

Review

Cytokine-based Cancer Immunotherapy: Challenges and Opportunities for IL-10

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Abstract. Cancer immunotherapy is an evolving field of research. Cytokines have been conceptualized as an anticancer therapy for longer than most other cancer immunotherapy modalities. Yet, to date, only two cytokines are FDA-approved: IFN- α and IL-2. Despite the initial breakthrough, both agents have been superseded by other, more efficacious agents such as immune checkpoint inhibitors. Several issues persist with cytokine-based cancer therapies; these are broadly categorised into a) high toxicity and b) low efficacy. Despite the only moderate benefits with early cytokine-based cancer therapies, advances in molecular engineering, genomics, and molecular analysis hold promise to optimise and reinstate cytokine-based therapies in future clinical practice. This review considers five important concepts for the successful clinical application of cytokine-based cancer therapies including: (i) improving pharmacokinetics and pharmacodynamics, (ii) improving local administration strategies, (iii) understanding context-dependent interactions in the tumour-microenvironment, (iv) elucidating the role of genetic polymorphisms, and (v) optimising combination therapies. IL-10 has been the focus of attention in recent years and is discussed herein as an example.

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Cytokines are soluble, low molecular weight proteins that mediate cell-to-cell communication. They are able to modulate the host immune response toward cancer cells and directly induce their apoptosis. Cytokine-based immunotherapy has been a promising area of research, yet to date, only IFN- α and IL-2 (1) have received FDA approval for cancer therapy. Moreover, these agents have been superseded by safer and more efficacious therapies such as immune checkpoint inhibitors (ICIs) (2).

Several issues persist with cytokine-based immunotherapies. These are broadly categorised into: a) high toxicity and b) low efficacy (2). The vast pleiotropism and redundancy in cytokine signalling, as well as dual immunosuppressive and immunostimulatory functions contribute to suboptimal safety and efficacy (3). Despite moderate benefits with early cytokine therapies, it remains an important field of research, especially considering progresses with IL-10.

This review addresses five obstacles in the clinical translation of next generation cytokine cancer immunotherapies. These include: (i) enhancing pharmacokinetics and pharmacodynamics; (ii) improving local administration; (iii) understanding context-dependent interactions in the tumour microenvironment (TME); (iv) elucidating the role of genetic polymorphisms; and (v) optimising combination therapy. We explore possible avenues in these domains listing biotechnological advances. IL-10 is used as a focused example.

IL-10 in Cancer Therapy: Between Pro-tumorigenic Inflammation and Anti-tumour Immunity – From Old to New

IL-10 is a pleiotropic cytokine with anti-inflammatory and immunostimulatory functions. In cancer, IL-10 may exert pro- or anti-tumour effects (4). Early research aimed to

neutralise IL-10 to mediate tumour rejection *via* T-cell stimulation. However, this approach has been limited by severe life-threatening inflammatory toxicities (4).

Current research focuses on increasing local IL-10 concentrations in the TME to mediate tumour regression since IL-10-dependent CD8⁺ T-cell stimulation induces tumour-specific immunity (4). *In vivo*, IL-10's anti-tumour mechanisms are well-documented (5, 6) and work synergistically with anti-PD-1 therapy (5, 7). Yet, a phase Ib trial, failed to show added benefit with the combination of IL-10 and pembrolizumab or nivolumab (8). Nevertheless, data could simply imply that IL-10 might not overcome ICI resistance in this setting (9).

Significant are also novel findings regarding the impact of receptor binding. IL-10R is composed of α and β subunits. Researchers engineered an IL-10 construct with potent immunomodulatory effects because it targeted IL-10R β with higher affinity (10). Thus, the relative affinity and avidity of IL-10 to receptor subunits may offer insight into lack of efficacy in certain settings.

Enhancing Pharmacokinetics and Pharmacodynamics

Chemical and physical instability limits cytokine therapies. Short half-life (11) and *in vivo* proteolytic enzymatic degradation necessitate high doses and frequent administration (12). Protein handling and storage, including freeze-thaw cycles and storage time, affect stability, causing denaturation and activity variations (12, 13).

IL-10 conjugation with polyethylene glycol (pegylation), creating pegilodecakin, increases serum half-life. *In vitro*, pegilodecakin inhibits tumour growth by oligoclonal expansion of tumour-specific CD8⁺ T-cells (14, 15). Clinically, pegilodecakin induces CD8⁺ immunity, elevating IFN- γ and granzyme B with acceptable toxicity (5, 6). Combination with anti-PD-1 (nivolumab) is safe and efficacious in phase I setting (8).

Conjugation to polyvinylpyrrolidone (PVP)-coated silver nanoparticles also improves pharmacokinetics (12). PVP-conjugates are retained in the blood increasing serum half-life (16). Silver (Ag) nanoparticles also mediate anti-inflammation (17, 18). Preclinically, conjugation of IL-10 to PVP-coated silver nanoparticles increased its anti-inflammatory characteristics (12). Furthermore, Ag-PVP conjugated IL-10 retained its effectiveness despite storage condition variations, compared to mouse recombinant IL-10, which showed decreased activity (12).

A further approach to improve pharmacokinetics is extracellular vesicle (EV)-loading. EVs are natural cell products and function as inter-cellular protein transporters (19). A recent study outlines a method of delivering IL-10 using EVs in a murine model of ischaemic acute kidney

injury (AKI) (20). EVs could be targeted to the kidney using adhesive EV surface components conferring desired therapeutic properties.

Antibody fusion creating immunocytokines, also known as bispecific antibodies (BsAbs), has also been investigated (21). Cetuximab-based IL-10 fusion protein CmAb-(IL10)2 developed by the conjugation of IL-10 with the epidermal growth factor receptor (EGFR) inhibitor cetuximab, demonstrated pre-clinical antitumor efficacy (8). CmAb-(IL10)2, increased half-life and reduced toxicity compared to systemic IL-10. CmAb-(IL-10) inhibits tumour growth, and when combined with anti-PD-1 and anti-CTLA-4, decreases CD8⁺ T-cell apoptosis. Bispecific antibodies have higher avidity and specificity as they interact with two surface antigens (22, 23).

Confining Effects to Tumour Location

Localising cytokine administration to the tumour may reduce toxicity and enhance efficacy of cytokine immunotherapy reducing systemic off-target proinflammatory effects and increasing local drug concentrations, respectively.

Although a potent anti-inflammatory cytokine, at high doses IL-10 has immunostimulatory effects on CD4⁺, CD8⁺, and/or NK cells increasing IFN- γ production (24), which induces antitumor activity (25). Injecting IL-10 directly into the tumour achieves sufficiently high drug concentrations to increase other cytokine levels such as IFN- γ , IL-4 and IL-18. IFN- γ upregulates MHC-I on tumour and dendritic cells improving CD8⁺ T-cell anti-tumour cytotoxicity (6).

Unlike recombinant IL-10 injection, gene therapy vectors offer tailored gene manipulation, fewer production limitations, and efficient protein production with appropriate post transcription modifications (26). Adenoviral-mediated expression of IL-10 has been used for immunosuppression in autoimmune conditions (27). However, safety limitations restrict adenovirus-based therapy to single administration (26).

Oncolytic viruses armed with IL-10 have been studied in murine pancreatic cancer models (28). Using a tumour-targeted oncolytic vaccinia virus containing IL-10, researchers observed twice as many survivors. Almost 90% of responding mice showed complete tumour clearance, compared to 40% treated with the unarmed virus. Increased macrophage infiltrate and MHC-II downregulation in the IL-10 condition indicate enhanced tumour rejection through innate and adaptive immunity modulation.

Nano-carriers selectively target cells for tumour eradication (29). Lipid nanoparticles enable substance loading (*e.g.*, drugs and mRNA) for vector transfection. Effective targeted delivery for selective mRNA-based protein expression could decrease off-target expression and enhance efficacy (26). Lipid nanoparticle mRNA delivery of IL-10 has been applied *in vivo* for inflammatory conditions (26) and could be expanded to cancers.

Understanding the Context-dependent Interaction Within the TME

The TME differs between each cancer: different tissues, different malignant cells-of-origin, and different stroma. Considering the distinct immune cells, contexts of stimulation, and concentrations of multiple factors involved is important (30). Therapeutic alteration of IL-10 in the TME produces paradoxical outcomes (30, 31).

The TME encourages chronic inflammation whilst suppressing acute inflammatory responses to maintain tumour growth. Tumours associated with worse prognosis exhibit higher tumour-associated macrophage (TAM) and T-regulatory cell (Treg) concentrations, and fewer tumour-infiltrating lymphocytes. IL-10 propagates pro- and anti-inflammatory processes maintaining homeostasis of anti-inflammatory Tregs and suppression of proinflammatory IL-17-expressing T cells (Th17) (4).

The anticancer effect of IL-10 is hypothesized to occur through: (i) reduction of tumour-promoting inflammation and (ii) stimulation of CD8⁺ T cells in the tumour milieu (4). IL-10 stabilises Tregs which dampen the immune response (32). It also promotes inflammation *via* pro-inflammatory cytokine induction such as IFN- γ and granzyme B which induce MHC-I/II for tumour antigen presentation. IL-10 induces CD8⁺ T-cell cytotoxicity (33) and, in its pegylated form, effectively induces tumour rejection (4).

Pro-tumorigenicity is mediated by diminished anti-tumour immunity (31). IL-10 negatively regulates proinflammatory IL-6 and IL-12/IL-23 signalling and reduces antigen presentation by downregulating MHC-II on APCs (34) and MHC-I on tumour cells (35) promoting tumour immune escape. TAMs secrete IL-10 hence levels correlate with tumour growth (31, 36). Tumours themselves release IL-10 contributing to immunosuppression [reviewed in (31)]. Concomitant IL-10R expression on tumours supports an autocrine signalling model (37). Although IL-10 correlates with tumour progression the multiple factors involved have prevented establishment of causality (31).

Elucidating the Role of SNPs

From the mid-2000s, 22 studies (38), spanning 13 malignancies (39), highlighted associations between IL-10 SNPs and cancer. While these were limited in cohort size and cause-and-effect relationships, they incited investigation. Given IL-10's importance in cancers (40-42), the impact of SNPs on molecular functioning is crucial.

At least 49 IL-10 SNPs have been reported within the likes of Ensemble Genome Browser databases (39). IL-10 variants induce differential protein expression. For example, IL-10-1082, -819, and -592 SNP haplotypes demonstrate differential expression *in vitro* (43). Genetic variation

accounts for up to 75% of interindividual differences (44). Differences in post-transcriptional regulation have been described: IL-10 mRNA stability varies indicating cell-specific interactions (43).

Contradictory findings have been reported on IL-10's role in carcinogenesis. Studies suggest SNPs are directly implicated in breast cancer. IL-10-593C>A polymorphisms modify disease free and overall survival in lymph-node-positive cancer after adjusting for clinical parameters including estrogen and progesterone receptor status (45). SNPs such as -1082A/G predispose to breast cancer, potentially, by conferring an additional transcription factor binding site in the promoter region (46, 47). Yet, meta-analyses are conflicting on IL-10 rs1800896 and rs1800871 polymorphisms and breast cancer susceptibility (38, 48, 49). Equally, studies across prostate (50-52), skin (53, 54), lung (55), gastric (56) and cervical cancers (57, 58) have not been consistent.

Optimising Combination Therapy

As aforementioned, IL-10-armed oncolytic viruses doubled the rate of complete responses in pancreatic cancer models (28). Gorby *et al.* showed that their engineered IL-10 construct improved CAR-T-cell activity *in vitro* compared to CARs cultured with wild type or no IL-10 (10). Pegilodecakin mediates a sustained increase in serum IL-18 (5), which enhances efficacy and durability of CAR-expressing CD8⁺ T cells (59, 60). Combinations with ICIs stem from the understanding that IL-10 mediates antitumour effects through CD8⁺ T cell stimulation in the tumour milieu while ICIs reinvigorate exhausted CD8⁺ T cells by suppressing inhibitory immune signals (61). Anti-CTLA-4 plus anti-PD-1 tumour regression also depends on IL-7 and IFN- γ (62), which are inducible by pegilodecakin (5). In a recent article, Guo *et al.* provide preclinical evidence that IL-10-Fc-based therapy is safe and highly effective in combination with many existing immunotherapies, such as ICIs, cancer vaccines, and adoptive T cell transfer, potentially complementing and synergizing with these for enhanced efficacy and response rates (63). By promoting oxidative phosphorylation, independently of progenitor exhausted T cells, IL-10-Fc directly and potently enhanced expansion and effector functioning of terminally exhausted CD8⁺ tumour-infiltrating lymphocytes, which pose a major challenge in lack of response to ICIs and most other immunotherapies. Established solid tumours were eradicated and durable cures achieved in most treated mice (63). A phase 2 study in advanced melanoma linked increased baseline IL-10 to tumour responses with PD-1 inhibition (64). Moreover, combination of pegilodecakin with pembrolizumab or nivolumab demonstrated a favourable adverse event profile (8). Conversely, the combination of

pegilodecakin with FOLFOX has failed to improve efficacy as second-line therapy in metastatic pancreatic cancer (65), despite promising early phase data (66, 67).

Conclusion

Cytokines have proven to be an effective anti-cancer therapy, however due to their pleiotropic effect, short half-life and systemic toxicity, they have been surpassed by other targeted agents. New approaches that improve cytokine targeting and alter their pharmacokinetics are being developed. This has resulted in a number of ongoing trials (NCT03193190, NCT04165967), the results of which are eagerly awaited.

Conflicts of Interest

The Authors declare that they have no competing interests in relation to this study.

Authors' Contributions

K.S.R.: conceptualization, supervision, reviewing the literature, drafting and revising the article, and final approval of the version to be published. A.E.C., H.D., A.M.G., and S.M.K.: reviewing the literature, drafting and revising the article, and final approval of the version to be published. M.S. and B.S.: supervision, revisions and final approval of the version to be published.

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