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Common Gamma Chain Cytokines in Combinatorial Immune Strategies against Cancer

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Abstract

Common γ chain (γ C) cytokines, namely IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 are important for the proliferation, differentiation, and survival of lymphocytes that display antitumor activity, thus stimulating considerable interest for the use of cytokines in cancer immunotherapy. In this review, we will focus on the γ C cytokines that demonstrate the greatest potential for immunotherapy, IL-2, IL-7, IL-15, and IL-21. We will briefly cover their biological function, potential applications in cancer therapy, and update on their use in combinatorial immune strategies for eradicating tumors and hematopoietic malignancies.

Keywords

Cancer Immunotherapy; Gamma Chain Cytokines; Hematopoietic Malignancies; Solid Cancers; Immunostimulation

1. Introduction

The myriad of cytokines that are produced in the tumor microenvironment play a profound role not only in cancer pathogenesis but also in cancer elimination when presented with

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appropriate immune stimulatory conditions. Cytokines that are secreted during inflammation aid in developing the host's innate and adaptive anti-tumor responses by enhancing tumor-recognition and stimulating lymphocyte effector function [1]. On the other hand, cytokines secreted by stromal and/or immune cells may also assist in tumor growth and promotion [2–6]. Therefore, understanding the relationship of cytokines with tumor cells as well as host immune cells will provide new opportunities for immunotherapy.

Several studies have shown the effectiveness of cytokines to increase the potential of cytotoxic lymphocytes capable of decreasing tumor size or sustaining remission. Cytokines that often display such effects belong to the γ -chain family consisting of interleukins IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [7–9]. These cytokines signal through a receptor complex sharing a common γ chain subunit (γ C) generally implying signaling redundancy [8]. IL-2, IL-7, IL-15, and IL-21 demonstrate the greatest potential for immunotherapy [10–12]; however, the use of these cytokines in combination with other therapeutics allowing for potential synergism will be the focus of this review. We will briefly cover the normal physiological function of these cytokines, potential role in immunotherapy, and the outcome of the most recent clinical trials on the use of these cytokines in combinatorial therapy involving the treatment of hematological malignancies and solid cancers.

2. Normal Physiological Functions of γ Chain Cytokines

2.1. IL-2

Predominately produced by activated CD4⁺ T cells under normal biological conditions, IL-2 is characterized for its ability to induce the expansion of CD4⁺ and CD8⁺ T cells [13,14], promote growth and differentiation of activated B cells [15], and encourage the cytotoxic activity of natural killer (NK) cells [16]. Both IL-2 and IL-4 are required for optimal CD4⁺ development, whereby IL-2 maintains transcriptional accessibility to a portion of the *IL4* gene that in turn facilitates the differentiation of CD4⁺ T cells [17]. IL-2 also induces the expression of IL-4R α which further contributes to CD4⁺ differentiation [18]. However, as a method to eliminate over-stimulation of T cells that may result in autoimmunity, IL-2 also induces Fas-mediated apoptosis in CD4⁺ T cells [19]. Analysis of the gene promoter regions uncovered binding sites for various transcription factors including nuclear factor of activated T-cells (NFAT), NF- κ B, and activator protein 1 (AP-1) [20] suggesting IL-2 is highly regulated transcriptionally and via mRNA stability. The IL-2 receptor (IL-2R) is a heterotrimeric complex consisting of IL-2R α , IL-2R β , and γ C subunits. As an additional mechanism of regulating immunity, IL-2R α is highly expressed in CD4⁺CD25⁺FoxP3⁺ regulatory T (Treg) cells [21] that suppresses potentially deleterious activities of T helper (Th) cells and maintains self-tolerance [22]. IL-2R α does not contain a cytoplasmic signaling subunit; however, it is pertinent for high affinity binding of IL-2 to its receptor [23]. IL-2R β and γ are needed for signal transduction where IL-2R β associates with Janus tyrosine kinase-1 (Jak1) and γ C is associated with Jak3 [24]. Upon activation of the IL-2R, Jak1 and Jak3 phosphorylate Signal Transducer and Activator of Transcription-5 (STAT5) [25]. Activation of Jak proteins also induces other signaling proteins such as phosphoinositide 3-kinase (PI3K) [26] and Src [27]. Further data indicate IL-2 activates STAT1 and STAT3 binding to IL-2R β in a different subdomain than STAT5, and this

binding is independent of tyrosine residues [28]. IL-2-induced dimerization of STAT5a/b molecules results in the expression of pro-survival genes and FoxP3 transcription factor, master regulator of transcription program in Treg cells [29]. Also, formation of STAT5a/b di- or tetramers can alternate transcriptional programs depending on recruitment of transcription regulators with opposite action, histone acetyltransferase or methyltransferase as well as nuclear corepressor complex [30].

Further, IL-2 is linked to the suppression of Th17 differentiation that may be due to epigenetic suppression by competitive STAT5 binding to the IL-17 promoter [31]. Mutation within the IL-2 gene [32] or the IL-2R α [33] are known to cause autoimmune diseases; while, defects of signaling proteins such as γ C are found in severe combined immunodeficiency disease (SCID) [34]. Paradoxically, IL-2 and IL-2R are expressed in cancer cells also. Reichert et al. documented increased expression of endogenous IL-2 and receptor subunits β and γ , when carcinoma cells are in the G2/M phase of the cell cycle as opposed to the G0/G1 phase. They were able to show IL-2 decreases expression of p27 and p21, negative regulators of cellular proliferation, thereby controlling cell cycle progression [35]. Despite this finding, it is important to stress that the mechanism of action of γ C cytokines is not based on direct anticancer effects, but depends on immune-mediated pathways regulating the proliferation and survival of lymphocytes targeting tumor that will be discussed further in this review.

2.2. IL-7

A key homeostatic cytokine, IL-7 is essential for the proper function of the immune system, required for the growth and survival of T cells [36–38], and regulates the quality and quantity of an immune response. IL-7 utilizes two mechanisms to promote naive T cell survival: 1) inhibition of the apoptosis through upregulation of anti-apoptotic proteins, B-cell lymphoma 2 (Bcl-2) and myeloid cell leukemia 1 (Mcl-1) [39] and 2) maintenance of cellular trophic state and glucose metabolism [40]. T cells do not produce IL-7, but it is produced by non-immune stromal cells such as lymphatic endothelial and epithelial cells, bone marrow epithelial, a subpopulation of epithelial cells in the intestines [41]. IL-7 and its receptor are expressed by tumor cells such as colorectal [42], hematopoietic, lung, and brain cancers [43]. Unlike IL-2R, the IL-7R is a heterodimer consisting of IL-7R α and γ C that may lead to activation of Src kinases [44], PI3K [45]; and similarly to IL-2, Jak1, Jak3 and STAT5 [46]. IL-7 protects T cells from tumor-induced dysfunction by inhibiting CD27/CD28 loss while maintaining proliferative capabilities, and IL-2 production [47]. IL-7 may play a role in restoring T cells following T cell depletion [48]. Mutations of the IL-7R α have been shown to lead to a T cell-deficient, B cell and NK cell-replete SCID phenotype [49].

2.3. IL-15

Expressed across a myriad of cells, IL-15 effects extend to nearly all hematopoietic cells and various non-immune cells. Discovered almost simultaneously by Burton [50] and Grabstein [51] in 1994, IL-15 has been shown to play a role in the generation of CD8⁺ memory T cells [52], homeostasis of natural killer T (NKT) cells [53], and survival of NK cells [54]. Additionally, IL-15 inhibits apoptosis in T cells by inducing anti-apoptotic protein Bcl-2 [55,56]. Studies have indicated IL-2, IL-7, and IL-15 leads to the generation of

Foxp3⁺CD25⁺CD4⁺ Treg cells that possess a higher affinity for self-peptides than that of a majority of CD4⁺ T cells produced in the thymus. These cells would be typically eliminated in the thymus, but later functions to suppress the immunological responses of CD4⁺CD25⁻ T cells [57]. Interestingly, IL-15 is not expressed in T cells like IL-2; however, IL-15 share IL-2/15R β and γ C thereby implicating similar effects [58]. For high affinity binding to the IL-15R, an additional subunit, IL-15R α , is required forming a heterotrimeric receptor [59]. Unlike other γ C cytokines that exert their effects as a soluble protein, IL-15 functions as a bound cytokine on cells expressing IL-15R α such as activated monocytes. The IL-15R α presents IL-15 to target cells expressing IL-2/15R β and γ C such as CD8⁺ T cells. This mode of action is termed trans-presentation [60,61]. Despite similar biological effects with IL-2, IL-15 does not share homology on a protein level with IL-2 [51] and IL-15 has its own unique effects. For example, IL-2 regulates the expansion and differentiation of activated T cells, and the regulation of T cell tolerance. On the other hand, IL-15 is involved in the survival of memory and naive CD8⁺ T cell and NK cell without any affect on Treg cells [62]. Additionally, low levels of IL-15 stimulated the production of all T cell subpopulations, especially the T cell receptor (TCR)- $\gamma\delta$ T cells in the fetal thymic organ culture; yet, these results were not observed in the presence of IL-2. Additionally, high concentrations of IL-15 shifted from the production of TCR $\alpha\beta$ cells to NK cells, and to a higher degree than high concentrations of IL-2 [63]. Phenotypic differences produced by IL-2 and IL-15 such as cell size are not attributed to signal transduction but duration and strength of the signal as well as distribution of the receptor [62,64]. IL-2R α is predominately repressed in T and B cell; conversely, IL-15R α reside on monocytes and DCs [62]. Despite the ability of IL-2 and IL-15 to activate the same downstream signaling pathways, the distinct differences between the functions of IL-2 and IL-15 are considerable in recruiting the appropriate response for therapeutic uses. Previous study indicated the IL-15 gene may be regulated at the level of translation and secretion [65]. The effect of IL-15 in cancer pathogenesis remains controversial. Some studies suggest that it improves immune response to cancers while others indicate that it promotes tumorigenesis; both of which are reviewed in subsequent sections.

2.4. IL-21

Predominately produced by CD4⁺ T cells, IL-21 is the most recently added member of the γ C family. IL-21 stimulates B cells, memory T cells, and bone marrow progenitors. It also increases the cytotoxicity of CD8⁺ T cells and NK cells while having the capacity to suppress Tregs [66,67]. It also shows autocrine effects promoting Th17 differentiation and IL-17 production [68] that also may play a role in oncogenesis. Interestingly, activated T cells stimulated with α -TCR and α -CD28 secrete alternatively spliced forms of soluble γ C resulting in inhibition of γ C signaling and promote inflammation by increasing Th17 differentiation in mice [69]. IL-21 induces apoptosis in DC that may contribute to immune tolerance, and this induction was inhibited by granulocyte-macrophage colony-stimulating factor (GM-CSF) due to competition among STAT binding [70]. Unlike IL-2, calcium alone may be able to stimulate induction of IL-21 mRNA in activated T cells [71]. The IL-21R is a heterodimer consisting of IL-21R α and γ C [72]. Similar to previous cytokines, IL-21 signaling induces Jak1, Jak3, STAT1, STAT3 [68], and STAT5 [73] proteins in addition to PI3K [74] and mitogen-activated protein kinase (MAPK) [75] proteins. IL-21 exerts its

effects in T cells mainly through activation of STAT3, basic leucine zipper transcription factor, JUN, and interferon regulatory factor 4 (IRF4) [67,76]

3. Use of γ Chain Cytokines in Solid Cancers

3.1. IL-2

Preclinical—As one of the most studied cytokines for immunotherapy, modified forms of IL-2 are being developed to ensure longevity and precise targeting to the tumor site. Miki et al. demonstrated that IL-2 micelles not unmodified IL-2, enhanced DC vaccination in EG7 lymphoma-bearing mice. DC vaccination along with IL-2 micelles decreases tumor volume significantly 12 days following inoculation. This is mainly attributed to the short half-life of IL-2 in serum. The development of IL-2 micelles enabled long term-high serum levels of IL-2 that remained in serum 48 hours after inoculation compared to unmodified IL-2 that was completely abolished within 6 hours. The IL-2 micelles increased the number of cytotoxic T lymphocytes (CTL) in the spleen and the activity of antigen-specific CTL into the tumor [77]. An IL-2 “superkine” was developed that has a high affinity for the IL-2R β eliminating the requirement of IL-2R α . IL-2 “superkine” demonstrated an increase in cytotoxic T cells, antitumor capabilities, and a proportional decrease in Tregs in IL-2R α knockout mice when compared to IL-2 [78]. By inducing variants within the superkine that weakens the binding to γ C, IL-2 signaling can be harnessed in a controllable manner by fine-tuning the amplitude of the signal. These IL-2 variants function as “receptor signaling clamps.” When partial agonist conditions are achieved, modulation of the signaling output during immunotherapy is rendered without over or under stimulation of immunity [79].

Clinical—IL-2 was initially tested in a clinical trial in the mid 1990s [80]. To date, IL-2 (Aldesleukin/Proleukin) is FDA approved for treatment of melanoma and renal cell carcinoma with complete remission in a subset of patients [81]; however, various clinical trials are underway for the use of IL-2 in other cancers (see Table 1). Yang et al. compared high-dose (720,000 U/kg) and low-dose (72,000 U/kg) of IL-2 indicating 21% and 13% response rates, respectively [82]. Initial clinical trials of renal carcinoma indicate that 12% of the patients respond to treatment and of that 28% remain in remission [83,84]. Early trials for metastatic melanoma patients indicate objective responses in approximately 1/3 of patients when treated with tumor-infiltrating lymphocytes and high-dose IL-2 (720,000 U/kg) with or without cyclophosphamide [85]. Therefore, IL-2 has proven to be relatively efficient at high doses in renal carcinoma and melanoma. Moreover, pharmacokinetic studies show that the vehicle of IL-2 administration affects the outcome. A phase I clinical trial in women with metastatic breast cancer was performed utilizing an anti-CD3/anti-HER2 bispecific antibody, anti-CD3 activated T cells with low dose IL-2 (300,000 IU/m²) and GM-CSF. This combination displayed anti-tumor effects with an increase in Th1 cytokines and IL-12 production. After 14.5 weeks, 59% of patients saw stabilization in their disease while 41% displayed progression in the disease. IL-2 played an essential role in continued expansion of T cells that infiltrated the tumor [86]. Immunocytokine, F16-IL2 with doxorubicin has also shown anti-tumor effects in a phase I trial in solid tumors and a phase II trial in metastatic breast cancer [87]. F16 is an antibody specific to the A1 domain of tenascin-C, which is virtually undetectable in normal tissues but is strongly expressed

upon tissue injury and in the stromal compartment of cancers from various origins [88,89]. Adverse toxicities in this trial were controlled with no treatment-related deaths. This study demonstrated the safe and potential efficacy of this combinational treatment with a 57% and 67% response rate for Phase I and II respectively [87]. Due to the short half-life of IL-2, development of optimal drug delivery systems may improve the outcomes of IL-2 immunotherapy. A phase III trial on the efficacy and safety of adjuvant immunotherapy with cytokine-induced killer (CIK) cells stimulated with IL-2 in 230 hepatocellular carcinoma patient post curative treatment displayed an increase in overall survival and impeded recurrence by 14 months compared to the control group [90]. CIK cells are capable of eradicating hematological and solid malignancies. Activated CIK cells are a heterogeneous population of T cells sharing phenotypic and functional properties of NK cells such as expressing natural killer group 2, member D (NKG2D), granule exocytosis, cytokine secretion, and cytotoxicity [91]. There was an increase in adverse events in the immunotherapy-treated group than the control, but serious events did not significantly differ [90]. Thus, IL-2 may act as a viable adjuvant to other therapeutics in the treatment of solid tumors.

3.2. IL-7 and IL-15

Preclinical—Yuan et al. showed in mice injected with TM40D breast cancer cell line that IL-7 plasmids decreased tumor growth through induction of CD8⁺ T cells and subsequent IFN- γ production. Blockade of IFN- γ abolished the cytolytic activity of CD8⁺ T cells [92]. Additionally, IFN- γ along with IL-7 abolished 75% of pre-established intracerebral N32 rat glioma tumors, and prolonged survival in a more aggressive RG2 glioma tumors by increasing the activity of circulating T cells [93]. Gou et al. evaluated the antitumor efficacy of IL-7 and oxaliplatin in a female BALB/c mice injected with CT26 colorectal carcinoma cells. This combination reduced the growth of lung and bone metastasis by 2.5-fold when compared to the untreated group. This was attributed to an increase in CD8⁺ T cells and a decrease in Treg cells [94]. Of note, IL-7 in combination with IFN- γ appears to be vital for antitumor functions.

Berger et al. demonstrated IL-15 can be safely administered and has the capacity to increase the function of peripheral lymphocytes such as CD8⁺, CD4⁺ T cells, and NK cells in nonhuman primates [95]. Administration of IL-15 and antibodies against programmed death-ligand 1 and CTL-associated protein 4 increased survival rates, activity of T lymphocytes, and IFN- γ secretion in a metastatic murine colon carcinoma model [96]. In mice, IL-7/IL-7R, were also able to promote lung cancer growth through inducing cyclin D1 gene expression via AP1-dependent pathway. The authors also correlated low cyclin D1 with increased survival in non-small cell lung carcinoma patients [97]. Gattinoni et al. proved that in established B16 melanoma in C57BL/6 mice the increased efficacy of lymphodepletion after adoptive T cell therapy was not due to increased population but function of anti-tumor T cells due to their exposure to homeostatic cytokines IL-7 and IL-15. Therefore, lymphodepletion eliminates immunosuppressive cells that cause cytokine sinks and thereby, enhances the anti-tumor activities of transferred T cells via the increased availability of cytokines [98]. Conversely, a previous study indicates that lymphodepletion

before adoptive T cell transfer shows marked improvement in therapeutic efficacy in patients with metastatic melanoma [99].

Preclinical studies utilizing IL-15/IL-15R α /Apo A-1 fusion protein allowed for prolonged half-life of the cytokine and successful treatment of metastatic tumors such as lung metastasis of melanoma and liver metastasis of colon cancer in C57BL/6 mice [100]. Additionally in mice, Kermer et al. generated a trifunctional fusion protein, IL-15/IL-15R α /4-1BBL, that did in fact stimulate T cell proliferation and cytotoxicity and was highly efficient in eliminating metastasis [101]. These studies suggest that in the form of fusion proteins, IL-15 is able to target the tumor, and it also has potential to eliminate metastasis of aggressive tumors.

Clinical—The first clinical trial of IL-7 by Rosenberg et al. in lymphopenic patients with metastatic melanoma or metastatic sarcoma demonstrated a dose dependent increase in CD4⁺ and CD8⁺ lymphocytes while decreasing Treg cells [102]. In a subsequent phase I trial with adults diagnosed with incurable non-hematologic malignancies, treatment with recombinant human IL-7 displayed a similar increase in the repertoire of peripheral lymphocytes and a decrease in Treg [103]. This suggests that IL-7 has the potential of influencing the tumor microenvironment and extending the life of patients with refractory malignancies.

The Waldmann group published the results of the first in-human clinical trial of recombinant IL-15. Metastatic renal and melanoma patients were treated with varying concentration of rhIL-15 for 12 days. The authors noted an initial decline in NK and memory CD8⁺ T cells; however, a significant increase in these cells occurred shortly thereafter before returning to baseline. There was no remission observed in the patients but best response being stable disease. Some patients presented a decrease in marker lesions and experienced clearance of lung lesions. Yet, serum levels of proinflammatory cytokines IL-6, TNF- α , and IFN- γ were increased in addition to grade 3 toxicities observed at doses above 1 μ g/kg [104]. These results indicate the safe administration of IL-15 at low doses; nevertheless, post-treatment side effects may limit its ability at certain doses. Conversely, elevated circulating levels of IL-7 and IL-15 are found in early stage prostate cancer patients versus patients that displayed benign prostatic hyperplasia implying chronic inflammation promoting the onset of the disease [105] implying dual roles of these cytokine in cancer progression.

3.3. IL-21

Preclinical—IL-21 has sparked interest as a strong potential immunotherapeutic agent since its influence on NK cell-mediated antitumor function was observed [106]. Mice with established subcutaneous melanoma treated with IL-21 demonstrated an increase in circulating anti-tumor T cells 4–7 days after chemoimmunotherapy [107]. However, recent findings suggest IL-21 promote Th17 differentiation and IL-10 production, but it may also suppress pathogenic T cell subset Th1/17 that co-produce IFN- γ and GM-CSF. [108]. IL-21 shows its greatest potential when targeted directly to the tumor via a tumor-targeting vehicle. Tumor targeting molecules such as *Salmonella typhimurium* have been combined

with IL-21 to promote suppression of tumor growth in B16F10 melanoma inoculated C57BL/6 mice [109].

Zoon et al. demonstrate the significance of synergism within the γ C family. B16 melanoma inoculated C57BL/6 mice were subjected to pretreated splenocytes or lymph node cells cultured in IL-2, IL-21, IL-7/15 or IL-7/15/21. Both IL-21 and IL-7/15/21 increased the production of CD8⁺ cells compared to IL-2 or IL-7/15. CD8⁺CD44⁺CD62L^{high} central-memory T cells stimulated by IL-7/15/21 conditions were more effective at reducing metastases than IL-2 [110].

Peripheral mononuclear cells treated with IL-21 and IL-2 has demonstrated synergistic enhancement of CD4⁺ and CD8⁺ T cell proliferation compared to IL-2 alone. The presence of IL-21 decreased IL-2 and TGF β -driven Treg expansion related to persistence of active STAT3, but IL-21 did not suppress Treg activity [111]. This was further demonstrated in IL-21R^{-/-} mice whereby the authors were able to show that IL-21 may indirectly decrease the availability of IL-2 leading to Treg suppression [112]. The benefits of combinatorial therapy with IL-2/IL-21 are more effective than IL-2 and may decrease the toxic effect of high dose IL-2. This combination is associated with delayed tumor progression and increases survival in C56BL/6 mice with established B16F10 melanoma tumors but to a lesser extent for 3LL Lewis lung carcinoma. Impressively, 46% of the melanoma inoculated mice treated with IL-21/IL-2 exhibited complete tumor regression. This was attributed to higher circulating CD8⁺ T cells of both memory and effector phenotypes [113]. These data reiterated the importance the addition of IL-21 may have in an immunotherapy regimen in generating an appropriate levels and subtype of lymphocytes.

Clinical—Initial trials involving IL-21 in 24 melanoma patients indicated mild to moderate adverse effects with one patient experiencing complete remission, 11 stable diseases, and 12 patients with progressive disease. Of the 19 renal carcinoma patients, an overall disease control rate of 89% was achieved [114]. IL-21 is overexpressed in the gut mucosa of patients with ulcerative colitis (UC) that amplifies inflammatory conditions. Therefore, blockade of IL-21 may be beneficial to prevent UC-associated colon carcinoma [115]. Recently, a phase I/II study in renal cell carcinoma involving rIL-21 and sorafenib demonstrated tolerance with low toxicities. Antitumor effects were observed in 21% of the recipients in whom disease control was observed in 82% [116]. A trial in stage IV melanoma indicates IL-21 may regulate NKT cells. Despite constitutively low expression of NKT cells in these patients, IL-21 possibly enhanced cytotoxicity [117]. These findings suggest differential effects of IL-21 in various conditions may limit its use in the treatment of specific cancers.

4. Use of γ Chain Cytokines in Hematopoietic Cancer

4.1. IL-2

Preclinical—Unlike solid tumors, the presence of γ C cytokines may promote the progression of many hematopoietic cancers. In theory, suppressing the biological effects of γ C cytokines inhibits the propagation of potential leukemic lymphocytes. Yet, studies indicate γ C cytokines have contradictory effects in hematopoietic disorders. IL-2 in

combination with cytarabine has shown anti-leukemic activity in mice models with acute myeloid leukemia (AML). Specifically, combinatorial therapy of F16-IL2 with cytarabine in AML relapse patients after allo-hematopoietic stem cell transplantation (HSCT) displayed favorable results including blast reduction, transient remission, and immune cell infiltration with manageable side effects. The use of this combination extends the life of the patient; however, it does not provide a cure for relapsed AML [118]. Mutations with the IL-2 “superkine” that weakens the binding to the γC prolonged survival in a model of graft-versus-host disease and blocked spontaneous proliferation of smoldering/chronic adult T cell leukemia [79]. Further studies are warranted to expand combinatorial use of IL-2 with other chemotherapeutics for leukemias.

Clinical—Early clinical trials with IL-2 in AML patients were unsuccessful in retaining remission [119]. Analysis of cytokine production in newly diagnosed AML and acute lymphoblastic leukemia (ALL) showed a significant increase in circulating levels of IL-2 and IL-4 in AML patients when compared to ALL patients [120]. Due to high toxicity of IL-2 and the risk of capillary leak syndrome, regulating the IL-2R in hematological malignancies may be a better option. CD25 (IL-2R α) is expressed in 13% of AML patients and may be a predictive marker for risk assessment of AML [121,122]. Additionally, CD25 expression was significantly higher in adult and pediatric precursor B acute lymphoblastic leukemia (B-ALL) patients with the Philadelphia (Ph) chromosome translocation t(9;22) compared to Ph⁻ B-ALL [123–125]. CD25 expression is proving to be a specific and relatively sensitive marker for the identification of Ph⁺ B-ALL. In phase I/II trials, high doses (up to 8 mg/kg) of anti-CD25 (daclizumab) were well tolerated in human T-cell leukemia virus 1 associated adult T-cell leukemia/lymphoma (ATL) and induced a partial response in a subset of patients with smoldering/chronic ATL [126]. From these data, it appears IL-2 is not a plausible immunotherapeutic for leukemia but it may be a viable target and potential biomarker for diagnosis.

4.2. IL-7

Preclinical—Since leukemia such as AML promotes an immunosuppressive microenvironment [127,128], the ability of IL-7 to inhibit tumor-induced suppressive activity of CD27^{low} and CD28^{low} T cells may be beneficial [47]. However, other studies indicate that IL-7 promotes human T cell acute lymphoblastic leukemia (T-ALL) proliferation in a xenotransplant mouse model by inducing p27Kip1 downregulation and Bcl-2 upregulation [129]. As these studies indicate, IL-7 displays pleiotropic characteristics in cancer.

Mutations in the IL-7R α result in gain-of-function mutations that act as drivers of pediatric T-ALL [130]; however, this is not observed in adults [131]. This mutation is associated with an in-frame insert creating unpaired cysteine residues that, in turn, form a disulfide bridge leading to ligand-independent homodimerization. A recent study showed that the FDA-approved reducing agent, N-acetylcysteine, was able to reduce disulfide bonding and downstream IL-7R-mediated leukemogenic signaling in T cell leukemia cell line, DND-41. Treatment with N-acetylcysteine also significantly inhibited the progression of DND-41

cells engrafted in immunodeficient mice [132]. This reiterates the need to revisit approved drugs for alternative applications.

Clinical—Since IL-7 signaling results in developing lymphocytes of both the B cell and T cell lineages, IL-7 could potentially serve as a stimulant for lymphoid malignancies. IL-7 and thymic stromal-derived lymphopoietin (TSLP) share the IL-7R α . In patient samples diagnosed with ALL, TSLP is able to stimulate proliferation and overcome the effects of mTOR inhibitors that induce anti-tumor effects [133].

4.3. IL-15

Preclinical—Transgenic C57BL/6 mice with a chronic overexpression of IL-15 (100–150 pg/mL and 600–800 pg/mL of serum IL-15) caused fatal leukemic transformation exhibited by the IL-15R α -dependent expansion of CD8 $^{+}$ T cell [134]. Overexpression is also found in ALL, and ATL [135] suggesting that IL-15 may play a role in hematopoietic pathogenesis. On the other hand, IL-15 may promote cytokine-induced killer cells (CIK) by increasing the number of CD3 $^{+}$ CD56 $^{+}$ NKT cells while decreasing Treg cells and immunosuppressive cytokine IL-35 in K562 myeloid leukemia cells. The IL-15-induced CIK were more potent at creating the cytotoxic environment than IL-2-induced CIK implying IL-15 use is more favorable than IL-2 [136]. Furthermore, due to IL-15 induction of NKG2D receptor on NK and CD8 $^{+}$ T cells [137], it is believed that IL-15 may increase NK cell-mediated cytotoxicity toward cancer cells [138].

Aberrant histone deacetylase (HDAC) activity has been observed in acute leukemia [139]; thus sparking the interest of HDAC inhibitors as cancer therapy [140]. Some in the field speculate IL-15 may contribute to the effects of HDAC inhibitors in acute leukemias. The author hypothesized the reported anticancer effect of HDAC inhibitors is partially due to the modulation of IL-15 and IL-15R α . IL-15, in turn, modulates the expression of cell surface and soluble receptors of major histocompatibility complex class I-related chain A/B that regulates cytotoxic activity of NK and CD8 $^{+}$ T cells [141]. This premise is still yet to be determined.

Clinical—In a phase 1 trial in patients with T cell large granular lymphocytic leukemia, the use of a human monoclonal antibody that targets the shared IL-2/IL-15R β subunit, Hu-Mik β 1, was able to block the trans-presentation of IL-15 to the heterodimeric IL-15 receptor (IL-15R β / γ C), but it did not block the response of IL-15 in cells that expressed the heterotrimeric IL-15 receptor (IL-15R α /IL-15R β / γ C). All nine patients showed stable disease without reduction in the quantity of peripheral leukemic cells. However, three patients did display a transient increase in neutrophils [142]. However, IL-15-activated peripheral blood mononuclear cells or NK cells from health donors enhance rituximab-dependent toxicity against leukemic cells from CLL patients mainly mediated by NK cells [143]. These results galvanized the controversy surrounding IL-15 as an immunotherapeutic.

4.4. IL-21

Preclinical—As the most recently discovered γ C cytokines, exploration of IL-21 in leukemia has gained considerable interest. IL-21R is expressed in myeloid and lymphoid

cells having pro- or anti-apoptotic effects depending on level of differentiation and other contributing factors. For instance, IL-21 stimulates apoptosis in CLL cells via B-cell lymphoma 2 interacting mediator of cell death, BIM [144] and granzyme B production [145]. However, other studies indicate IL-21 in combination with IL-4 promote proliferation of CLL and IL-21 is elevated in chemotherapy-resistant populations [146]. The growth-stimulating role of IL-21 is evident in other hematological disorders such as multiple myeloma and Hodgkin's lymphoma [67].

Yet, studies have indicated IL-15 and IL-21 synergistically increase the expression and function of CD8⁺ T [147] and NK cells [148] resulting in tumor regression. In BALB/c mice injected with RLmale1 mouse lymphoma cell line, transfection with genes for IL-15 and IL-21 attained complete regression and elimination of metastatic foci. This was seen in 80% of the treated mice. The cooperative effect of IL-15 and IL-21 also augmented NK activity [149]. These preclinical studies upheld IL-21 as a viable candidate for clinical trials.

Clinical—Pascutti et al. demonstrated that T cells and B cell signaling in patients with CLL stimulate leukemic proliferation with the assistance of IL-21 [150]. Sun et al. showed that alterations in the cytokine profile of a newly diagnosed AML patient significantly differ from an AML patient in complete remission except IL-21 and IL-35. Increased levels of IL-21 correlated with clinical observations such as increase in white blood cells, neutrophils, and serum LDH levels [151]. Despite these finding a phase I trial with rIL-21 and rituximab in relapsed B-cell small lymphocytic lymphoma/chronic lymphocytic leukemia achieved a 42% response rate in 19 patients displaying a reduction in tumor burden. This study demonstrates that in 33% of patients presenting rituximab-resistance, IL-21 may have contributed to the response rate [152]. Contradicting results of IL-21 in clinical and preclinical trials still questions the value of this cytokine as a potential therapy in hematopoietic malignancies prompting continuing studies.

5. Potential Limitations in the Use of γ Chain Cytokines in Immunotherapy

Patients treated with high-dose IL-2 displayed clinical manifestations of capillary leak syndrome [153], hypotension, nausea, vomiting, septic shock and death [154]. Therefore, low dose IL-2 regimen may be adequate if used in combination with other anti-tumor agents. However, a phase III randomized trial showed that chemotherapy with low-dose IL-2 (3,000,000 IU/day) failed to provide significant improvement in advanced non-small-cell lung carcinoma patients compared to chemotherapy alone [155]. With varying response rates in solid tumors to IL-2, and with an increased risk of toxicity, there is a need for exploring the efficacy of other cytokines, in combination with other therapies. Studies have indicated that the response of renal cell carcinoma cancer patients to IL-2 is decreased if the patient is, for example, exposed to other forms of therapy prior to IL-2, suggesting that IL-2 is most effective as the front-line treatment [156]. To further complicate its use, another dominant side effect of IL-2 therapy is fever. Interestingly, a recent study by Kostner et al. demonstrated that melanoma patients treated with IL-2 and IFN α that displayed a peak temperature of 39.5°C achieved an overall survival of 15.2 months in comparison to patients below 39.5°C with a survival of 8.7 months. These data indicate that fever may be a prognostic biomarker for survival in IL-2/IFN α treated patients with melanoma [157].

Despite the enormous advances IL-2 has made in advancing immunotherapeutic options, its use is often hampered in determining which patients will respond to IL-2 or how to alter the therapy to optimize antitumor activity. Thus, biomarkers to identify which patient population may benefit more from IL-2 therapy should be pursued.

Many of the drawbacks of IL-2 are evident in other γ C cytokines as well. IL-2, IL-7, IL-15, and IL-21 expand distinct T cell sub-populations and to varying magnitudes; this may limit their antitumor potency. Studies have indicated that IL-15 may enhance cell motility in some cancers as well serve as an angiogenic factor and powerful anti-apoptotic factor; thus posing concerns on its antitumor effects. Additionally, IL-15 induces IL-10, an immunosuppressive cytokine [94] that may impact lymphocyte response to the tumor. IL-15 fusion proteins that target the cancer cells are showing some favorable preclinical outcomes, thus galvanizing the necessity of combinatorial therapy with IL-15. Like other γ C cytokines, IL-21 is associated with dose-limiting side effects such as granulocytopenia and liver toxicities [158]. Its ability to stimulate proinflammatory cytokines such as IL-6 and IL-17 that promote tumor growth may limit its clinical use [159].

6. Current Clinical Trials Involving γ C Cytokines

Despite the limitations of cytokine therapy discussed above, the Cancer Immunotherapy Trials Network categorizes T cell growth factors (IL-7, -15, and -21) as priority agents for study among top academic immunologists due to their promising potential in treating cancer. This exemplifies the critical contributions of common γ C cytokines in immunotherapy. IL-2, IL-7, IL-15, and IL-21 are currently in clinical trials for the treatment of various cancers as summarized in Table 1 (clinicaltrials.gov).

Due to FDA approval, IL-2 is heavily utilized as combinational therapy with various chemotherapeutic drugs and lymphocyte transfer therapies. A simple search of IL-2 and cancer in clinicaltrials.gov rendered 600 studies. These trials incorporate high and low doses of IL-2, before, after and during chemotherapy treatment in various cancers indicating optimization of its use in different cancers under distinct therapeutic regimens. The use of high dose IL-2 (720,000 IU/kg) and NK cell infusion following chemotherapy did not result in an objective response in 7 melanoma and 1 renal cell carcinoma patients (NCT00328861). Despite the lack of adverse effects, the patient population is too low to establish conclusions concerning this therapeutic regimen. The initial outcome of an ongoing phase II trial utilizing chemotherapy followed by a booster vaccine and low dose IL-2 in patients with mantle cell lymphoma that were stable or responding disease 12 months post treatment indicate 15 of the 23 had an increase in IFN- γ secretion with no severe adverse effects. Event free survival was extended in 9 patients between 5 to 18 months. The overall survival rate is still to be determined (NCT00101101).

One trial targeting Ewing's sarcoma, rhabdomyosarcoma and neuroblastoma in which patients were treated with cyclophosphamide and fludarabine versus IL-7, is currently suspended (NCT00923351). Both groups underwent DC vaccination and lymphocyte depletion. However, the clinicians noted out of the 13 patients treated with IL-7, 6 had evidence of a positive immune response and less serious adverse event compared to the DC

vaccinated group. Due to the low patient population and other factors leading to early termination of the study, no clear conclusions can be made but it does provide hope for therapy for rare cancers. Although current IL-7 trials in cancer are few, the role of IL-7 in HIV/AIDS progression is gaining recognition as a potential therapy [160–162].

A study utilizing combinational therapy with IL-2, lymphocyte transfer, rituximab and chemotherapy in patients with relapsed Non-Hodgkin lymphoma and CLL investigated whether tumor-infiltrating NK cells were stimulated during treatment. Of the six patients that participated in the clinical trial, none displayed circulating NK cell count greater than 100 cells/ μ L. However, 4 patients presented an overall response to therapy. Only half of those patients who initially responded to treatment experienced disease progression within 6 months of therapy (NCT00625729). This study was also prematurely terminated due to the lack of NK cell expansion. A majority of IL-15 trials involving hematopoietic malignancies occurs in combination with NK cells capitalizing on the belief that IL-15 increases tumor-specific NK cell-mediated cytotoxicity [138]. A clinical trial (NCT01369888) for IL-15 therapy of metastatic melanoma patients following chemotherapy and lymphocyte transfer was terminated due to severe autoimmune reaction. However, this trial only consisted of 3 patients.

For IL-21, clinicaltrials.gov have an impressive quantity of completed studies involving IL-21 in combinational therapy for a wide array of cancers including ovarian, renal, breast carcinomas, melanoma, and leukemia. A Phase II study of IL-21 versus dacarbazine in patients with melanoma (NCT01152788) showed dacarbazine extended survival, the response rate, and decreased severe adverse events better than IL-21. Collectively, these clinical trials demonstrate the progress and setbacks posed by the use of γ C cytokines to treat cancer. They also render hope for those who suffer from these devastating diseases.

7. Concluding Remarks

Due to the ability of γ C cytokines to impact T cell, NK cell, and macrophage development and function, they present hope to patients with immunodepletion following harsh chemotherapy treatment. However, pleiotropic responses, redundancy of cytokine signaling, and the two-edged function of many cytokines to induce immune activation as well as promote cancer growth reiterate the need of exploring the complexity of cytokines as a therapeutic option. Discerning the roles of cytokines in the regulation of tumors and development of anti-tumor immune response is critical to the development of effective immunotherapeutic strategies against cancer.

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Biographies

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HIGHLIGHTS

- γ C cytokines are essential for the normal biological function of the immune system.
- γ C cytokines in the tumor-bearing host influence antitumor lymphocyte functions.
- Antitumor effects of γ C cytokines impact both solid and hematological tumors.
- γ C cytokines may synergistically increase the effectiveness of cancer therapies.
- Clinical trials are currently ongoing for all γ C cytokines in combinatorial therapies.

Figure 1. History of γ Chain Cytokine Discovery and Induction into Clinical Trials. This timeline details key historical events in the discovery of common γ chain cytokines and their development for use in clinical applications; IL: interleukin; FDA: Food and Drug Administration.

A.

B.

C.

D.

Figure 2.
Photos of Authors. A) Stephanie R. Pulliam, B) Dr. Roman V. Uzhachenko, C) Dr. Samuel E. Adunyah, D) Dr. Anil Shanker

Table 1

Ongoing Clinical Trials of γ Chain Cytokines[†]

Cytokine	Combinational Therapy	Phase	Cancer type	Clinical Identifier Number	Estimated Completion (mth-yr)
IL-2*	F16-IL2 and Paclitaxel	Ib/II	Solid Tumors Ovarian, Fallopian Tube or Primary	NCT01134250	May-16
	TIL and Low Dose IL-2	I	Peritoneal Cancer	NCT01883297	Dec-23
	Ipilimumab, ACT, TIL, and High Dose IL-2	I	Melanoma	NCT01701674	Dec-15
	Autologous IL-2, CD40+ Tumor Cells, Lenalidomide	I/II	B-cell Chronic Lymphoblastic Leukemia	NCT01604031	Jun-19
	TIL with IL-2	II	Metastatic Melanoma	NCT02360579	Sep-19
	TIL, Low Dose IL-2, Cyclophosphamide and Fludarabine	II	Melanoma	NCT01883323	Dec-23
	Stereotactic Ablative Radiation and High Dose IL-2	II	Metastatic Renal Carcinoma	NCT01896271	Dec-17
	Cyclophosphamide, Fludarabine, IL-2, TIL	I/II	Pleural Mesothelioma	NCT02414945	Nov-25
	IL-2 with Various Chemotherapeutics	I	Solid Tumors or Lymphomas	NCT02130869	Sep-20
	PSA/IL-2/GM-CSF Vaccine	Ia/Ib	Recurrent Prostate Cancer	NCT02058680	Dec-15
IL-7	Vemurafenib, ACT, TIL, High Dose IL-2	II	Metastatic Melanoma	NCT01659151	Aug-16
	Hu3F8 and IL-2	I	Neuroblastoma and GD2 ⁺ Solid Tumors	NCT01662804	Aug-15
	L-19-IL2 and Dacabazine	I/II	Metastatic Melanoma	NCT02076646	Aug-16
	Stereotactic Ablative Radiation and L19-IL2	II	Oligometastatic Solid Tumors	NCT02086721	Oct-16
	High Dose IL-2 and SBRT	II	Metastatic Renal Cancer	NCT02306954	Jun-25
	Sipuleucel-T with CYT107	II	Castration-Resistant Prostate Cancer	NCT01881867	Unknown
	Recombinant IL-7	II	Post Chemotherapy Solid Tumors	NCT01339000	Apr-17
	IL-15 with NK cells	II	Acute Myeloid Leukemia	NCT02395822	Jan-19
	Continuous Infusion of rhIL-15	I	Advanced Cancer	NCT01572493	Apr-16
	IL-15 with Haploidentical Donor NK Cells	I	Acute Myeloid Leukemia	NCT01385423	Oct-15
IL-15	Rituximab with ALT-803	I/II	B Cell Non-Hodgkin Lymphoma	NCT02384954	Jun-23
	IL-15 with NK Cells	I	Refractory Solid Tumors	NCT01875601	Nov-22
	Bacillus Calmette-Gerin with ALT-803	Ib/II	Non-Muscle Invasive Bladder Cancer	NCT02138734	Oct-18
	IL-21 Expanded NK cells, Cytatbine, Fludarabine	I/II	Acute Myeloid Leukemia	NCT01787474	Unknown

[†] Data collected (May 28, 2015) from clinicaltrials.gov using cancer and respective interleukin as the search phrase in recruiting status or open studies only.

* Due to FDA approval, IL-2 is utilized heavily in clinical trials resulting in 451 search results. Listed is only a representation of the diversity.

ACT: adoptive cell transfer; TIL: tumor infiltrating lymphocytes; CYT107: glycosylated IL-7; ALT-803: recombinant IL-15 complex; SBRT: Stereotactic Body Radiation