Flip the coin: IL-7 and IL-7R in health and disease

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Abstract

Interleukin (IL)-7 and its receptor (IL-7R) are critical for T- and, in the mouse, B-cell development, as well as differentiation and survival of naïve T-cells, and generation and maintenance of memory T-cells. They are also required for innate lymphoid cell development and maintenance, and consequently for the generation of lymphoid structures and barrier defense. Here, we discuss the central role of IL-7 and IL-7R in the lymphoid system and highlight the impacts of its deregulation, placing a particular emphasis on its 'dark side' as a promoter of cancer development. We also explore therapeutic implications and opportunities associated with either positive or negative modulation of the IL-7/IL-7R signaling axis.

Introduction

Interleukin (IL)-7 is a 25 kDa secreted soluble globular protein encoded by the *IL7* gene. Its receptor (IL-7R) is a heterodimeric complex consisting of IL-7Rα (encoded by *IL7R*) and the common gamma chain (encoded by *IL2RG*), shared with the receptors for IL-2,-4,-7,-9,-15 and -21. While it was first characterized as a growth or survival factor for murine T- and B-cell precursors, its central regulatory role throughout the lymphoid system has become increasingly evident. IL-7 is a critical developmental cue at every stage of T-cell development, for both αβ and γδ lineages, and for development and survival of naive T-cells as well as generation and maintenance of CD4 and CD8 memory. More recently, the discovery of the innate lymphoid cell family was accompanied by recognition that IL-7 was also essential for development and maintenance of many of its members and consequently for the generation of lymphoid structures and barrier defense mechanisms mediated by these cells. Thus, IL-7 is tightly interwoven in the very fabric of the entire lymphoid system. It is perhaps unsurprising, then, that IL-7 and IL-7R are also implicated in the pathogenesis of different disease states mediated by dysfunctions of lymphoid function. In this review, we examine the physiology and pathophysiology of IL-7 and IL-7R, with particular emphasis on their involvement in the promotion of cancer, for which there is increasing evidence, and consider current opportunities and prospects to either exploit or target IL-7R-mediated signaling for therapeutic benefit.

IL-7: expression of ligand and receptor

Insights into the functions of IL-7 are gained by examining where, and by which cells, the cytokine and its receptor are made. Although *Il7* mRNA was first detected at high levels in the thymus, more recent studies, and the generation of a number of *Il7* reporter systems, reveal that expression occurs in a broad range of tissues. While there is incomplete agreement between different reporter strains about where *Il7* can be detected, this likely relates to the relative

sensitivity of individual reporters coupled with variation in the extent to which IL-7 is actually produced (reviewed in [1]). The highest levels of *Il7* production appear to be in lymphoid organs such as thymus and lymph nodes (LNs). Perhaps surprisingly they are considerably lower in spleen and bone marrow (BM). However, expression is also readily detectable in numerous non-lymphoid sites, such as intestine, lung, liver[2] and skin (**Fig. 1**). In terms of specific cellular sources, early BM chimeras experiments strongly suggested a non-hematopoietic source of IL-7 and this proves to be the case. Cells of both epithelial and endothelial origin were identified as sources. Thymic epithelial cells (TECs) and fibroblastic reticular cells (FRC) in LNs are important sources[3, 4], whereas lymphatic endothelia in LNs[5], afferent lymphatics[6] and bronchus-associated lymphoid tissue of the lung[7] may account for the high levels of IL-7 found in lymphatic fluid and ultimately blood serum. *Il7* is also constitutively expressed by keratinocytes in the skin while expression is inducible in gut epithelia by IFNγ signaling. There are also reports of IL-7 expression in the brain, produced by neuronal progenitor cells. Similarly, IL-7Rα is broadly expressed throughout the lymphoid system: on B-cell progenitors (but not mature B-cells), innate lymphoid cells, and throughout T-cell development and maturation - though notably absent on selecting thymocytes and effectors cells[8, 9]. There are also emerging reports of IL-7R expression in the development of fetally-derived macrophages (http://dx.doi.org/10.1101/534859), while outside of the hematopoietic system, lymphatic endothelial cells express IL-7Rα[6] that is required to drive lymphatic expansion during formation of tertiary lymphoid structures[10].

Developmental functions and mechanisms of IL-7

As suggested by IL-7-deficient mice, IL-7 signaling is critical for development and maintenance of the entire lymphoid compartment, including T-lymphocytes, B-lymphocytes, and innate lymphoid cells. IL-7 plays an important role throughout the process of hematopoiesis, facilitating key lineage fate decisions (**Fig. 2**). While some controversy over the identity of the

earliest lymphoid progenitor cell remains, the so-called common-lymphoid progenitor is in part characterized by its expression of IL-7Rα[11], and IL-7 signaling is thought to be important for lymphoid lineage specification, directing cells toward B- rather than T-lymphocyte fate[12]. While mature B-cells do not express the IL-7R, development of pre-pro-B cells from common-lymphoid progenitors (CLPs), and subsequent B-cell development in the BM, absolutely requires IL-7 in the mouse (reviewed in [13]), whereas in humans IL-7 appears to have a less critical role.

Lymphoid progenitors leave the BM and migrate to the thymus giving rise to numerous lymphoid lineages whose development is dependent on, or influenced by, IL-7 signaling. Thymic NK cells, in contrast to conventional NK cells, depend on IL-7[14]. Pre-T cell progenitors lack expression of CD4 and CD8 surface antigens and are termed double-negative (DN). In the mouse, DN cells are subdivided into four populations (DN1-4) on the basis of expression of CD44 and CD25. Development of γδ T-cells from DN2 thymocytes is absolutely dependent on IL-7, and strong IL-7 signaling is thought to specifically favor γδ over αβ T-cell lineage choice[15]. Developing αβ T-cells also require IL-7 at DN3 and DN4 stages, whose function can be substituted only in part by Bcl2 overexpression[16] and PI3K activation[17]. IL-7 signaling appears to facilitate proliferation and differentiation of these DN stages by regulating cell growth genes such as CD98, and blocking expression of the transcriptional repressor, Bcl-6, in a Stat5-dependent process[16]. In contrast, positive selection of CD4 CD8 double-positive (DP) thymocytes does not require IL-7 signaling, and these cells lack functional IL-7R expression[18]. In mice, IL-7R functional inactivation is achieved by the near complete loss of IL-7Rα surface expression coupled with high expression levels of SOCS proteins that inhibit cytokine signal transduction[19]. Cells that successfully pass selection re-express IL-7R in a multistep process dependent upon TCR signaling[8], and transcription factors Foxo1[20], Ets-1[21] and NF-kB[22].

More recently, a family of lymphoid cells, innate lymphoid cells (ILCs), has been described that plays important roles in inflammation, immune defense, tissue development and remodeling[23].

There are three broad classes of ILCs (groups 1, 2 and 3) whose regulation of effector function parallels that of conventional T-cells, namely Th1, Th2 and Th17-type. IL-7 is critical for development of both ILC2 and ILC3 and IL-7-deficient mice completely lack these subsets. Aside from T and B lymphopenia, it was later recognised that IL-7 and IL-7R deficient mice also exhibited profound defects in secondary lymphoid structures, lacking normal sized LNs and Peyer's patches in the gut[24]. It is now recognized that development of these lymphoid structures requires a subset of Rorc-dependent ILC3: lymphoid tissue-inducer cells (LTi). LTi are required to recruit LN organizer cells of stromal origin, together collaborating in the formation of mature lymphoid structures. In IL-7-deficient mice, these structures initially form normally but fail to develop into LNs after birth. It appears that IL-7 signaling in the LTi is required for the final stage of LN formation[25, 26]. Indeed, IL-7-driven expansion of LTi is sufficient for ectopic development of lymphoid structures[27].

Control of immune homeostasis by IL-7

In contrast to B-cells, T-cells continue to express IL-7R in both naive and memory states, and IL-7 signaling is critical for long-term maintenance of all T-cell populations. IL-7 promotes cell survival by modulating the intrinsic pathway of apoptosis[28]. The idea that competition for homeostatic resources limits the size of the T-cell compartments is as attractive as it is enduring[29], and there is evidence from mice lacking one or other CD4 or CD8 lineage that IL-7 constitutes one of the key cues controlling the size of the T-cell compartment.

Following activation, T-cells rapidly lose expression of IL-7Rα. Maintenance of IL-7R expression in peripheral T-cells is dependent on the activity of the FOXO1 transcription factor[20]. The activity of FOXOs is negatively regulated by Akt-mediated phosphorylation that is activated via PI3K by upstream TCR or IL-2 signaling[30]. This effectively uncouples effector cells from IL-7-dependent homeostatic control, relying rather on TCR and IL-2 signaling for their survival and

proliferation. Following the response, while most effector cells will undergo apoptosis, some survive to become long-term memory cells. IL-7 signaling plays a key role in the formation of this memory population. In the absence of antigen, TCR-dependent repression of IL-7Rα expression is reversed, and effector T-cells can re-express IL-7Rα. In some settings at least, cells that re-express IL-7Rα are the precursors of the long-term memory cells, suggesting that IL-7 could be instructing effectors to develop into memory cells[31]. However, there is also evidence that effector T-cells are far more predisposed to undergo apoptosis than naïve or memory T-cells. While re-expression of IL-7R can delay their death, the effector pool still undergoes a significant contraction because the effector cells are less able to compete and survive in response to IL-7 than other T-cells[9]. Nevertheless, IL-7 signaling represents a key gateway into the memory T-cell pool, ensuring only the fittest effectors persist. Following establishment of memory, both CD4[32] and CD8 memory T-cells[33] continue to be dependent on IL-7 for their long-term survival, although CD8 memory cells also have a strong reliance on IL-15. Tissue-resident memory cells were recently recognized as important mediators of immunity in non-lymphoid organs, where they reside and are maintained. These populations do not recirculate and so their dependence on IL-7 for survival[34] likely explains why epithelial barriers have local sources of IL-7 production. Indeed, dendritic epidermal γδ T-cells in the skin are also dependent in part on IL-7 for their survival[35].

There is a growing body of evidence that ILC survival is IL-7-dependent[36, 37] and that this accounts for some features of immune homeostasis that rely on IL-7. ILCs appear to be particularly sensitive to IL-7 levels. Competition for IL-7 with T-cells appears to greatly restrict the size of the ILC compartment[37], and it is notable that blockade of IL-7 *in vivo* affects ILCs more rapidly and to a greater extent than other IL-7-dependent populations such as T-cells[36]. Blockade of IL-7 during citrobacter infection compromises the intestinal barrier, and IL-7 induction during infection drives ILC3 expansion required for normal barrier function[38]. Loss of ILC3 in LNs following IL-7 ablation also reveals a hitherto unappreciated role for these cells in regulating lymphocyte

migration[36]. Thus, ILC function in immune homeostasis appears highly susceptible to perturbations in IL-7 function.

IL-7/IL-7R in the context of chronic inflammation and autoimmunity

As highlighted above, lack of IL-7/IL-7R-mediated signaling compromises lymphoid development and homeostasis. Its role in supporting normal immunity is evident in mechanisms that promote antiviral immune responses and limit organ toxicity[39]. However, abnormally high or unregulated levels of IL-7 and IL-7R also associate with immunopathology. IL-7 is thought to support aberrant immune activity in autoimmune diseases such as diabetes and multiple sclerosis[40], and chronic inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis and inflammatory bowel disease[41-44]. Patients with Crohn's disease and ulcerative colitis display high IL-7 and IL-7R levels, and evidence of colon-specific increased mucosal IL-7R signaling, with colonic IL-7Rα expression predicting resistance to anti-TNF therapy[42, 45]. Notably, antibody-mediated IL-7R blockade reduces human T-cell homing to the gut and colitis in humanized mouse models[45].

IL-7 and IL-7R in hematological cancers

IL-7 in T-cell acute lymphoblastic leukemia

Can IL-7R-mediated signaling promote proliferation to such an extent that it contributes to the development of lymphoid tumors? The *in vitro* evidence that IL-7, including that produced by BM and thymic stromal cells, can induce survival and proliferation of T-cell acute lymphoblastic leukemia (T-ALL) cells is extensive (reviewed in [46]). In fact, more than 70% of T-ALL patients

present with IL-7R-positive blasts[47] and the response correlates with IL-7Rα expression in leukemia cells[47, 48]. In contrast to normal thymocytes, IL-7 appears to stimulate T-ALL cells irrespectively of their maturation block, including at the DP stage[49], although frequency of response may be higher in more immature cases[48]. Correlative data from AKR/J mice, whose thymocytes naturally overexpress IL-7Rα and tend to develop thymic T-cell lymphomas[50], and from T-ALL patients, whose blasts present with evidence of IL-7 consumption[51], suggest that IL-7-mediated signaling contributes to leukemia development *in vivo*. Importantly, findings from IL-7 transgenic mice[52, 53] and xenotransplant of human T-ALL cells into IL-7-deficient mice[51] support this possibility.

Several mechanisms increase IL-7Rα expression in T-ALL: NOTCH1, a major T-cell oncogene, activates *IL7R* transcriptionally, which in turn is involved in Notch-mediated T-ALL cell maintenance[54]; ZEB2 translocation leads to *IL7R* transcriptional upregulation[55]; RPL10 R98S mutation upregulates IL-7Rα and downstream signaling elements[56]; mutations in DNM2[57], which regulates IL-7Rα trafficking and surface availability, may impact IL-7R-mediated signaling; and SOCS5 epigenetic silencing upregulates IL-7Rα and JAK/STAT signaling[58].

IL-7 in B-cell acute lymphoblastic leukemia

IL-7 also induces proliferation *in vitro* of B-ALL cells[59] and promotes B-cell tumorigenesis in IL-7 transgenic mice[52], whereas high levels of *IL7R* have been associated with CNS involvement and relapse in pediatric B-ALL[60]. In addition, IL-7Rα is fundamental for B-ALL arising in mice from combined STAT5 activation and PAX5 haploinsufficiency[61], and B-ALL development in the context of infection exposure and PAX5 deficiency involves a pre-leukemic state that is hypersensitive to IL-7, subsequently originating leukemia by acquiring JAK3 and JAK1 mutations[62]. BLNK/SLP-65 inactivation occurs in human B-ALL, and mouse BLNK-deficient leukemia cells display constitutive JAK3/STAT5 signaling triggered by autocrine IL-7[63].

Accordingly, BLNK-deficiency cooperates with constitutive activation of STAT5, a major IL-7 signaling effector, in promoting mouse B-cell leukemogenesis[63]. Ikaros (encoded by *IKZF1*) is a critical tumor suppressor, often inactivated in B-ALL, whose role in pre-B-cell differentiation relies largely on turning-off IL-7-dependent gene expression[64], likely by directly repressing *IL7R* and competing with STAT5 DNA-binding[65]. SH2B3 binds JAK3 and regulates pro-B cell homeostasis by decreasing IL-7-triggered JAK/STAT5 signaling. Accordingly, pre-leukemic B-cell progenitors with combined loss of *SH2B3* and *Tp53* are hyper-responsive to IL-7 on their path to B-ALL[66].

Idiosyncrasies of IL-7-mediated signaling in healthy and leukemic cells

We will now focus on T-ALL to consider why IL-7 may preferentially benefit leukemia cells at the expense of their normal counterparts (**Fig. 3a-d**). For one, IL-7 promotes the expansion of T-ALL cells, but does not overcome their differentiation block[67]. This means that IL-7 positive effects on developmentally-arrested malignant precursors can be 'perpetuated' in the absence of ensuing differentiation checkpoints. By contrast, normal thymocytes benefit from IL-7 transiently but inevitably differentiate and undergo stringent processes of selection that are at the core of T-cell development, against which T-ALL cells are largely protected by their maturation arrest. Additionally, the activation of oncogenes that strongly promote proliferation, such as c-MYC, can often trigger apoptosis – an effect that can be counterbalanced by external cues. MYC is frequently activated in T-ALL[68], although its ability to drive malignant transformation *per se* is debated[69]. Pre-leukemic cells with aberrant MYC activation will thus benefit from an IL-7-rich environment that protects them against oncogene-induced apoptosis, offering time for the acquisition of additional mutations. Oncogenic insults suffered by (pre)malignant cells may also shape how they respond to microenvironmental cues. As noted above, different oncogenic insults upregulate IL-7Rα in T-ALL and pre-leukemic B-cells often are IL-7-hypersensitive. This is especially relevant

given that (pre)leukemic cells may actively instruct stromal cells to downregulate IL-7 production in specific niches, decreasing IL-7 availability for normal precursors[70].

IL-7 binding promotes IL-7Rα and γc heterodimerization, leading to JAK1 and JAK3 activation and consequent downstream signaling, most notably STAT1/3/5, PI3K/Akt/mTOR and MEK/ERK pathways. However, T-ALL cells appear to differ from normal T-cells in the recruitment of signaling pathways towards particular cellular responses to IL-7 (**Fig. 3e**; reviewed in [29]). PI3K signaling is involved in IL-7-induced cell cycle progression[71] without impacting cell survival or Bcl-2 expression[72] in naïve T-cells. Contrarily, PI3K/Akt/mTOR signaling is essential for IL-7-mediated proliferation and survival of T-ALL cells by promoting both $p27^{kip1}$ downregulation as well as Bcl-2 upregulation[73, 74]. In agreement, IL-7-mediated Bcl-2 upregulation in T-ALL cells appears to be STAT5-independent[75] – again in opposition to the fact that IL-7-mediated STAT5 activation in naïve T-cells associates with survival[71], suggesting that IL-7-mediated Bcl-2 upregulation occurs via STAT5 in normal T-cells. Overall, although normal and leukemic T-cells recruit the same signaling axes downstream from IL-7/IL-7R, they may exploit them to achieve different functional outcomes. These differences have potential therapeutic relevance, since PI3K/Akt/mTOR pathway inhibitors should preferentially eliminate IL-7-dependent T-ALL cells while being merely cytostatic for peripheral T-cells.

IL7R gain-of-function mutations

IL7R gene amplification was reported in T-ALL[76], although the consequences for leukemogenesis are unknown. In contrast, *IL7R* mutational activation is a driver of ALL[77-82]. Somatic gain-of-function mutations in IL-7Rα lead to constitutive activation of the receptor promoting cell transformation *in vitro* and tumor formation *in vivo*. They were identified in around 10% of T-ALL cases and more rarely in B-ALL[77, 78], where they are enriched in particular subgroups, especially in CRLF2-rearranged, Ph-like, IKZF1 N159Y, iAMP21 and PAX5 P80R

cases[78, 83]. Although enriched in patients overexpressing *HOXA* and *TLX*[77] and frequent in early T-cell precursor ALL (ETP-ALL)[79], *IL7R* mutations occur in all T-ALL subtypes[82].

Mutations in *IL7R* are somatic, heterozygous and affect either exon 5 or 6, falling into four classes (**Fig. 4**). Type 1a mutations are the most frequent, occur in exon 6, and are insertions or insertions-deletions that include an unpaired cysteine in the extracellular juxtamembrane-transmembrane (EJMT) portion of IL-7Rα, leading to disulfide bond-dependent mutant receptor homodimerization and constitutive, ligand-independent signaling[77]. Type 1b mutations also affect exon 6 but insert non-cysteine residues within the transmembrane region closer to the cytosolic domain, including tryptophans, SxxxG-related motifs, or other residues that are suspected or formally shown to lead to ligand-independent IL-7Rα dimerization[77, 84]. Type 1c mutations impact the EJMT region, similar to type 1a; however, they result in insertion of positively-charged aminoacids that promote γc heterodimerization and IL-7 hyperresponsiveness[85]. Contrary to the others, type 2 mutations are exclusive to B-ALL, affect exon 5 and lead to S185C substitution in the extracellular domain. They confer cytokine-independence, but require TSLPR/CRLF2 and can increase responsiveness to TSLP[78].

The ability of mutant IL-7R *per se* to originate T- or B-ALL *in vivo* when expressed in hematopoietic progenitors is yet to demonstrate. Nonetheless, retroviral expression of mutant IL-7Rα in hematopoietic stem and progenitor cells accelerated Notch-induced T-ALL, whereas in CLPs mutant IL-7Rα alone caused mature B-cell leukemia/lymphoma - but not B-ALL[81]. In a different study, mutant *IL7R* overexpression in DN thymocytes with inactivation of the *Ink4*/*Arf* tumor suppressor, from $Arf^{-/-}$ mice, induced thymocyte development arrest at an immature stage, eventually leading to ETP-ALL-like disease[80]. Likewise, mutant *IL7R* cooperated with *NRAS* G13D in inducing T-ALL from transplanted DN thymocytes[86]. Interestingly, mutant *IL7R* alone caused multisystemic inflammatory disease rather than leukemia[86].

IL-7R-mediated signals in resistance to therapy

IL-7/IL-7R-mediated signaling may be critical for resistance to conventional chemotherapy and to targeted therapeutics. In T-ALL cells, exogenous IL-7 stimulation or mutational activation of IL-7R signaling elements (including the receptor itself) induce glucocorticoid resistance, which can be bypassed using the JAK inhibitor ruxolitinib or MEK, AKT, mTOR, or dual PI3K/mTOR inhibitors[76, 87]. Notably, *IL7R* mutation associates with very poor prognosis in relapsed pediatric T-ALL cases[88]. Additionally, IL-7 promotes resistance to imatinib in Philadelphia-positive B-ALL cells *in vitro*[89] and likely contributes to the same phenomenon *in vivo*[90]. IL-7 may also mediate resistance to the mTOR inhibitor rapamycin[91], which may be of relevance also for T-ALL[73].

IL-7 signaling in other hematological tumors

An oncogenic role of the IL-7-IL-7R signaling axis may extend to other lymphoid tumors. For example, locally-produced IL-7 promotes proliferation, residence and expression of c-Myb and Bcl-2 in cutaneous T-cell lymphoma (CTCL) cells[34]. Other hematological tumors include chronic lymphocytic leukemia, T-cell prolymphocytic leukemia, and Hodgkin's lymphoma[59, 92].

Potential tumor suppressor functions of IL-7 signaling

Whereas excessive IL-7/IL-7R signaling is oncogenic, it is possible that, within particular spatial and developmental scenarios, diminished IL-7R-mediated signaling compromises homeostasis to a degree that paradoxically potentiates malignancy. In support of this notion, IL-7Rα deficiency potentiates lymphomagenesis in *p53*-null mice by exacerbating telomere erosion in T-cell precursors[93]. In such a scenario, *IL7R* appears to act more in the lines of a tumor suppressor than an oncogene. Likewise, IL-7R signaling prevents premature AID activation during B-cell development[94], protecting the B-cell genome and potentially preventing leukemogenesis. However, the alternative is that by preventing AID activity and excessive genomic instability, while

potentiating viability and proliferation, the IL-7/IL-7R axis actually creates the perfect milieu for transformation.

IL-7 and IL-7R in solid tumors

IL-7 stimulates multiple immune-mediated mechanisms that contribute to the eradication of tumors. We will, however, continue to explore the pathological role of IL-7/IL-7R-mediated signaling, which may extend beyond hematological cancers (**Fig. 5**). Epithelial cancers can aberrantly express IL-7Rα and IL-7 and may thus benefit from their activity[95-98]. Evidently, effects on the immune system, including promoting inflammation, that indirectly contribute to the generation of a pro-tumoral milieu are likely to occur[99]. The involvement of IL-7 and IL-7R in colon chronic inflammation agrees with the observation that patients with metastatic colorectal cancer display high IL-7 levels[42] and that the cancer tissue itself can secrete IL-7[100]. Notably, high IL-7 and IL-7R expression in tumor tissues of breast and lung cancer patients correlates positively with LN metastasis and poorer survival[95, 96, 101, 102]. Likewise, high IL-7 expression in prostate cancer[103] associates with poor prognosis[104]. These data are incompatible with increased T-cell anti-tumoral activity and rather suggest IL-7/IL-7R pathophysiologically-relevant activation loops in solid tumors. In agreement, *IL7R* gains were reported in lung[105], pancreatic[106] and esophageal[107] carcinomas. Notably, network analysis of recurrently mutated genes revealed IL-7-mediated signaling activation during clonal evolution of breast cancer[108].

Can IL-7 directly stimulate non-hematopoeitic cancer cells? *In vitro* studies using cell lines suggest so. IL-7 promoted proliferation[101], epithelial-mesenchymal transition (EMT) and metastasis[97] of human breast cancer cells. Likewise, IL-7 stimulated prostate and bladder cancer cell migration, invasiveness and EMT[104, 109]. In lung cancer, IL-7 stimulated the proliferation of tumor cells via cyclin D1 upregulation and appeared to promote tumor growth also in a subcutaneous xenotransplant mouse model[110]. The expression of IL-7R and cyclin D1 in the tumor

tissue of lung cancer patients correlated positively, and the latter associated with poor prognosis[110]. In a murine orthotopic breast cancer model, IL-7-expressing cancer-associated fibroblasts promoted tumor growth and maintenance of cancer stem-like cells[98]. IL-7 may also be critical in brain tumors, possibly contributing to resistance to cisplatin treatment in glioma[111]. IL-7 contribution to tumor development may further involve promotion of lymphangiogenesis [102] and osteoclastogenesis[112].

IL-7 traction for therapeutic purposes

IL-7 administration in cancer patients

Animal experiments consistently showed that IL-7 administered pharmacologically could induce T-cell expansion with little toxicity, suggesting IL-7 could have a therapeutic benefit in humans. Over 400 patients have now been treated with IL-7 with few toxicities other than a transient injection site reaction when administered subcutaneously (s.c.). In the earliest trials using non-glycosylated IL-7, anti-IL-7 antibodies were elicited, but this response has apparently been alleviated using glycosylated IL-7 produced by Revimmune (formerly Cytheris Corp).

Cancer patients were the first humans to be treated with IL-7. Although there were no apparent anti-cancer benefits of IL-7, dramatic immunological effects were reported. The first trial included twelve metastatic melanoma and sarcoma patients[113]. Administration s.c. showed a serum half-life of about 12hrs. Injected 8 times, 3 days apart, the highest IL-7 dose elicited a 3-7-fold increase in circulating naïve T-cells, no increase in Tregs and, in some patients, an increase in B-cell progenitors in BM. A subsequent trial in 16 subjects with refractory cancer[114] confirmed the increase in naïve T (but not Tregs), showed this was likely due to increased cycling and Bcl-2-associated resistance to apoptosis, and demonstrated increased TCR diversity. Decreased Treg frequency was an important distinction from treatments with IL-2, a related γc cytokine, which had the opposite effect. A study in lymphopenic breast cancer patients showed that IL-7 treatment

before (but not during) chemotherapy increased CD4 counts[115]. A trial in pediatric sarcoma patients combined a tumor vaccine with IL-7, confirming IL-7 induced an increase in T-cells and and decreased Treg frequency, but without anti-cancer benefit[116]. A trial combining IL-7 with the vaccine Provenge in prostate cancer (CITN-03) showed a doubling of PSA-reactive T-cells that could predict a benefit in a sufficiently powered study. IL-7-Fc (NeoImmune Tech/Genexine) was reported to be well-tolerated and to increase T-cells in healthy subjects (AACR 2019 abstract), is in phase 1b/2a trials combined with Temozolomide in glioblastoma, and with the checkpoint inhibitor Atezolizumab (anti-PD-L1) in skin cancers, melanoma, Merkel Cell carcinoma and cutaneous squamous cell cancer, and with Pembrolizumab (anti-PD-1) in triple negative breast cancer. IL-7-Fc will be combined with CAR-T in a trial in acute lymphoblastic leukemia. A trial that will combine Revimmune IL-7 with Atezolizumab in urothelial cancer (NCT03513952) will begin accruing soon.

In hematopoietic stem cell transplant patients, recovery of host T-cells after ablation was increased 2-fold by IL-7 in a trial of 12 patients treated for three weeks. Increases were largely in memory T-cells with augmented TCR diversity and *in vitro* CMV responses[114]. A trial of IL-7 in cord blood transplantation (RevImmune/MD Anderson NCT03600896) is underway.

IL-7 administration in AIDS and other infectious diseases

AIDS was the first infectious disease to be investigated with IL-7 therapy. In 13 HIV-infected patients who were concurrently treated with anti-retrovirals but had relatively low CD4 T-cell counts, IL-7 increased CD4 and CD8 T-cells[117]. Increases, mainly in naïve and central memory T-cells, were up to 7-fold and counts remained elevated up to 45 weeks after discontinuation of IL-7. In 25 HIV-infected patients who were treated with anti-retrovirals, increased central memory T-cells were seen 14 days after a single injection s.c. of IL-7[118, 119]. Concerns that IL-7 could promote HIV replication were not confirmed, since only a transient increase in viral transcripts was

observed in a fraction of patients. Mucosa-associated invariant T-cells are deficient in HIV patients despite anti-retroviral-treatment. Increases of these cells by 4-5-fold were reported 12 weeks following three s.c. IL-7 injections[119]. IL-7 also elevated T-cell counts in idiopathic (non-HIV) CD4-deficient patients[120]. There are discussions of testing IL-7 in AIDS patients with mycobacterial infection and in patients with multi-drug resistant bacterial infections.

Progressive multifocal leukoencephalopathy (PML) is a severe, rare central nervous system infection caused by JC virus in immunodeficient patients. A single patient with idiopathic CD4 deficiency and PML, after an initial treatment with antivirals, was treated with IL-7 showing a doubling of blood CD4 T-cells and disappearance of viral sequences in cerebrospinal fluid[121]. Subsequent case reports of compassionate use of IL-7 in PML reported CD4 increases and patient improvement[122].

Sepsis is frequently fatal despite treatment with antibiotics. Recent studies have shown that much of this morbidity results not from the initiating pathogen but from loss of CD4 T-cells and a subsequent rise in secondary infections[123]. IL-7 *ex-vivo* was shown to greatly increase IFNγ production by T-cells from septic patients[124]. A Phase 1 trial of IL-7 in septic patients showed significant improvement in CD4 counts, although subject numbers were too few to determine whether there was a clinical benefit[125]. A larger trial of IL-7 given IV in sepsis patients is planned (NCT03821038). At NCI, IL-7-Fc will be combined with a set of pathogen vaccines in aging patients with cancer to determine if expansion of T-cell repertoire has functional effects.

IL-7R pathway antagonists in the clinic

As mentioned above, IL-7/IL-7R signaling has been implicated in autoimmune and chronic inflammatory diseases, as well as in cancer. Therapeutic targeting of the pathway could occur at several levels[126] and a number of clinical trials are underway and planned.

Anti-IL-7R monoclonal antibodies

Blocking IL-7R with Mabs could blunt effector T-cells but spare Tregs in autoimmune disease. An antagonist anti-IL-7Rα Mab (Pfizer), tested in cynomolgus monkeys, blocked IL-7 in T-cells *ex-vivo*[127] and is in Phase I trials in type 1 diabetic and multiple sclerosis patients. Another antagonist anti-IL-7Rα Mab (GSK), in trial in multiple sclerosis, was halted prematurely for undisclosed reasons. However, a Phase IIa study in primary Sjogren's syndrome is underway. An antagonist anti- IL-7Rα Mab (OSE Immunotherapeutics) was tested in baboons, showed inhibition of a recall DTH response without T-cell depletion[128] , and blocked human T-cell homing to the gut in humanized mice[45]. A Phase I clinical trial is planned in healthy volunteers, aiming for Phase II evaluation in IBD and Sjogren's syndrome. Cytotoxic anti-IL-7Rα Mabs, shown to be effective in translational studies in T-cell acute lymphoblastic leukemia[129], have been developed at NCI (Hixon et al submitted for publication) and will be evaluated in clinical trials by Fannin LLC.

Pharmacological signaling inhibitors

The IL-7R pathway offers new druggable targets in ALL[126] that are beginning to be explored clinically. As mentioned above, ruxolitinib showed therapeutic benefit in translational studies of T-ALL [77, 130], especially in ETP-ALL cases [48, 87]. St Jude Children's Research Hospital will incorporate ruxolitinib into front-line therapy for ETP-ALL (NCT03117751). Probably a much broader set of T-ALL (and B-ALL) patients would benefit from treatment with inhibitors of IL-7R signaling.

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Author Contributions

JTB, SKD and BS wrote the manuscript and approved its final version.

Conflict of interest disclosures

SKD is a co-inventor on the patent application "IL-7R-alpha specific antibodies for treating acute lymphoblastic leukemia" filed by the NIH (DHHS). U.S. Patent Application No. 62/238,612. The other authors declare no conflict of interest.

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Figure Legends

Figure 1. Atlas of IL-7 production. IL-7 is produced in both primary (bone marrow and thymus) and secondary (lymph nodes and spleen) lymphoid organs where it supports development and maintenance of lymphocyte populations. However, production is also detected in non-lymphoid sites such as mucosal and epithelial layers, where it is important for maintaining intact barrier, as well as other sites such as the brain, where function is less clear.

Figure 2. Developmental functions of IL-7. During hematopoiesis, IL-7 signaling is important in supporting development of multiple lymphoid lineages. IL-7 signaling promotes commitment of progenitors to B-cell fate, but is also essential for development of group 2 and group 3 ILCs in the bone marrow. In the thymus, IL-7 signaling promotes γδ T cell lineage development but is also essential to support expansion of DN4 αβ thymocytes as they proliferate and fill the thymus with DP thymocytes ready for selection.

Figure 3. Potential mechanisms promoting the outgrowth of leukemia cells in the context of IL-7 stimulation. Several scenarios may explain how a limited resource such as IL-7 may benefit more (pre)leukemic cells than normal lymphoid precursors. **(a)** Maturation arrest is a characteristic of both B- and T-ALL cells that protects them from stringent selection steps that necessarily occur as a lymphoid precursor differentiates in the bone marrow or thymus, while fully benefiting from the survival and proliferative signals arising from IL-7 signaling. **(b)** Oncogenic hits such as MYC activation often lead to cancer development only if complemented by secondary hits that counterbalance oncogene-induced apoptosis. IL-7 provides non-cell-autonomous pro-survival cues that should allow (pre)leukemic cells to thrive, buying time for subsequent fully-transforming lesions to occur and subsequent (more aggressive) leukemia development. **(c)** Different genetic

lesions such as intracellular Notch (ICN1), ZEB2 translocation (tZEB2) or mutations in ribosomal genes (RPLmut) in T-ALL, or SH2B3 or PAX5 deficiency in B-ALL, lead to IL-7Rα upregulation and consequent IL-7 hypersensitivity. **(d)** On the other hand, (pre)leukemic cells can downregulate the production of IL-7 by stromal cells in such a way that normal precursors fail to fully access an essential factor for their development, while full transformation or hyperresponsiveness to IL-7 may allow for expansion of the malignant cells in low IL-7-containing niches. **(e)** IL-7 signaling differs subtly between normal T-cells and T-ALL cells. STAT5 and PI3K/Akt/mTOR signaling are both absolutely required for T-ALL cell survival and cell cycle progression - although, in contrast to normal T cells, STAT5 does not appear to upregulate Bcl-2 downstream from IL-7 stimulation. IL-7 promotes cell cycle progression in normal T-cells via PI3K/Akt/mTOR signaling and viability via STAT5.

Figure 4. Classes of *IL7R* gain-of-function mutants in leukemia. (a) IL-7Rα mutants found in leukemia patients fall into 4 classes, affecting either exon 6 (Type 1) or, less frequently, exon 5 (Type 2). The latter are all S185C variants affecting the trasmembrane domain and occur exclusively in B-ALL patients. Mutations affecting exon 6 are: type 1a) insertions or insertions-deletions introducing an unpaired cysteine in the extracellular juxtamembrane-transmembrane (EJMT) region; type 1b) lie deeper in the transmembrane domain region and include tryptophans, SxxxG-related motifs, or other residues; type 1c) occur in the EJMT region but lead to the introduction of negatively-charged amino acids such as arginine (R) or histidine (H). **(b)** Specificities on receptor dimerization and signaling resulting from each class of mutations.

Figure 5. Potential pro-tumoral functions of IL-7R-mediated signaling in solid cancers. *In vitro* and *in vivo* studies suggest that the IL-7/IL-7R axis is implicated in promoting proliferation, epithelial-mesenchymal transition (EMT), migration, invasion and metastasis, as well as resistance

to chemotherapy of cancer cells, while also stimulating osteoclastogenesis in bone metastasis and lymphangiogenesis. In agreement, *IL7R* is amplified in different cancers and high levels of IL-7 and/or IL-7R can be associated with poor prognosis.

Figure 1

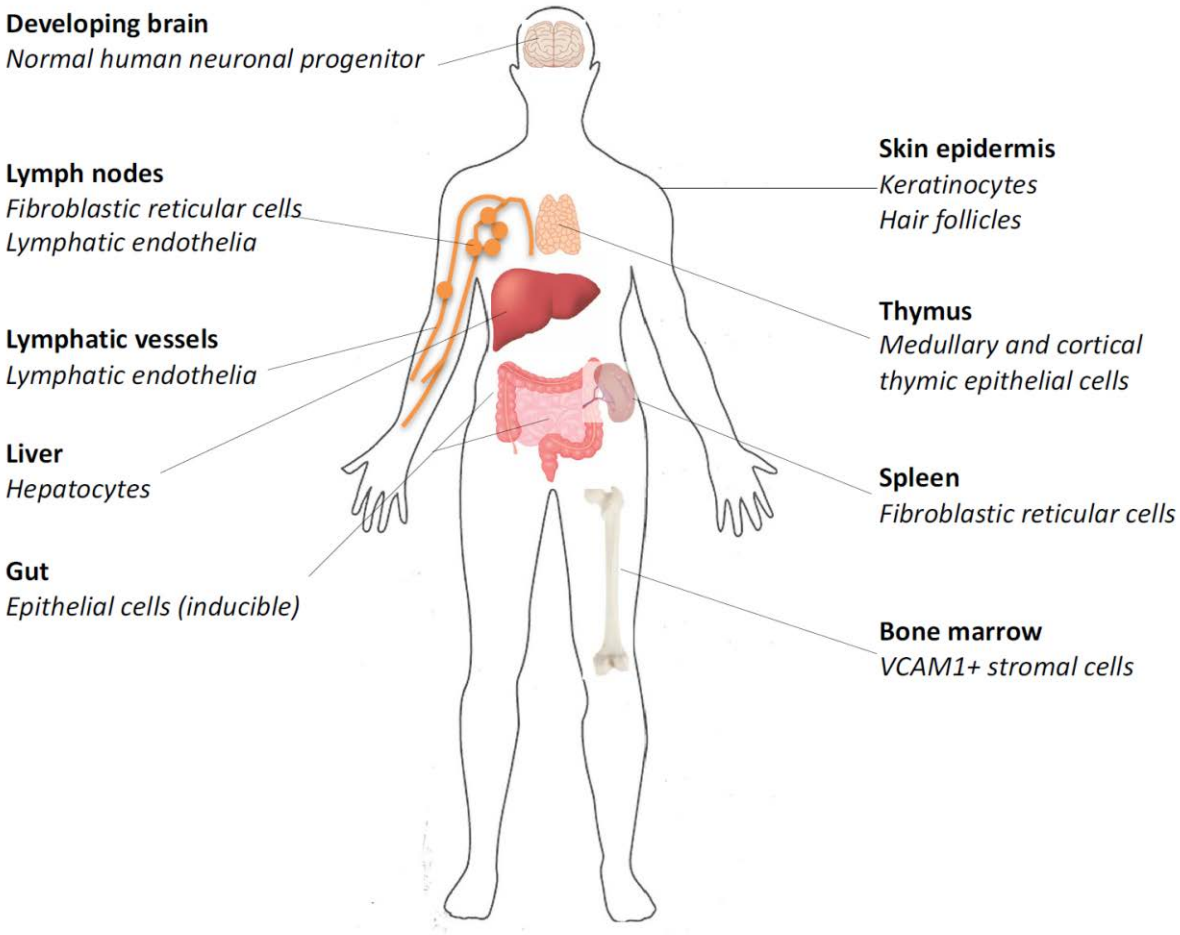

Developing brain
Normal human neuronal progenitor
Lymph nodes
Fibroblastic reticular cells
Lymphatic endothelia
Lymphatic vessels
Lymphatic endothelia
Liver
Hepatocytes
Gut
Epithelial cells (inducible)
Skin epidermis
Keratinocytes
Hair follicles
Thymus
Medullary and cortical
thymic epithelial cells
Spleen
Fibroblastic reticular cells
Bone marrow
VCAM1+ stromal cells

Figure 2

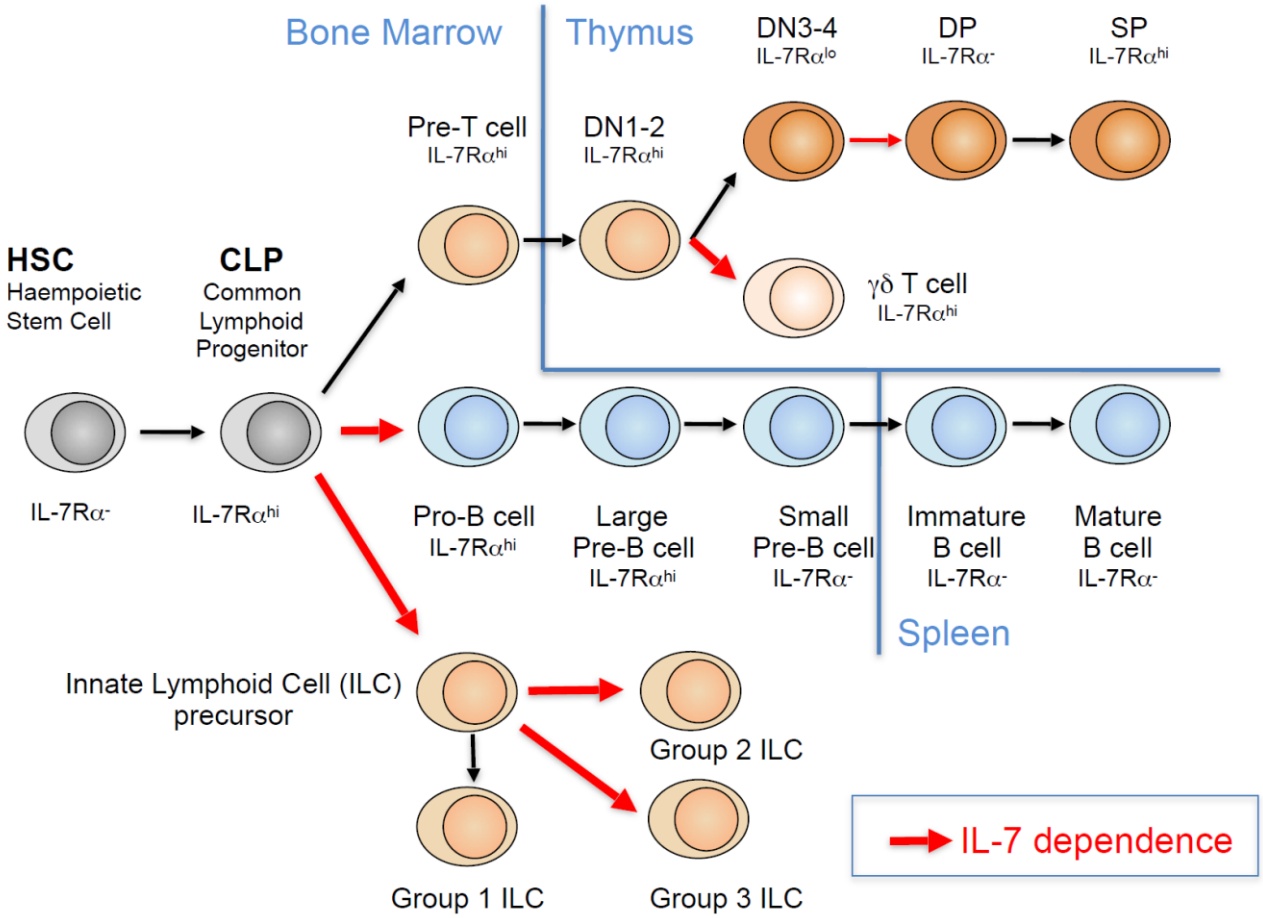
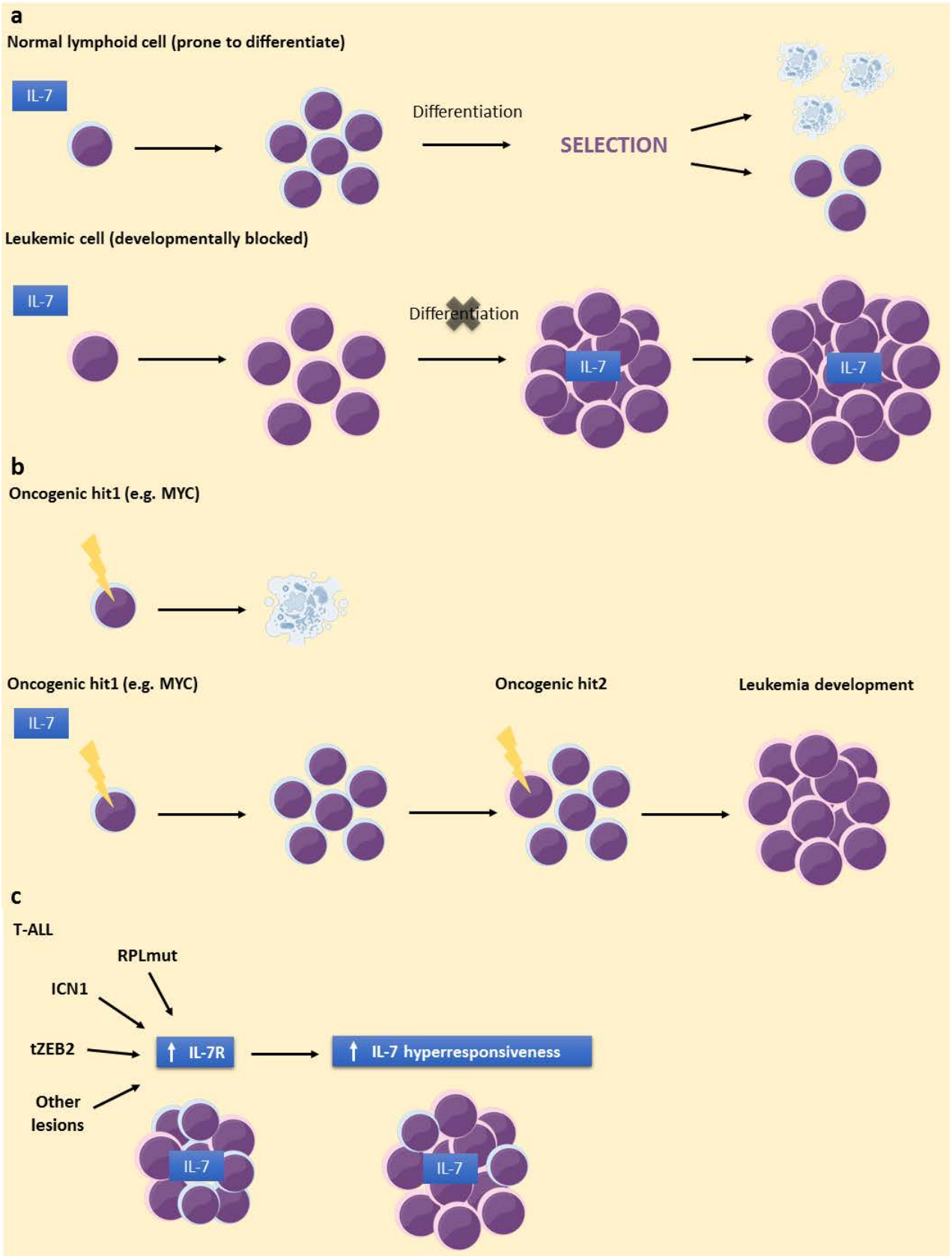
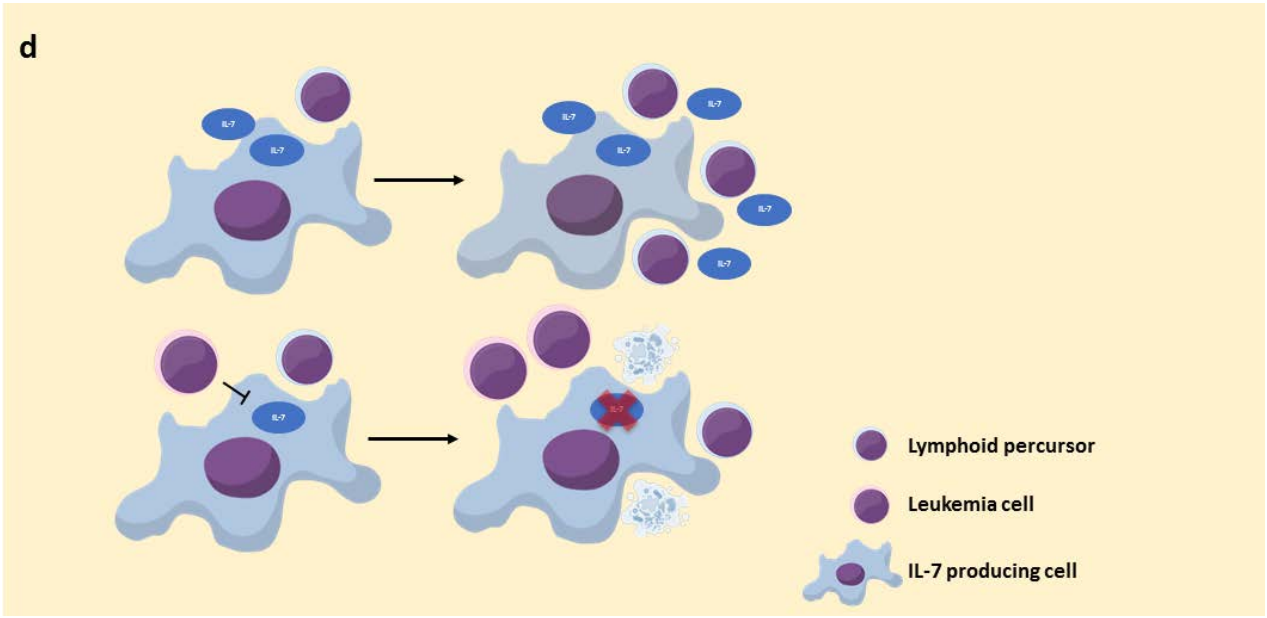

Bone Marrow
Thymus
DN3-4
IL-7Rα^{lo}
DP
IL-7Rα^{-}
SP
IL-7Rα^{hi}
Pre-T cell
IL-7Rα^{hi}
DN1-2
IL-7Rα^{hi}
HSC
Haempoietic
Stem Cell
CLP
Common
Lymphoid
Progenitor
γδ T cell
IL-7Rα^{hi}
IL-7Rα^{-}
IL-7Rα^{hi}
Pro-B cell
IL-7Rα^{hi}
Large
Pre-B cell
IL-7Rα^{hi}
Small
Pre-B cell
IL-7Rα^{-}
Immature
B cell
IL-7Rα^{-}
Mature
B cell
IL-7Rα^{-}
Spleen
Innate Lymphoid Cell (ILC)
precursor
Group 2 ILC
Group 1 ILC
Group 3 ILC
IL-7 dependence

Figure 3


a
Normal lymphoid cell (prone to differentiate)
IL-7
Differentiation
SELECTION
Leukemic cell (developmentally blocked)
IL-7
Differentiation
IL-7
IL-7
b
Oncogenic hit1 (e.g. MYC)
Oncogenic hit1 (e.g. MYC)
Oncogenic hit2
Leukemia development
IL-7
c
T-ALL
RPLmut
ICN1
tZEB2
IL-7R
IL-7 hyperresponsiveness
Other lesions
IL-7
IL-7


d
IL-7
Lymphoid percursor
Leukemia cell
IL-7 producing cell

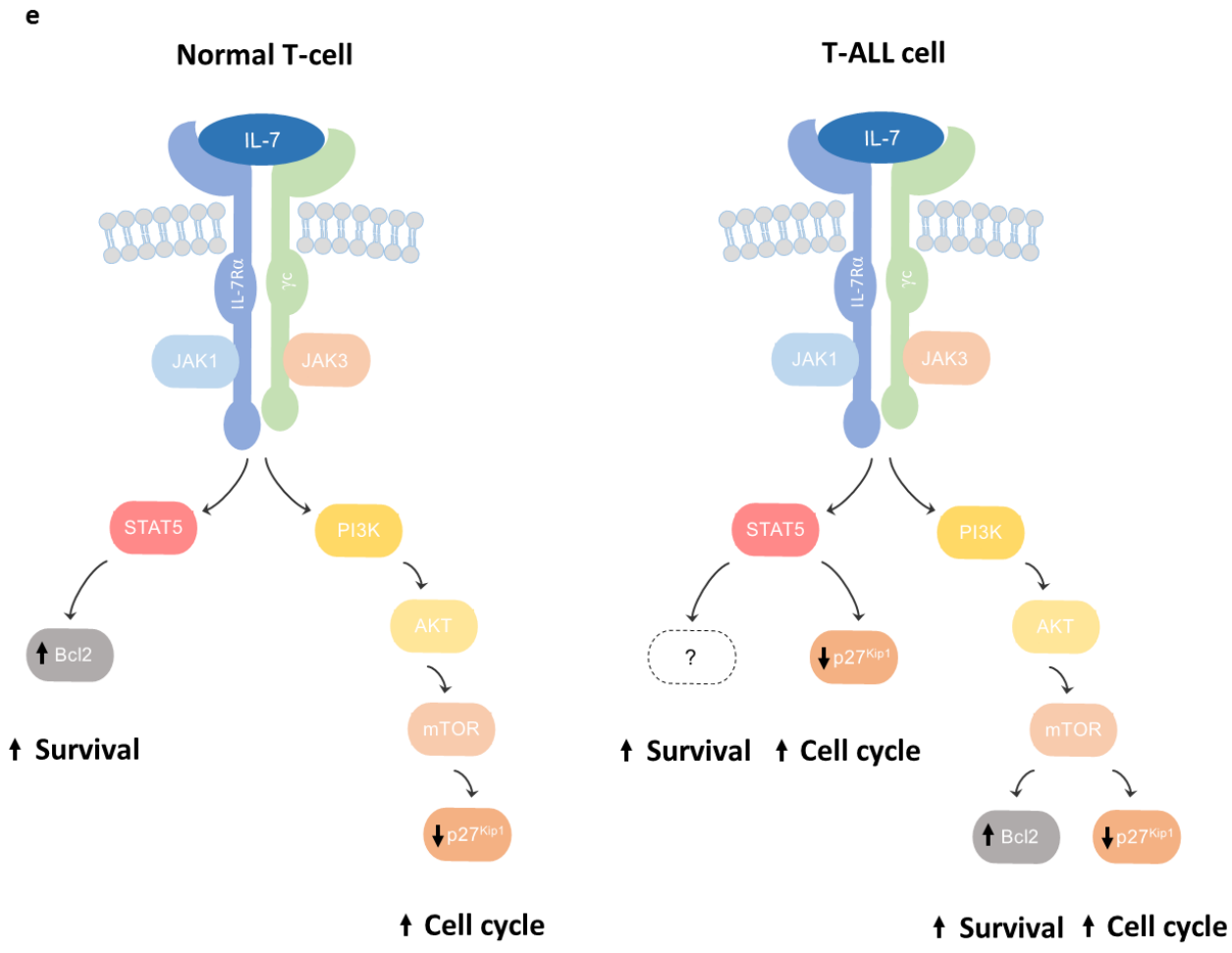

e
Normal T-cell
T-ALL cell
IL-7
IL-7Rα
γc
JAK1
JAK3
STAT5
PI3K
AKT
mTOR
Bcl2
p27Kip1
?
↑ Survival
↑ Cell cycle

Figure 4

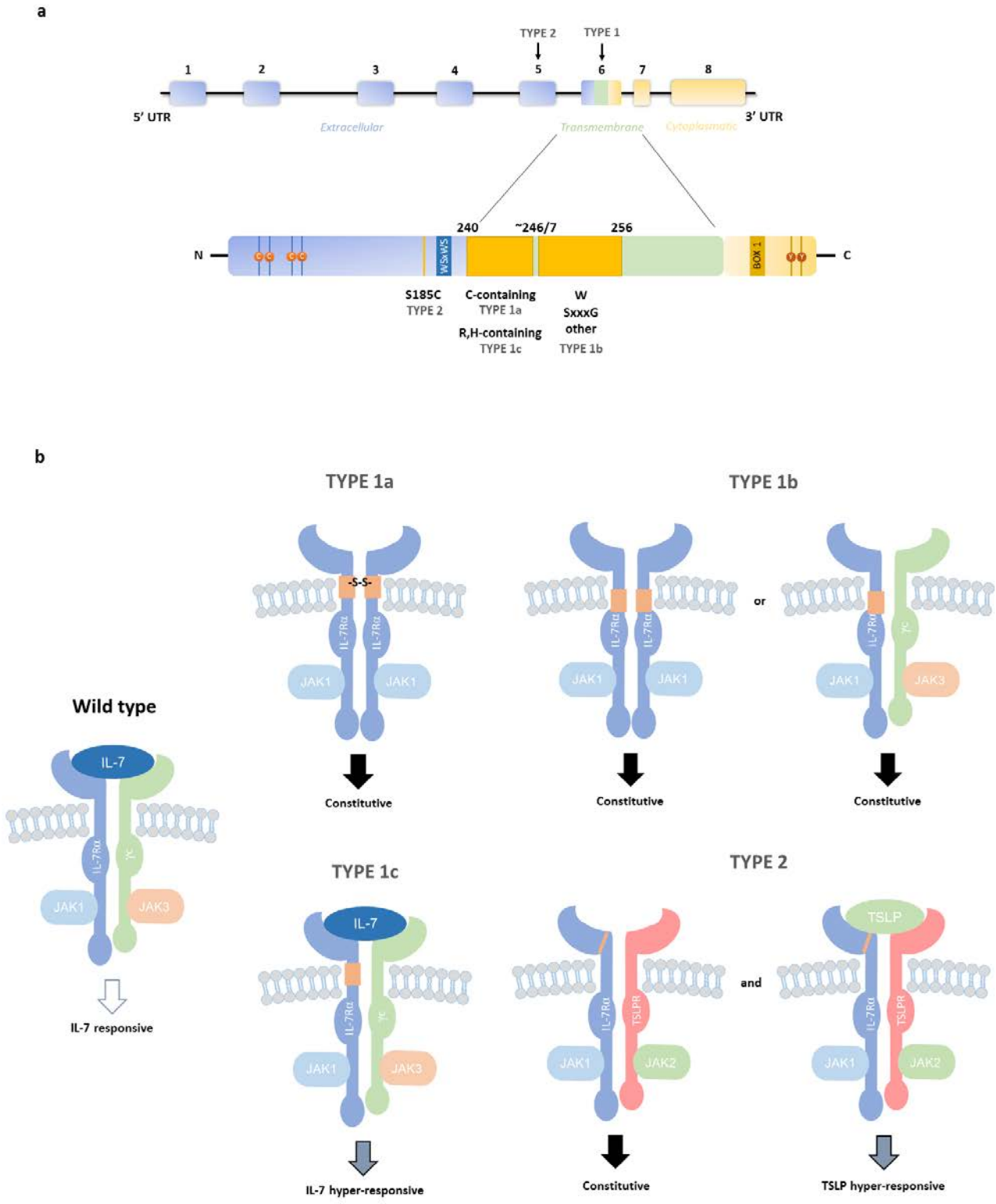

a
TYPE 2
TYPE 1
1
2
3
4
5
6
7
8
5' UTR
3' UTR
Extracellular
Transmembrane
Cytoplasmatic
240
~246/7
256
N
C
WSxWSA
BOX 1
S185C
TYPE 2
C-containing
TYPE 1a
R,H-containing
TYPE 1c
W
SxxxG
other
TYPE 1b
b
TYPE 1a
TYPE 1b
-S-S-
IL-7Rα
JAK1
JAK3
or
Constitutive
Wild type
IL-7
IL-7 responsive
TYPE 1c
TYPE 2
IL-7 hyper-responsive
TSLP
TSLPR
JAK2
and
TSLP hyper-responsive

Figure 5

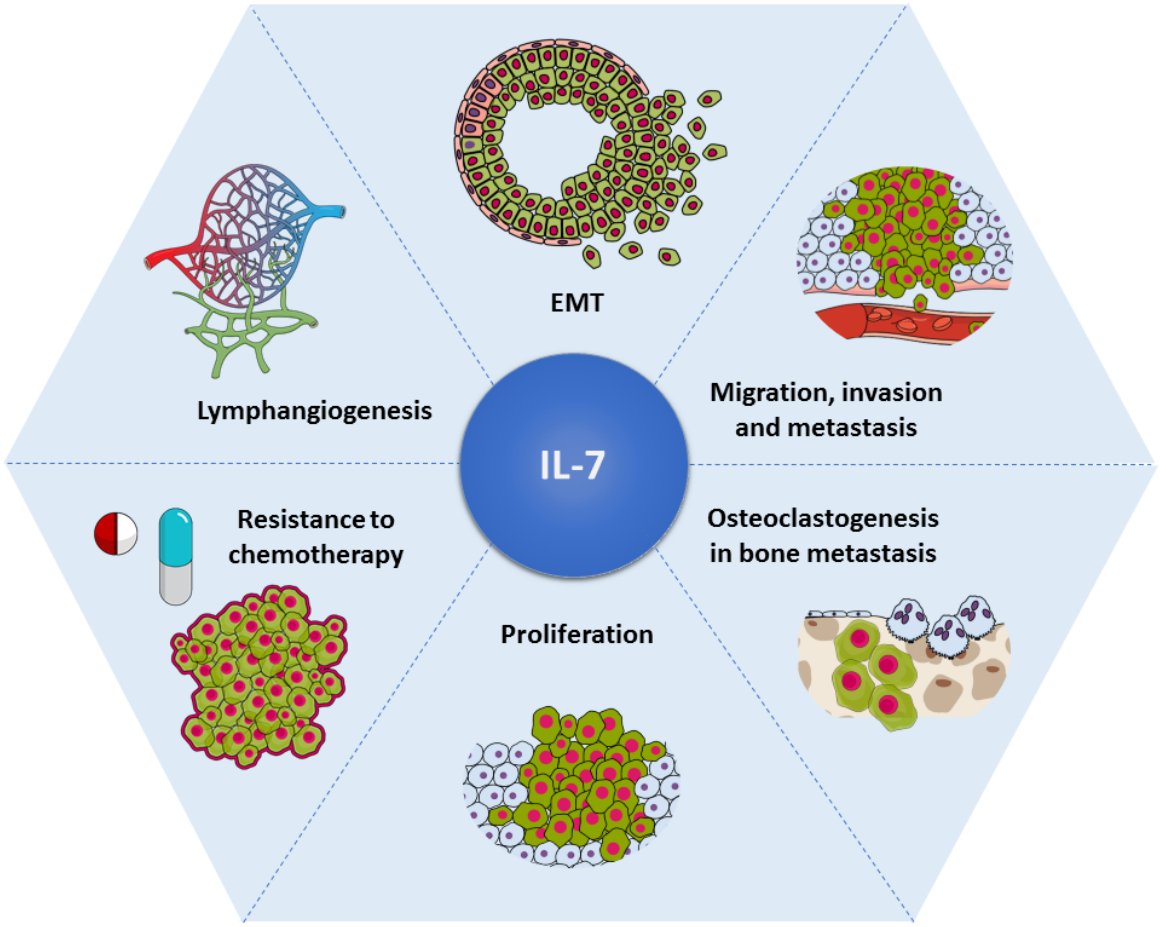

EMT
Migration, invasion and metastasis
Osteoclastogenesis in bone metastasis
Proliferation
Resistance to chemotherapy
Lymphangiogenesis
IL-7

Table 1. IL-7/IL-7R agonists and antagonists in human subjects

Product	Source	Disease	Current/future trials
IL-7	Revimmune (Cytheris)	Cancers	CITN-03, NCT03513952
		HSCT	NCT03600896
		HIV	
		CD4 deficiency	
		Myco, MDR	future
		PML	
		Sepsis	NCT03821038
IL-7-Fc	NeoImmune Tech	Cancers	current and future
		Elderly	plus vaccine - future
αIL-7R	Pfizer	T1D, MS	current
	GSK	Sjogren's	current
	OSE	IBD, Sjogren's	future
	NCI/Fannin	ALL	future

Abbreviations: Hematopoietic stem cell transplantation (HSCT), human immunodeficiency virus (HIV), mycobacterial infection in AIDS patients (Myco), multi-drug resistant bacterial infections (MDR), progressive multifocal leukoencephalopathy (PML), type 1 diabetes (T1D), multiple sclerosis (MS), inflammatory bowel disease (IBD), acute lymphoblastic leukemia (ALL).