the complete remission of established immunologically cold mouse tumours**. *Nat Biomed Eng* 2020, **4**:531–543.
The authors engineer an IL-12 cytokine fused to a collagen-binding domain for enhanced tumor accumulation and high anticancer efficacy in murine melanoma and mammary carcinoma models.

66. ** Williford JM, Ishihara J, Ishihara A, Mansurov A, Hosseinchi P, Marchell TM, Potin L, Swartz MA, Hubbell JA: **Recruitment of CD103+ dendritic cells via tumor-targeted chemokine delivery enhances efficacy of checkpoint inhibitor immunotherapy**. *Sci Adv* 2019, **5**:1–16.
The authors fuse CCL4 to a collagen-binding domain and analyze the effect of increased CCL4 tumor localization on recruitment of $CD103^+$ dendritic cells and $CD8^+$T cells.

67. Lasek W, Zagożdżon R, Jakobisiak M: **Interleukin 12: still a promising candidate for tumor immunotherapy?** *Cancer Immunol Immunother* 2014, **63**:419–435.

68. ** Momin N, Mehta NK, Bennett NR, Ma L, Palmeri JR, Chinn MM, Lutz EA, Kang B, Irvine DJ, Spranger S, *et al.*: **Anchoring of intratumorally administered cytokines to collagen safely potentiates systemic cancer immunotherapy**. *Sci Transl Med* 2019, **11**:1–14.
The authors develop a fusion protein in which cytokines (IL-2 and IL-12) are fused to lumican, a collagen binding domain. This approach significantly increases local retention of cytokines, safety, and antitumor immunity compared to non-anchoring versions.

69. Charych DH, Hoch U, Langowski JL, Lee SR, Addepalli MK, Kirk PB, Sheng D, Liu X, Sims PW, VanderVeen LA, *et al.*: **NKTR-214, an engineered cytokine with biased IL2 receptor binding, increased tumor exposure, and marked efficacy in mouse tumor models**. *Clin Canc Res* 2016, **22**:680–690.

70. Arenas-Ramirez N, Zou C, Popp S, Zingg D, Brannetti B, Wirth E, Calzascia T, Kovarik J, Sommer L, Zenke G, *et al.*: **Improved cancer immunotherapy by a CD25-mimobody conferring selectivity to human interleukin-2**. *Sci Transl Med* 2016, **8**: 1–13.

71. Markley JC, Sadelain M: **IL-7 and IL-21 are superior to IL-2 and IL-15 in promoting human T cell-mediated rejection of systemic lymphoma in immunodeficient mice**. *Blood* 2010, **115**: 3508–3519.

72. Yeku OO, Purdon TJ, Koneru M, Spriggs D, Brentjens RJ: **Armored CAR T cells enhance antitumor efficacy and overcome the tumor microenvironment**. *Sci Rep* 2017, **7**:1–14.

73. Kunert A, Chmielewski M, Wijers R, Berrevoets C, Abken H, Debets R: **Intra-tumoral production of IL18, but not IL12, by TCR-engineered T cells is non-toxic and counteracts immune evasion of solid tumors**. *OncoImmunology* 2018, **7**:1–12.

74. Krenciute G, Prinzing BL, Yi Z, Wu MF, Liu H, Dotti G, Balyasnikova IV, Gottschalk S: **Transgenic expression of IL15 improves antiglioma activity of IL13Rα2-CAR T cells but results in antigen loss variants**. *Cancer Immunol Res* 2017, **5**: 571–581.

75. Chmielewski M, Abken H: **CAR T cells releasing IL-18 convert to T-Bethigh FoxO1low effectors that exhibit augmented activity against advanced solid tumors**. *Cell Rep* 2017, **21**: 3205–3219.

76. Ma X, Shou P, Smith C, Chen Y, Du H, Sun C, Porterfield Kren N, Michaud D, Ahn S, Vincent B, *et al.*: **Interleukin-23 engineering improves CAR T cell function in solid tumors**. *Nat Biotechnol* 2020, **38**:448–459.

77. Stephan MT, Moon JJ, Um SH, Bersthteyn A, Irvine DJ: **Therapeutic cell engineering with surface-conjugated synthetic nanoparticles**. *Nat Med* 2010, **16**:1035–1041.

78. Tang L, Zheng Y, Melo MB, Mabardi L, Castaño AP, Xie YQ, Li N, Kudchodkar SB, Wong HC, Jeng EK, *et al.*: **Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery**. *Nat Biotechnol* 2018, **36**:707–716.

79. Carmenate T, Pacios A, Enamorado M, Moreno E, Garcia-Martínez K, Fuente D, León K: **Human IL-2 mutein with higher antitumor efficacy than wild type IL-2**. *J Immunol* 2013, **190**: 6230–6238.