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REVIEW

Cytokines Focus

Effects of interleukin-2 in immunostimulation and immunosuppression

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Historically, interleukin-2 (IL-2) was first described as an immunostimulatory factor that supports the expansion of activated effector T cells. A layer of sophistication arose when regulatory CD4⁺ T lymphocytes (Tregs) were shown to require IL-2 for their development, homeostasis, and immunosuppressive functions. Fundamental distinctions in the nature and spatiotemporal expression patterns of IL-2 receptor subunits on naive/memory/effector T cells versus Tregs are now being exploited to manipulate the immunomodulatory effects of IL-2 for therapeutic purposes. Although high-dose IL-2 administration has yielded discrete clinical responses, low-dose IL-2 as well as innovative strategies based on IL-2 derivatives, including "muteins," immunocomplexes, and immunocytokines, are being explored to therapeutically enhance or inhibit the immune response.

Background

Clues supporting the existence of IL-2 emerged in 1976. That year, after successfully expanding T cells in vitro, Morgan et al. (1976) reported the presence of a T cell growth factor in the conditioned media of phytohemagglutinin-stimulated blood lymphocytes. In the early 1980s, human IL-2 was firmly identified as a variably glycosylated ∼15.5-kD protein (Robb and Smith, 1981), thereafter purified (Smith et al., 1983), and finally cloned (Taniguchi et al., 1983). At the same period, the IL-2 receptor (IL-2R) was discovered (Kuribayashi et al., 1981; Robb et al., 1981), thus solving the first type I cytokine/receptor complex.

By permitting a prolonged culture of T cells, the discovery of IL-2, initially called "T cell growth factor," facilitated molecular and cellular investigations that precipitated, for example, the characterization of the TCR and its function (Allison et al., 1982; Haskins et al., 1983), or the identification of the first human retrovirus: human T cell leukemia virus (HTLV-1; Poiesz et al., 1980). Initial studies performed in vitro concluded to a critical role of IL-2 in the development of effector T lymphocytes. Moreover, experimental investigations conducted in a chicken model of autoimmune thyroiditis revealed a pro-autoimmune effect of IL-2 and IL-2R–expressing T lymphocytes (Krömer et al., 1985), an observation that was mechanistically explained by the capacity of IL-2 to reverse anergy of self-reactive T cells in mice (Gonzalo et al., 1993) and simultaneously validated by clinical studies in humans showing that cancer patients treated with high-dose (HD) IL-2 frequently developed autoimmune thyroiditis (Krouse et al., 1995).

However, in vivo studies conducted in the 1990s in mouse strains lacking IL-2 or IL-2R subunits led to a revision of the concept that the IL-2/IL-2R system would be solely involved in immunostimulatory circuities. Indeed, rather than harboring an immunodeficiency, these animals demonstrated lymphadenopathy, uncontrolled proliferation of peripheral activated T cells, and signs of autoimmunity (Sadlack et al., 1993; Suzuki et al., 1995; Willerford et al., 1995). Such observations unveiled the existence of immunosuppressive mechanisms critically relying on IL-2 and later attributed to regulatory CD4⁺ T cells (Tregs; Sakaguchi et al., 1995; Malek et al., 2000, 2002).

The immunomodulatory effects of IL-2, mainly on effector and regulatory T lymphocytes, have been exploited for treating various pathologies, though with limited clinical benefits so far. In this line, a recombinant human IL-2 called aldesleukin (brand name: Proleukin) was approved for the treatment of kidney cancer and melanoma as early as 1992 and 1998, respectively (Alva et al., 2016).

After introducing some fundamental aspects of IL-2 biology, the present review will summarize current strategies to introduce IL-2 into the immunotherapeutic armamentarium.

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Biology of IL-2

TCR signaling and IL-2 production

IL-2 is mainly produced by $CD4^+$ T lymphocytes (naive, memory, and T helper [Th] 1) following antigenic stimulation, by type 2 and 3 innate lymphoid cells in the small intestine, and to a lesser extent by activated $CD8^+$ T cells, B cells, and by other innate immune entities such as natural killer (NK) and NKT lymphocytes, dendritic cells (DCs), monocytes, or mast cells (Malek, 2008; Wojciechowski et al., 2009; Hershko et al., 2011; Zelante et al., 2012; Zhou et al., 2019). In naive T lymphocytes, the engagement of the TCR and co-stimulatory molecules (e.g., CD28) within an immunological synapse activates activator protein 1 (AP-1), NFκB, and NFAT (Fig. 1). In cooperation with constitutive factors, these transcription factors promote the expression of the *IL2* gene (Serfling et al., 1995). *IL2* transcription occurs within 30 min after stimulation but is transient, declining to background levels within 24–48 h. Additionally, post-transcriptional regulatory mechanisms further restrict the availability of IL-2 mRNAs, the levels of which usually peak at 4–8 h after stimulation (Jain et al., 1995). The turnover of IL-2 mRNAs is mostly controlled by proteins interacting with an AU-rich cis element (ARE) in their 3′-untranslated region. Among these trans-acting factors figure nuclear factor 90 (NF90) and tristetraprolin. NF90 is activated by protein kinase (PK) B (best known as AKT) upon CD28 co-stimulation, or by PKC upon re-stimulation with PMA, and then exported from the nucleus to the cytosol. There, NF90 binds to ARE and stabilizes IL-2 mRNAs, thus allowing their translation (Pei et al., 2008; Zhu et al., 2010). In contrast, tristetraprolin is expressed in T lymphocytes following activation and plays a critical role in the rapid decay of IL-2 mRNAs, as its interaction with ARE promotes its degradation (Ogilvie et al., 2005; Yang et al., 2015). Altogether, these transcriptional and post-transcriptional mechanisms control the magnitude and duration of IL-2 production by activated T cells.

IL-2R signaling

Once secreted, IL-2 is consumed in an autocrine/paracrine manner by neighboring cells that harbor its receptor, IL-2R (Fig. 2). The latter consists of a hetero-complex of up to three subunits: α, β, and γ, also known as CD25, CD122, and CD132, respectively. Although each receptor subunit may independently bind IL-2 with a weak affinity (Kd: ∼10^{-8}–10^{-7} M), only the intermediate-affinity βγ dimeric (Kd: ∼10^{-9} M) and the strong-affinity αβγ trimeric IL-2R (Kd: ∼10^{-11} M) mediate intracellular signal transduction (Flynn and Hartley, 2017). The γ subunit is ubiquitously expressed on most hematopoietic cells. It is also referred to as the "common" chain (labeled "γ_c"), as it is shared with the receptors for IL-4, -7, -9, -15, and -21. The β subunit appears constitutively expressed at various levels on T and NK(T) lymphocytes. In contrast, the α chain/CD25 is detected on early thymocytes, absent on naive/memory T cells (particularly in mice, but detectable on a minor fraction of human naive/memory T cells), transiently exposed on activated/effector T lymphocytes, and preferentially/more stably expressed on Tregs (Kmieciak et al., 2009; Flynn and Hartley, 2017). Within the myeloid compartment, monocytes display the intermediate-affinity βγ receptor, whereas DC subtypes can present the three subunits of IL-2R (Bosco et al., 2000; Herr et al., 2014; Kitashima et al., 2018). Interestingly, DCs may also supply the α chain in trans, thus supporting high-affinity binding of IL-2 to naive T lymphocytes that undergo antigen priming (Wuest et al., 2011). Additionally, some non-hematopoietic cell types may harbor either (i) dimeric βγ receptors, as this applies to intestinal epithelial cells, dermal fibroblasts, or fibroblast-like synoviocytes; or (ii) the high-affinity αβγ IL-2R, as reported for endothelial cells, proximal tubular epithelial cells, or gingival fibroblasts. As an aside, IL-2R expression has also been documented for malignant cells such as melanoma and cervical tumor cells (Valle-Mendiola et al., 2016).

Binding of IL-2 to IL-2R activates JAK1/3. In turn, these kinases ignite the phosphoinositide-3-kinases (PI3Ks)/phosphatidylinositol 3,4,5-trisphosphate (PIP3)/AKT/mechanistic target of rapamycin (mTOR)/$p70^{S6K}$, and Ras/Raf/mitogen-activated protein kinase kinases 1 and 2 (MAP2K1/2, also known as MEK1/2)/extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling cascades and phosphorylate STAT5. Activation of mTOR, $p70^{S6K}$, AKT, and ERK1/2 modulates the activity and de novo expression of multiple downstream regulators involved in protein synthesis, autophagy, cell metabolism, survival, proliferation, and differentiation. Concomitantly, activated STAT5 determines the fate of the cell by transactivating numerous target genes (Fig. 2). While these three signaling cascades are stimulated in effector T cells, it is important to note that the STAT5 pathway is predominantly triggered in Tregs (Cheng et al., 2011). In T lymphocytes, depending on their subtype, STAT5-stimulated genes can encode the following: various cytokine receptors including IL-2Rα and IL-2Rβ (positive feedback), IL-4Rα or IL-12Rβ; diverse proteins involved in cell proliferation and survival (e.g., PIM1, MYC, cyclins, and B cell lymphoma (BCL) 2); some effector molecules such as granzyme B, CD178 (best known as Fas-ligand), and some cytokines like IFN-γ, TNF-α, or IL-4; and some regulators of immune cell functions such as the suppressor of cytokine signaling 1 and 2 (SOCS1/2), and the transcription factor forkhead box P3 (FOXP3). IL-2-activated STAT5 also represses the expression of particular effector genes such as *IL17A* or *BCL6* (Kovanen et al., 2005; Grange et al., 2013; Knosp et al., 2013; Kanai et al., 2014; Ross and Cantrell, 2018). Phenotypic changes consecutive to IL-2 stimulation are described in the following paragraph.

Pleiotropic action of IL-2

IL-2 is a pleiotropic cytokine with immunostimulatory or immunoinhibitory activity depending on the target cell. Its effects on nonhematopoietic and innate immune cells remain poorly deciphered (Valle-Mendiola et al., 2016). Above all, IL-2 stands out as a well-established regulator of T cell development and homeostasis.

$CD4^+FOXP3^+CD25^+$ Tregs are responsible for maintaining immunological self-tolerance and for down-regulating inflammatory and adaptive immune responses. As opposed to conventional T cells (Tconv cells), Tregs do not produce IL-2 but critically rely on its presence for their differentiation from immature single-positive $CD4^+$ T cells in the thymus (referred to as

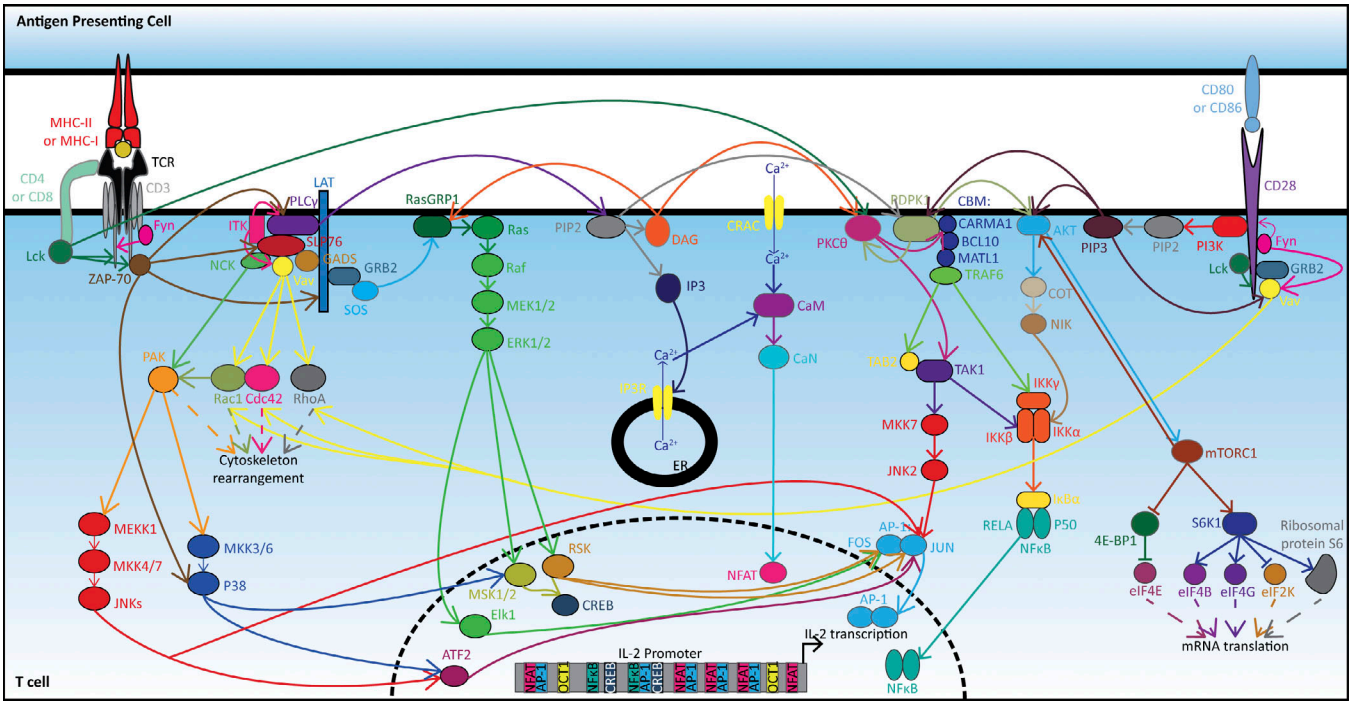

Figure 1. **IL-2 transcription following stimulation of the TCR and CD28 signaling cascades.** Interactions between the CD4 (or CD8)/TCR complex at the surface of a T lymphocyte and an MHC-II (or MHC-I) molecule coupled to a cognate antigen at the surface of an APC triggers several intracellular signaling pathways leading to IL-2 production. Precisely, upon CD4/CD8 binding to MHC, lymphocyte-specific protein tyrosine kinase (Lck) is carried to the TCR proximity and is autophosphorylated and activated. Lck can phosphorylate CD3, which recruits the protein zeta-chain–associated protein kinase 70 (ZAP-70). ZAP-70 undergoes trans-autophosphorylation, leading to its activation, and phosphorylates the linker for the activation of T cells (LAT). Then, LAT plays as an adaptor protein supplying docking sites for: (i) growth factor receptor-bound protein 2 (GRB2); (ii) GRB2-related adaptor protein 2 (also known as GADS); and (iii) phospholipase C gamma1 (PLCγ). GRB2 binds to the son of sevenless protein (SOS), which activates Ras guanyl-releasing protein 1 (RasGRP1). RasGRP1 promotes the release of guanosine diphosphate (GDP) and the binding of guanosine triphosphate (GTP) on membrane-associated protein Ras, a small GTPase. GTP-bound Ras recruits the rapidly accelerated fibrosarcoma protein (Raf) and promotes its dimerization and activation by autophosphorylation. Activated Raf recruits and phosphorylates the mitogen-activated protein kinase kinases 1 and 2 (best known as MEK1/2), which in turn phosphorylate ERK1/2. Phosphorylated ERK1/2 translocates to the nucleus and activates ETS Like-1 protein (Elk1), mitogen and stress activated protein kinases 1 and 2 (MSK1/2), and p90 ribosomal S6 kinases (RSKs). The transcription factor Elk1, together with RSKs, promotes the expression of the gene *FOS*, while MSK1/2 activates c-AMP response element-binding protein (CREB). RSKs also phosphorylate JUN, enhancing the transcriptional activity of AP-1, which is constituted of a heterodimer of JUN and FOS. Both AP-1 and CREB translocate to the nucleus, bind to promoter of the *IL2* gene, and stimulate its transcription. SH2 domain containing leukocyte protein of 76 kD (SLP-76) is recruited to GADS and phosphorylated by ZAP-70. Phosphorylated SLP-76 recruits PLCγ, the noncatalytic region of tyrosine kinase (NCK) adaptor protein, and the proteins Vav and interleukin-2-inducible T cell kinase (ITK). Fyn phosphorylates Vav, a guanine nucleotide exchange factor, which can activate Ras-related C3 botulinum toxin substrate 1 (Rac1), cell division control protein 42 homologue (Cdc42), and Ras homologue gene family, member A (RhoA), which are involved in cytoskeleton rearrangement. NCK recruits the p21-activated serine/threonine kinase (PAK) at the membrane, and the Cdc42/Rac1 complex activates PAK by disrupting PAK homodimerization and allowing its autophosphorylation. This leads to the activation of mitogen-activated protein kinase kinase kinase 1 (MEKK1) and dual specificity mitogen-activated protein kinase kinases (MKKs) 3 and 6 (MKK3/6). MEKK1 can further phosphorylate MKK4/7, and activated MKK4/7 activates in turn JNKs. Then, JNKs phosphorylate JUN leading to an enhanced transcriptional activity of the AP-1 complex. JNKs also activate the activating transcription factor 2 (ATF2) that phosphorylates and promotes the transcription of JUN. MKK3/6 phosphorylates P38 mitogen-activated kinases, which further activate ATF2 and MSK1/2. The recruitment of PLCγ by SLP-76 leads to its phosphorylation by ITK and ZAP-70. PLCγ detaches from SLP-76, re-translocates to the plasma membrane, binds to phosphatidylinositol 4,5-bisphosphate (PIP2), and hydrolyses PIP2 to produce diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). IP3 binds to its receptor, IP3 receptor (IP3R), leading to the release of Ca^{2+} from ER, which activates calmodulin (CaM). This cytosolic influx of Ca^{2+} from ER stores ignites a prolonged opening of Ca^{2+} release–activated Ca^{2+} channels (CRAC) located at the plasma membrane, a requirement for sustaining T cell functions. Then, CaM binds to calcineurin (CaN), leading to its activation. CaN dephosphorylates NFAT, which can translocate into the nucleus. In the meantime, DAG can recruit to the plasma membrane and activate RasGRP1, leading to the downstream signaling pathway of Ras/Raf. Additionally, DAG also recruits the protein kinase C theta (PKCθ), leading to a conformational change and its phosphorylation by Lck. Simultaneously, the 3-phosphoinositide-dependent protein kinase 1 (PDPK1) is translocated to the membrane by binding to PIP2 or PIP3. Then, PDPK1 binds to caspase recruitment domain-containing protein 11 (CARMA1) and phosphorylates PKCθ; the latter autophosphorylates to achieve full activation. PKCθ phosphorylates CARMA1, which undergoes oligomerization and recruits BCL10. Afterwards, BCL10 oligomerizes and is phosphorylated by the receptor-interacting serine/threonine-protein kinase 2, leading to the recruitment of the mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MATL1). In turn, MATL1 oligomerizes and binds to the TNF receptor-associated factor 6 (TRAF6). Then follows TRAF6 oligomerization, activation of its ubiquitin ligase activity, and auto-polyubiquitination. Ubiquitinated TRAF6 recruits the mitogen-activated protein kinase kinase kinase 7-interacting protein 2 (TAB2), which binds the mitogen-activated protein kinase kinase kinase 7 (best known as the transforming growth factor β-activated kinase 1 [TAK1]). The complex TAB2/TAK1 is ubiquitinated by TRAF6, and TAK1 undergoes autophosphorylation. Then, TAK1 phosphorylates MKK7, which activates JNK2. Then follows the JNK2-mediated phosphorylation of JUN, which associates with FOS to form the AP-1 complex. Activated TAK1 also phosphorylates NFκB inhibitor kinase subunit β (IKKβ), while TRAF6 ubiquitinates the γ subunit IKKγ (also known as NFκB essential modifier). Simultaneously, PDPK1 phosphorylates PKB (best known as AKT), allowing the latter to activate the mitogen-activated protein kinase kinase kinase 8 (also known as cancer osaka thyroid oncogene [COT]).

COT activates the NFκB-inducing kinase (NIK, also known as MAP3K14), which phosphorylates IKKα. It results in an activated IKK complex composed of IKKα/β/γ. The NFκB inhibitor α (best known as IκBα), which otherwise sequesters the transcription factor NFκB in the cytoplasm, is phosphorylated by the IKK complex and then undergoes ubiquitination and degradation. The released NFκB, a heterodimer constituted of p50 (also known as NFκB subunit 1) and p65 (also known as v-Rel avian reticuloendotheliosis viral oncogene homologue A [RELA]), can therefore translocate to the nucleus. Ultimately, together with constitutive transcription factors like the octamer-binding protein 1 (OCT1, also known as POU domain class 2 transcription factor 1), the nuclear translocation of AP-1, NFκB, NFAT, and CREB, which all dispose of cis-regulatory elements within the promoter of the *IL2* gene, will initiate its transcription. Moreover, AKT phosphorylates mTOR within the mTORC1 complex, composed of Raptor, proline-rich AKT substrate of 40 kD, DEP domain-containing mTOR-interacting protein, and mammalian lethal with SEC13 protein 8. mTORC1 can phosphorylate the eukaryotic translation initiation factor (eIF) 4E-binding protein 1 (4E-BP1), thus abrogating its inhibitory sequestration of eIF4E. mTORC1 also activates the ribosomal protein S6 kinase β-1 (S6K1). S6K1 phosphorylates and activates thereafter the ribosomal protein s6 as well as the translation initiation factors eIF4B and eIF4G, while inactivating eIF2K by phosphorylation. Altogether, these events up-regulate mRNA translation. Concurrent with the activation of the TCR signaling, a co-stimulatory signal, consecutive to the interaction of B7-1 (CD80) or B7-2 (CD86) on an APC with CD28 on the T cell, is triggered. The intracellular downstream signaling pathway starts with the phosphorylation of CD28 by Lck and Fyn, allowing the recruitment to CD28 of PI3Ks, GRB2, and GADS. PI3K can further phosphorylate PIP2 to PIP3, leading to the recruitment of PDPK1 and AKT to the membrane. The resulting stimulation of the AKT downstream signaling pathway activates NFκB and promotes the up-regulation of mRNA translation by mTORC1. In the meantime, Vav is recruited to the membrane through binding to GRB2 or PIP3 and phosphorylated by Fyn. Thereafter, Vav can stimulate Rac1, Cdc42, and RhoA-related cytoplasmic events. Sources: Reactome; KEGG pathway (Huse, 2009; Courtney et al., 2018).

natural/thymic Tregs) or from naive CD4⁺ T cells in the periphery (referred to as peripheral Tregs), when they acquire FOXP3, and hence for their expansion, survival, and immunoregulatory functions (including the secretion of the immunosuppressive cytokines IL-10 and TGF-β) in the periphery (Kündig et al., 1993; Sadlack et al., 1993; Suzuki et al., 1995; Willerford et al., 1995; Malek et al., 2002; D'Cruz and Klein, 2005; Fontenot et al., 2005; Burchill et al., 2007; Zheng and Rudensky, 2007; Malek, 2008; Tang et al., 2008; Campbell and Koch, 2011; Goldstein et al., 2013; Lin et al., 2013; Chinen et al., 2016; Ross and Cantrell, 2018).

Additionally, IL-2 regulates lineage commitment of CD4⁺ Th cell subsets. Th1 lymphocytes promote cellular immune responses against intracellular microbes and cancer cells. Th1 cell differentiation is triggered by IL-2–mediated expression of IL-12Rβ in naive CD4⁺ T cells. Then, together with IL-12 produced by APCs, IL-2 coordinates the expression of the transcription factor T-Box21 (TBX21, best known as T-bet), which orchestrates Th1 differentiation and the production of type 1 cytokines like IFN-γ (Reem and Yeh, 1984; Shi et al., 2008; Liao et al., 2011). Th2 lymphocytes regulate humoral immunity to extracellular parasites and bacteria, as well as allergic inflammation. In the presence of IL-4, IL-2 sensitizes cells to the Th2 program by up-regulating IL-4Rα expression and by inducing epigenetic changes that boost IL-4 secretion (Ben-Sasson et al., 1990; Zhu et al., 2003; Cote-Sierra et al., 2004; Le Gros et al., 2008; Liao et al., 2008). Th17 cells coordinate the adaptive defense against extracellular pathogens, mediate tissue inflammation, and participate in many autoimmune pathologies. IL-2 inhibits the differentiation of Th17 cells through various mechanisms including repression of retinoic acid receptor–related orphan nuclear receptor γt, IL-6Rα, and IL-17A. These three factors are required for the development and the proinflammatory activity of Th17 cells (Laurence et al., 2007; Liao et al., 2011; Yang et al., 2011). Accordingly, it has been reported that IL-2 consumption by Tregs facilitates Th17 differentiation in *Candida albicans* infection (Pandiyan et al., 2011). Similarly, IL-2 inhibits the differentiation of T follicular helper (Tfh) CD4⁺ T cells, which control B cell responses and germinal center formation. Thus, IL-2 activates the expression of positive regulatory domain zinc finger protein 1 (PRDM1, best known as B lymphocyte–induced maturation protein 1 [BLIMP-1]) which in turn trans-represses gene expression of BCL6, a transcriptional regulator required for Tfh development (Johnston et al., 2009; Ballesteros-Tato et al., 2012). Interestingly, in an influenza infection model, high levels of IL-2 prevented the development of T follicular regulatory (Tfr) cells in the course of the infection. However, once the virus was eliminated and the response resolved, some Tregs down-regulated IL-2Rα/CD25 and up-regulated BCL6 before differentiating into Tfr cells. These Tfr cells migrated to B cell follicles to prevent clonal expansion of self-reactive B lymphocytes (Botta et al., 2017).

In naive CD8⁺ T lymphocytes, IL-2 mediates the acquisition of the effector cytotoxic phenotype following antigen encounter by promoting the secretion of IFN-γ, TNF-α/β, granzyme B, and perforin. The intensity of the IL-2/IL-2R signaling activity shifts the fate of CD8⁺ T cells toward a short-lived effector or a long-lived memory phenotype. De facto, high levels of IL-2 stimulate the synthesis of BLIMP-1, which controls the effector transcriptional program and inhibits the expression of central memory markers such as BCL6, IL-7Rα/CD127, and CD62L. On the contrary, low levels of IL-2 impair the synthesis of effector molecules, while authorizing the (re)expression of BCL6, IL-7Rα/CD127, and CD62L, thus driving activated CD8⁺ T cells toward the memory compartment (Manjunath et al., 2001; Williams et al., 2006; Kalia et al., 2010; Pipkin et al., 2010; Ross and Cantrell, 2018; Spolski et al., 2018). Similarly, low doses of IL-2 drive a memory or Tfh-like phenotype in CD4⁺ T lymphocytes (Boyman and Sprent, 2012). Importantly, BLIMP-1 induction in effector cells ultimately leads to a reduced production of IL-2, accompanied by a contraction of the effector alcove and the appearance of an exhausted state (Gong and Malek, 2007; Beltra et al., 2016; Zhu et al., 2017). Interestingly, this exhausted phenotype, characterized by surface exposure of programmed cell death protein 1 (PD-1) or up-regulation of IL-2Rβ, is reversible. As evoked in the next section, this peculiarity may be exploited in immunostimulatory treatments by combining IL-2 and anti-PD1.

Immunosuppressive and immunostimulatory IL-2 therapies

The exquisite sensitivity of both effector and regulatory T lymphocytes to IL-2 has designated this cytokine as a potential ally for treating immune-related diseases, spurring interest in both its immunosuppressive and immunostimulatory effects.

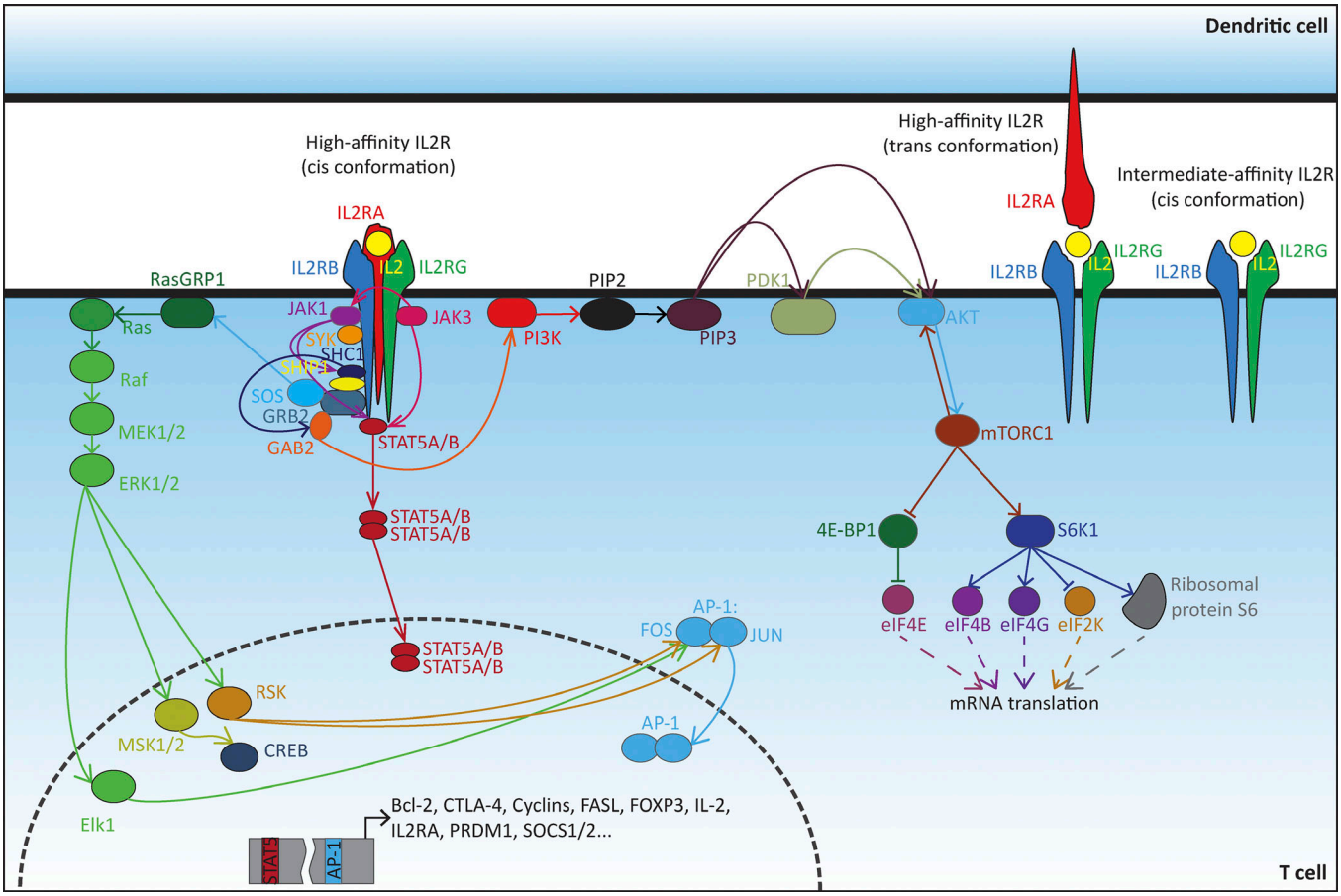

Figure 2. **IL-2R signaling and modulation of T cell activity.** IL-2R is composed of up to three subunits: α (IL2RA), β (IL2RB), and γ (IL2RG). The intermediate-affinity IL-2R is composed of the IL2RB and IL2RG subunits. The high-affinity receptor consists either of the cis gathering of the three subunits, for instance, on a T cell, or of the cis assembly of IL2RB and IL2RG complemented in trans with IL2RA located at the surface of a DC. IL2RB and IL2RG are respectively bound to the JAK1 and JAK3 and responsible for transducing intracellular signaling. Upon ligation of IL-2, JAK3 phosphorylates JAK1, which in turn recruits to IL2RB the spleen-associated tyrosine kinase (SYK) and phosphorylates it. JAK1 also phosphorylates IL2RB, leading to the recruitment of the STAT5A or its paralog STAT5B. STAT5A/B is further phosphorylated by JAK3, but JAK1 could also be involved. Phosphorylated STAT5A/B is then released from IL-2R and dimerizes in the cytosol. The dimer can finally translocate to the nucleus and regulates genes encoding immune-related factors such as IL2RA itself, or again FOXP3, Fas-ligand (FASL), positive regulatory domain zinc finger protein 1 (PRDM1), or suppressor of cytokine signaling 1 or 2 (SOCS1/2). The phosphorylation of IL2RB also creates binding sites for the SHC-transforming protein 1 (SHC1), which is phosphorylated probably by JAK1 or Lck (still unclear). SHC1 then recruits the Src homology 2 domain containing inositol polyphosphate 5-phosphatase 1 (SHIP1). The SHC1/SHIP1 complex is stabilized through interaction with GRB2, itself associated with GRB2-associated binder 2 (GAB2). SHC1 promotes the phosphorylation of GAB2, but the kinase involved is not clear. Phosphorylated GAB2 can further recruit PI3Ks to the membrane, leading to the activation of the PI3K/PKB (best known as AKT)/mTOR pathway. JAK1 may also recruit PI3K. In the meantime, SHC1 recruits the complex GRB2/SOS. SOS interacts with, and activates, RasGRP1, resulting in the activation of the Ras/Raf pathway and the regulation of genes involved in T cell response. Sources: Reactome; KEGG pathway (Ross and Cantrell, 2018).

Fine-tuning of the balance between these two functionally contrasted T cell subsets is at the heart of IL-2–based immunotherapies. Immunoinhibitory IL-2 treatments aim at selectively expanding Tregs over effector T cells, whereas immunostimulatory IL-2 interventions should proceed inversely. To achieve these opposite goals, fundamental distinctions of the IL-2/IL-2R system within the targeted populations are being exploited. Thus, Tregs constitutively display the IL-2Rαβγ trimer, while activated effector T cells only transiently express IL-2Rα.

Strategies to improve immunosuppressive IL-2 therapy

When contemplating the addiction of Tregs to IL-2 and their preponderant role in maintaining immune tolerance and suppressing inflammation (Sakaguchi et al., 1995), it is a posteriori no surprise that inactivation of the *IL2*, *IL2RA*, *IL2RB*, *STAT5*, or *FOXP3* genes in mice led to Treg cell depletion/dysfunction and to multi-organ autoimmune and inflammatory syndromes (Sadlack et al., 1993; Suzuki et al., 1995; Willerford et al., 1995; Malek et al., 2002; Fontenot et al., 2003; Snow et al., 2003; Burchill et al., 2007; Yao et al., 2007). Importantly, similar clinical manifestations have been detected in patients affected by mutations in *IL2RA* or *FOXP3*, leading to the immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome (Bennett et al., 2001; Wildin et al., 2001; Moraes-Vasconcelos et al., 2008; Goudy et al., 2013). Based on these observations, reestablishing Treg cell functions thanks to the administration of recombinant human IL-2 has been considered for the treatment of autoimmune, inflammatory, or

graft-versus-host (GvHD) diseases (Krömer et al., 1985; Andreu-Sánchez et al., 1991; Koreth et al., 2011; Saadoun et al., 2011; Hartemann et al., 2013; Matsuoka et al., 2013; Castela et al., 2014; He et al., 2016).

For immunosuppression, the common strategy relies on the administration of low-dose (LD) IL-2 (as opposed to HD IL-2, which has been clinically approved for cancer therapy), based on the rationale that limited concentration of IL-2 restricts its capture to Tregs (rather than Tconv cells), as Tregs constitutively express high amounts of the high-affinity IL-2Rαβγ (Klatzmann and Abbas, 2015). The proof of concept of this approach was attained when LD IL-2 reverted and prevented experimental type 1 diabetes (T1D) in mice (Tang et al., 2008; Webster et al., 2009; Yu et al., 2009; Grinberg-Bleyer et al., 2010; Baeyens et al., 2013; Pérol and Piaggio, 2016). LD IL-2 also achieved disease control in other indications, but only when delivered chronically, like in Alzheimer's disease (Dansokho et al., 2016), or when combined with other drugs such as glucocorticoids in GvHD (Pérol and Piaggio, 2016) and *Trypanosoma cruzi* infection (González et al., 2015) or rapamycin in skin transplantation (Pérol and Piaggio, 2016). In the clinic, LD IL-2 has been evaluated for the treatment of T1D (Long et al., 2012; Hartemann et al., 2013; Todd et al., 2016), chronic GvHD (Koreth et al., 2011, 2016; Matsuoka et al., 2013; Kennedy-Nasser et al., 2014), systemic lupus erythematosus (SLE; He et al., 2016; Humrich and Riemekasten, 2016), alopecia aereata (Castela et al., 2014), and vasculitis associated with chronic hepatitis C virus infection (Saadoun et al., 2011). Overall, LD IL-2 was well tolerated, not only due to the dosing (<3 × 10^6 IU/d) but also due to a preference for s.c., rather than systemic, administration. In all these pathological conditions, LD IL-2 led to an increase in circulating Tregs, and some clinical responses were observed. Nevertheless, to increase clinical efficacy, improvements are needed as IL-2 has a short half-life, requires repeated administration, and can unwantedly activate NK cells (Ye et al., 2018).

The short half-life of IL-2 (10–85 min in serum) is intrinsic to its small molecular weight that falls beneath the glomerular filtration cutoff estimated at 30–50 kD (Konrad et al., 1990; Ruggiero et al., 2010). Hence, to maintain efficient IL-2 bioavailability, administration must be repeated at close intervals. Alternatively, prodrug versions of recombinant human IL-2 decorated with releasable polyethylene glycol (PEG) chains have been synthesized and allowed: (i) a sustained release of the cytokine; (ii) a prolonged stimulation of the IL-2R signaling pathway; and (iii) a biased binding of IL-2 either to IL-2Rα/CD25 or IL-2Rβ/CD122, depending on the sites of PEGylation (Charych et al., 2017). For instance, a PEGylated IL-2 named NKTR-358 demonstrates a lower affinity for IL-2Rβ than for IL-2Rα and preferentially activates Tregs over Tconv cells. NKTR-358 restored Treg function in murine and simian models of SLE and cutaneous hypersensitivity, respectively, encouraging its clinical evaluation in patients (Cully, 2017; Table 1).

IL-2/anti–IL-2 antibody immunocomplexes (referred to as "IL2Cxs") are also designed to increase the half-life of IL-2 (Létourneau et al., 2010). Moreover, depending on the fine specificity of the antibody, IL2Cxs can redirect IL-2 toward IL-2Rαβγ$^+$ Tregs (pro–Treg cell function) or IL-2Rβγ$^+$ NK and naive/memory CD8$^+$ T cells (pro-effector; Boyman et al., 2006). For instance, the mAb JES6-1 recognizes an epitope on murine IL-2 that contacts IL-2Rβ and γ. Interestingly, JES6-1 sterically blocks the interaction of IL-2 with IL-2Rβ and allosterically reduces IL-2 affinity for IL-2Rα. Experimentally, IL-2/JES6-1 selectively binds to and expands Tregs as they present sufficient IL-2Rα to displace the mAb (Spangler et al., 2015). Pro-Treg IL2Cxs have been successfully used in mouse models of experimental autoimmune encephalomyelitis (Webster et al., 2009), diabetes (Pérol and Piaggio, 2016), allergy (Smaldini et al., 2018), atherosclerosis (Dinh et al., 2012), and solid organ transplantation (Pérol and Piaggio, 2016). When complexed to recombinant human IL-2, the first clinical grade pro-Treg anti–IL-2, named F5111.2, demonstrated effectiveness in inducing T1D remission in diabetic mice, and in diminishing the severity of xeno-GvHD and experimental autoimmune encephalomyelitis (Trotta et al., 2018).

An alternative approach consisting in fusing IL-2 to the α chain of IL-2R has recently been evaluated in rodents (Ward et al., 2018). This IL-2–CD25 fusion protein exhibited an increased half-life and selectively expanded Tregs in vivo. In nonobese diabetic mice, administration of IL-2–CD25 enhanced Tregs in the endocrine pancreas and diminished the occurrence of diabetes. These results encourage the clinical evaluation of such a new class of IL-2 derivatives for treating autoimmunity and other pathologies that result from an exacerbated immune response (Ward et al., 2018).

Elucidation of the quaternary structure of IL-2 assembled to IL-2Rαβγ (Wang et al., 2005) has facilitated the engineering of a series of IL-2 "muteins" with variable affinity to IL-2Rα (Rao et al., 2003, 2005; Carmenate et al., 2013), IL-2Rβ (Liu et al., 2009; Levin et al., 2012; Mitra et al., 2015; Peterson et al., 2018), and IL-2Rγ (Liu et al., 2009; Mitra et al., 2015; Carmenate et al., 2018). These IL-2 analogues elicit graded (agonistic, mixed, or antagonistic) and differential signaling outputs downstream of IL-2Rαβγ and IL-2Rαβ, ultimately affecting the Treg/effector ratio. For instance, the IL-2 mutein H9-RETR (nine amino acid substitutions) was engineered to bind IL-2Rβ with a rather high affinity and block its heterodimerization with IL-2Rγ. H9-RETR antagonized signal transduction by native IL-2 and prevented ex vivo proliferation of pre-activated human CD8$^+$ T cells, as well as the cytolytic activity of NK cells (Mitra et al., 2015).

Like their WT counterpart, IL-2 muteins suffer from limited bioavailability in vivo. As a remedy, they are frequently fused with an mAb or a crystallizable fragment (Fc) of an antibody, thus generating so called "immunocytokines." Lately, a human IL-2 mutein harboring an N88D substitution responsible for a reduced affinity for IL-2Rβγ was fused to a nontargeted effector-function-silent human IgG1. In macaques, this long-lived IgG-$(IL\text{-}2N88D)_2$ fusion protein sustained preferential amplification of Tregs (Peterson et al., 2018). In a GvHD murine model, the stabilized antagonist H9-RETR-Fc4 remarkably extended survival (Mitra et al., 2015). Preclinically, several IL-2–Fc molecules demonstrated therapeutic ability to induce transplantation tolerance (Zheng et al., 2003; Millington et al., 2012; Jindal et al., 2015; Mitra et al., 2015) or to prevent autoimmune disorders

Table 1. **Examples of innovative immunosuppressive IL-2 therapies undergoing clinical evaluation**

IL-2 therapy				Clinical trial				
Type	**Agent (company)**	**Description**	**Delivery route**	**Indication**	**Co-therapy**	**Phase**	**Status**	**References**
Immunocytokine	AMG-592/efavaleukin-alpha (Amgen)	Fc–IL-2 mutein fusion protein	s.c.	SLE	–	1/2	Recruiting	NCT03451422 (Tchao et al., 2017)
			s.c.	RA	–	1/2	Recruiting	NCT03410056 (Tchao et al., 2017)
			s.c.	Chronic GvHD	–	1/2	Recruiting	NCT03422627 (Tchao et al., 2017)
PEGylated IL-2	NKTR-358 (Nektar Therapeutics)	PEG–IL-2 with IL-2Rα bias > IL-2Rβ bias	i.v.	SLE	–	1	Recruiting	NCT03556007

RA, rheumatoid arthritis.

(Zheng et al., 1999; Bell et al., 2015). An Fc–IL-2 mutein called AMG-592 (Amgen) was designed to have greater half-life than native IL-2, as well as an increased affinity for IL-2Rα. In human peripheral blood mononuclear cell cultures, AMG-592 preferentially expanded Tregs over effector T cells and lowered the production of pro-inflammatory cytokines in comparison to native IL-2. In an ongoing first-in-human trial, AMG-592 was well tolerated without severe adverse events and increased the Treg/Tconv cell ratio (Tchao et al., 2017). AMG is now being evaluated in clinical trials for the treatment of rheumatoid arthritis, SLE, and GvHD (Table 1). Another Fc–IL-2 mutein with increased affinity for IL-2Rα, named DEL-106 (Delinia-Celgene-BMS), is under development (Cully, 2017).

Strategies to improve immunostimulatory IL-2 therapy

Mathematical prediction models, validated by experimentations, demonstrated that Tregs locally outcompete Tconv cells in consuming IL-2, as long as its autocrine/paracrine level does not reach the T cell activation threshold. Beyond this point, activated T cells engage a positive feedback loop that will temporarily upregulate the high-affinity IL-2R, support their proliferation, and secure their effector program (Busse et al., 2010).

In line with these theoretical considerations, i.v. infusions of HD IL-2 (6–7.2 × 10^5 IU/kg/dose, 12–15 doses/d) have been conceived for the treatment of malignant and infectious diseases. In Western countries, HD IL-2 received approval for the care of metastatic renal cell carcinoma and melanoma in the 1990s (Alva et al., 2016). Seminal publications reported an overall response rate (ORR) of 14–23% (including 5–8% of complete responders [CRs]) in patients with kidney cancer (Fyfe et al., 1995, 1996) and a 16% ORR (6% CRs) in melanoma patients (Atkins et al., 1999). In the field of infectious disease, HD IL-2 has essentially been evaluated in AIDS as an adjuvant to antiretroviral therapy. A meta-analysis compiling data from 25 completed trials revealed an increase in the $CD4^+$ T cell count in the presence of IL-2. However, IL-2 supplementation did not reduce mortality or the risk of opportunistic infections, and even tended to increase the rate of severe adverse events, thus discouraging further investigations in HIV-positive patients (Onwumeh et al., 2017).

The mitigated success of HD IL-2–based cancer therapy can be attributed to the following: (i) its short bioavailability; (ii) an undesired expansion of Tregs that dampens antitumor immunity; and (iii) its dose-dependent toxicity. Indeed, elevated systemic (endocrine) levels of cytokines are nonphysiological (because cytokines are by definition paracrine factors) and hence accompanied by deleterious effects. Thus, systemic injection of IL-2 causes vascular leak syndrome due to bystander damage of IL-2Rαβγ$^+$ endothelial cells and due to the unwarranted release of pro-inflammatory cytokines from T and NK cells (Epstein et al., 2003; Boyman and Arenas-Ramirez, 2019).

On one hand, the deleterious side effects of HD IL-2 must be attenuated to enhance its therapeutic index. Lower dosages and alternative delivery routes, such as s.c., i.m., or intralesional injections, have been tested (Tang and Harding, 2019). Only intratumoral administration outperformed standard HD i.v. IL-2, reaching up to 62% CR in melanoma patients with skin and soft-tissue metastases (Konrad et al., 1990; Palmer et al., 1993; Ravaud et al., 2002; Radny et al., 2003; Yang et al., 2003; Geertsen et al., 2004).

On the other hand, the therapeutic index of HD IL-2 may be improved by the selective stimulation of effector cells instead of Tregs. Multiple groups are introducing IL-2 in combinatorial regimens alongside immunomodulatory regimens, with the hope of improving efficacy and eventually reducing IL-2 dosage. Thus, ≥45 trials are active, recruiting patients in diverse oncological indications (http://www.clinicaltrials.gov) to evaluate HD IL-2 in combination with surgery, chemotherapy, radiotherapy, adoptive T cell therapy, anti–PD-1 or anti–CTLA-4, tumor-targeting mAbs, or cancer vaccines.

In parallel, attempts are ongoing to modify the pharmacological properties of IL-2. For example, pro-effector IL2Cxs in which an anti–IL-2 antibody sterically impedes the interaction between IL-2 and IL-2Rα, but allosterically enhances IL-2 affinity for IL-2Rβ, are being developed (Spangler et al., 2015). Antibodies with such characteristics include S4B6 (anti-mouse IL-2), as well as Mab602 and NARA-1 (anti-human IL-2; Boyman and Arenas-Ramirez, 2019). Preclinical studies in melanoma-bearing mice revealed superiority of S4B6 IL2Cxs over free IL-2 because: (i) the IL-2 interaction with IL-2Rα$^+$ endothelial cells was disrupted and vascular leak syndrome prevented; and

(ii) naive IL-2Rβγ$^+$ CD8$^+$ T and NK cells were preferentially amplified over IL-2Rαβγ$^+$ Tregs. Encouragingly, IL2Cxs showed greater antitumor activity and some synergy with immune checkpoint inhibitors (Krieg et al., 2010; Létourneau et al., 2010; Arenas-Ramirez et al., 2016; Caudana et al., 2019).

A PEGylated IL-2 named NKTR-254 demonstrated a much more reduced affinity for IL-2Rα than for IL-2Rβ. A Phase 1/2 clinical trial has recently been completed in patients with late stage solid tumors (NCT02869295). Preliminary results indicate a favorable safety profile, a 10–30% shrinkage of the tumor burden in 23% of the patients, and an increase of tumor-infiltrating CD8$^+$ T and NK cells with minimal impact on Tregs (Marin-Acevedo et al., 2018). Additional Phase 1–3 trials are now enrolling patients (Table 2).

Based on in vitro evolution assays and protein crystallography, Levin et al. (2012) designed the IL-2 mutein H9 (the IL-2 agonist that sourced H9-RETR). H9 harbors five mutations affecting core residues (i.e., L80F, R80D, L85V, I86V, and I92F). These substitutions induce a natural conformational switch responsible for a much stronger interaction with IL-2Rβ and an optimal downstream signaling activation not requiring IL-2Rα (Levin et al., 2012). The so called "superkine" H9 favored the expansion of CD8$^+$ T and NK cells, demonstrated lower toxicity, and improved antitumor activity in several murine cancer models. Interestingly, IL-2 superkines demonstrated similar therapeutic activity as immunocomplexed WT IL-2 (Levin et al., 2012; Tang and Harding, 2019). In the clinic, the IL-2 mutein BAY50-4798 (Bayer) failed to demonstrate sufficient efficacy in advanced melanoma and renal cancer (ORR < 5%), leading to its discontinuation (Margolin et al., 2007). Retrospectively, dual introduction of an IL-2Rα bias plus the N88R mutation (which disfavors IL-2/IL-2Rβ interaction; Peterson et al., 2018) likely conferred an undesired pro-Treg cell activity to BAY50-4798.

Immunocytokines, which are hybrid proteins composed of WT or mutant IL-2 fused to Fc domains or tumor-targeting mAbs, are being developed. Signs of efficacy were observed in preclinical melanoma models treated with IL-2–anti-ganglioside GD2 (Becker et al., 1996a,b). The humanized version of such a construct, called *EMD273063* (Merck), demonstrated a weak efficacy in metastatic melanoma (ORR = 7.1%; Albertini et al., 2012). Additional immunocytokines already completed clinical evaluations such as an anti-EpCAM-IL-2 (EMD273066/tucotuzumab celmoleukin), an anti-single/double-stranded DNA-(D20T)IL-2 mutein (EMD521873), and a fibronectin-targeted L19 diabody-IL-2 (Darleukin), with no or only marginal responses (Johannsen et al., 2010; Eigentler et al., 2011; Albertini et al., 2012; Connor et al., 2013; Gillessen et al., 2013; Laurent et al., 2013; van den Heuvel et al., 2015). However, as a possible exception to this rule, intralesional therapy of metastatic melanoma with a combination of recombinant IL-2 protein with the human mAb fragment L19 (L19–IL-2) together with another similar protein in which IL-2 is replaced by TNF-α (L19-TNF-α) showed a 53.8% ORR and induced abscopal effects (Danielli et al., 2015). An alternative strategy consists in fusing IL-2 with IL-2Rα to sterically prevent interactions with the high-affinity receptor. Such a compound, ALKS4230, is being tested in two Phase 1/2 trials in combination with the anti-PD1 antibody pembrolizumab (Table 2).

Another procedure applied to chimeric antigen receptor T cell technology is exemplified by the ortho–IL-2/ortho–IL-2Rβ system. It consists of a mutated IL-2Rβ, ortho–IL-2Rβ, which no longer binds native IL-2 but its mutant form, ortho–IL-2. The expression of ortho–IL-2Rβ on chimeric antigen receptor T cells confers selective proliferation following ortho–IL-2 administration, avoiding bystander activation of other IL-2R$^+$ cells like Tregs (Sockolosky et al., 2018).

Finally, Garcia's team generated a computationally designed IL-2/15 hybrid molecule of 100 amino acids that differs from the natural IL-2 not only in sequence but also in topology, ultimately preserving its ligation to IL-2Rβγ but not to IL-2Rα. This so called "neoleukin" Neo-2/15 showed greater antitumor activity and tolerance than WT IL-2 in preclinical experiments (Silva et al., 2019).

Concluding remarks

Layer after layer, we keep uncovering the complexity of IL-2 biology and incrementing IL-2 therapy. Following decades of deceptions, we may finally meet broader success. In this line, standard therapy with nonmodified recombinant IL-2 protein still has room for improvement, as illustrated by its increased therapeutic index following an intralesional route. Regarding the innovative approaches embodied by IL-2 muteins such as superkines or antagonists, immunocomplexes, and immunocytokines, drawbacks have emerged. They include the intrinsic immunogenicity and poor bioavailability of muteins, the potential disassembly of immunocomplexes in vivo, or the unexpected depletion of T cell subsets targeted by IL-2 mutein-Fc immunocytokines (Vazquez-Lombardi et al., 2017). Additional rounds of preclinical research and clinical investigations will likely be necessary to unchain the full therapeutic potential of agents targeting the IL-2/IL-2R system. Most importantly, immunomodulatory combination therapies, particularly with immune checkpoint inhibitors, have proven preclinical efficiency. In the clinic, administration of HD IL-2 appears essential for the efficacy of cancer treatments relying on the adoptive transfer of ex vivo expanded autologous T cells (e.g., tumor-infiltrating lymphocytes, T cells with an engineered TCR, or a chimeric antigen receptor) with remarkable objective response rates witnessed in melanoma (34–56%), lymphoma (80–100%), and leukemia (67–100%; Rosenberg and Restifo, 2015; Boyiadzis et al., 2018). In this setting, IL-2–based therapeutics could emancipate as adjuvant agents that will enhance the efficacy of established immunotherapeutics.

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Table 2. **Examples of innovative immunostimulatory IL-2 therapies undergoing clinical evaluation**

IL-2 therapy				Clinical trial				
Type	**Agent (company)**	**Description**	**Delivery route**	**Indication**	**Co-therapy**	**Phase**	**Status**	**References**
Immunocytokine	ALKS 4230 (Alkermes)	IL-2–CD25 fusion protein	i.v.	Solid tumors	Pembrolizumab	1/2	Recruiting	NCT02799095 (Vaishampayan et al., 2017)
			s.c.	Solid tumors	Pembrolizumab	1	Recruiting	NCT03861793 (Vaishampayan et al., 2017)
	Darleukin (Philogen)	Anti-fibronectin diabody–IL-2 fusion protein	i.v. (?)	NSCLC	Radiotherapy; surgery	2	Not yet recruiting	NCT03705403 (Johannsen et al., 2010; Eigentler et al., 2011; Danielli et al., 2015)
	EMD273063/ hu14.18-IL2 (Merck)	Anti–GD2–IL-2 fusion protein	i.v.	Melanoma	Surgery	2	Active, not recruiting	NCT00590824
			i.t.	Melanoma	Radiotherapy; nivolumab; ipilimumab	1/2	Not yet recruiting	NCT03958383
			i.v.	Neuroblastoma	EEAHD NK cells	1	Recruiting	NCT03209869
	RG7461/ RO6874281 (Roche)	Anti–FAP–IL-2 mutein (F42A, Y45A, and L72G) fusion protein	i.v.	NSCLC; SCCHN; ESCC; cervical cancer	Atezolizumab; gemcitabine; vinorelbine	2	Recruiting	NCT03386721 (Klein et al., 2017)
			i.v.	Pancreatic cancer	Atezolizumab	1/2	Recruiting	NCT03193190 (Klein et al., 2017)
			i.v.	TNBC	Atezolizumab	1/2	Recruiting	NCT03424005 (Klein et al., 2017)
			i.v.	Melanoma	Pembrolizumab	1b	Recruiting	NCT03875079 (Klein et al., 2017)
			i.v.	Breast cancer; HNC; other solid tumors	Trastuzumab; cetuximab	1	Recruiting	NCT02627274 (Klein et al., 2017)
			i.v.	RCC	Atezolizumab; bevacizumab	1	Active, not recruiting	NCT03063762 (Klein et al., 2017)
	RG7813/ cergutuzumab amunaleukin (Roche)	Anti–CEA–IL-2 mutein (F42A, Y45A, and L72G) fusion protein	i.v.	Solid tumors	Atezolizumab	1	Active, not recruiting	NCT02350673 (Klein et al., 2017; Lo et al., 2017)
PEGylated IL-2	NKTR-214 (Nektar Therapeutics)	PEG–IL-2 with IL-2Rβ bias > IL-2Rα bias	i.v.	Melanoma	Nivolumab	3	Recruiting	NCT03635983 (Charych et al., 2016)
			i.v.	RCC	Sunitinib; nivolumab; cabozantinib	3	Recruiting	NCT03729245 (Charych et al., 2016)
			i.v.	Bladder cancer	Nivolumab	2	Recruiting	NCT03785925 (Charych et al., 2016)
			i.v.	Sarcoma	Nivolumab	2	Recruiting	NCT03282344 (Charych et al., 2016)
			i.v.	Melanoma; RCC; NSCLC; urothelial cancer; TNBC	Nivolumab; ipilimumab	1/2	Recruiting	NCT02983045 (Charych et al., 2016)
			i.v.	Skin cancers (melanoma, MCC); TNBC; ovarian cancer; RCC; CRC; urothelial cancer; sarcoma	NKTR-262; Nivolumab	1/2	Recruiting	NCT03435640 (Charych et al., 2016)

Table 2. **Examples of innovative immunostimulatory IL-2 therapies undergoing clinical evaluation (*Continued*)**

IL-2 therapy				Clinical trial				
Type	**Agent (company)**	**Description**	**Delivery route**	**Indication**	**Co-therapy**	**Phase**	**Status**	**References**
			i.v.	NHL	TAK-659	1	Recruiting	NCT03772288 (Charych et al., 2016)
			i.v.	NSCLC; bladder cancer; melanoma	Pembrolizumab; atezolizumab	1	Recruiting	NCT03138889 (Charych et al., 2016)
			i.v.	Prostate cancer	Nivolumab	1	Recruiting	NCT03835533 (Charych et al., 2016)
			i.v.	Solid tumors	Nivolumab	1	Recruiting	NCT03745807 (Charych et al., 2016)

CEA, carcinoembryonic antigen; CRC, colorectal cancer; EEAHD, ex vivo expanded and activated haploidentical donor; ESCC, esophageal squamous cell carcinoma; FAP, fibroblast activation protein-alpha; GD2, ganglioside D2; HNC, head and neck cancer; i.t., intratumoral; MCC, Merkel cell carcinoma; NHL, Non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; SCCHN, squamous cell carcinoma of the head and neck; TNBC, triple negative breast cancer.

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