A novel therapeutic strategy targeting multiple members of the γc-family cytokines; principles, relevance and potentially broad clinical applications.

Authors:

Yutaka Tagaya[1]

Nazli Azimi[2]

Affiliations:

[1] Cell Biology Lab, Division of Basic Science, Institute of Human Virology, University of Maryland School of Medicine, Baltimore MD 21201

[2] BIONIZ Therapeutics, Irvine, CA

Corresponding author:

Yutaka Tagaya

E-mail:
YTagaya@ihv.umaryland.edu

Abstract

Cytokines play diverse roles in normal and abnormal immunity. Dysregulated production of cytokines underlies a variety of disorders in humans by causing improper immune responses. Intervention to such abnormal cytokine action has been utilized in the clinical field as "anti-cytokine therapy" and proven effective as treatments. However the current anti-cytokine approaches lack effective and safe options for treating human diseases involving more than two cytokines as the pathogenic reason. This is an issue as the list of multi-cytokine diseases is expanding. To address this, a novel technology was developed by generating a novel class of multi-cytokine inhibitors (MCI). In short, cytokine-mimetic peptides were rationally designed each of which contains a motif shared only by the cytokines of the target and tested using specific biological assay. Peptides showing desired antagonistic activity were screened further for target specificity. The lead MCI, BNZ 132-1, specifically inhibits IL-2, -9 and -15 while it does not affect other γc-cytokines (IL-4, -7. and -21) or non-γc cytokines. This design ensures safety of the peptide upon clinical use by limiting off-target effects to the minimum, unlike small-molecule Jak kinase inhibitors which suppress a diverse array of cytokines and cause multiple adverse effects. Currently, we are conducting clinical trials involving BNZ 132-1 and saw expected transient decrease of select subsets of lymphocytes with minimum toxicity. BNZ 132-1 may provide a novel opportunity for treating many diseases such as myelopathy caused by human T-cell leukemia virus-1 (HTLV-1), non-viral human T-cell malignancies, autoimmunity (Alopecia areata, rheumatoid arthritis), graft-versus-host disease (GvH) upon transplant and cytokine release syndromes post microbial infections.

Introduction.

In the following sections, we will:

1. describe the basic nature of the γc-cytokines and their receptor which are primary therapeutic targets for us.

2. discuss about an emerging concept of "multi-cytokine diseases" in which functionally redundant cytokines from a single family are pathogenically involved.

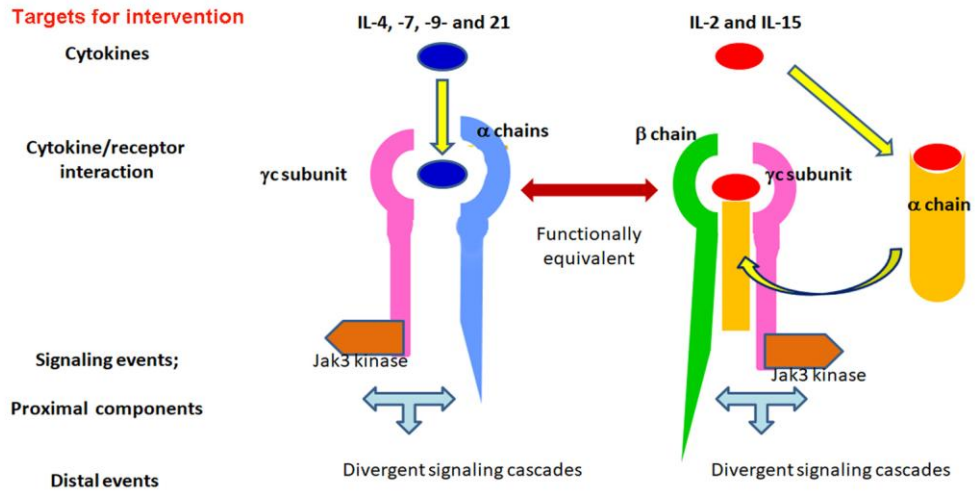
3. overview the currently available anti-cytokine strategy in the context of treating multi-cytokine diseases, which involves the special discussion on the limitations of current anti-cytokine strategy.

4. present our new BNZ technology and elaborate on the underlying concept, in vitro proof-of-concept data, its potential applications, preclinical data and on the on-going clinical trial involving the BNZ 132-1 peptide, the lead compound from our new technology.

1. The γc-family cytokines.

Since these are the intervention targets of our interest, we will quickly review the intriguing characteristics of this family of cytokines, as the function and receptor configuration of these cytokines directly connect with the objective, challenges and the uniqueness of our endeavor.

Figure 1. Anatomy of the γc-receptor system for the rational design of therapeutic interventions



The composition of multi-subunit receptors for γc-cytokines is depicted. In this family, IL-2 and IL-15 form a subfamily of their own and the receptor configuration (three subunits instead of two) illustrates the uniqueness of the subfamily. The α chains for IL-2 and IL-15 are not a signaling subunit, but mediate the unique trans-presentation for IL-15 (66). In the cascade of ligand to intracellular signaling components, the γc and Jak3 are the point of conversion and targeting these molecules is the logical choice to block multiple γc-cytokines by a single agent.

Cytokines are small polypeptides (Molecular weight ranging from 5 to 40kDa) that induce activation and differentiation of various hematopoietic cells (1, 2). Most, if not all, cytokines assume a distinct topology called "the four-helix bundles" structure (1) and the γc-family cytokines, namely IL-2, -4, -7, -9, -15 and -21, belong to this group. All γc-cytokines use the common gamma (γc, CD132) as a subunit in their multi-component receptor systems. Since γc is a signal transducing molecule, these cytokines naturally possess overlapping signaling pathways and display functional redundancy. The physiologic roles of each cytokine have been elucidated in detail using gene-targeting (Knock-out or KO) and transgenesis technologies in mice (3-19). Basically all 6 γc-cytokines are required for preparing various types of immune cells and participate in dictating the proper direction of the immune response upon microbial invasion and from other causes, as their biological relevance is well demonstrated by the phenotype of mice defective of the shared γc-receptor which have no T, B, NK cells and manifest severe immunodeficiency (20, 21) . We have a long history of studying the mechanisms, receptors, and biological functions of γc-cytokines because of our interest in the activation and development of T and NK cells.

2. Why blocking multiple-cytokines is a unique and worthy scientific goal?

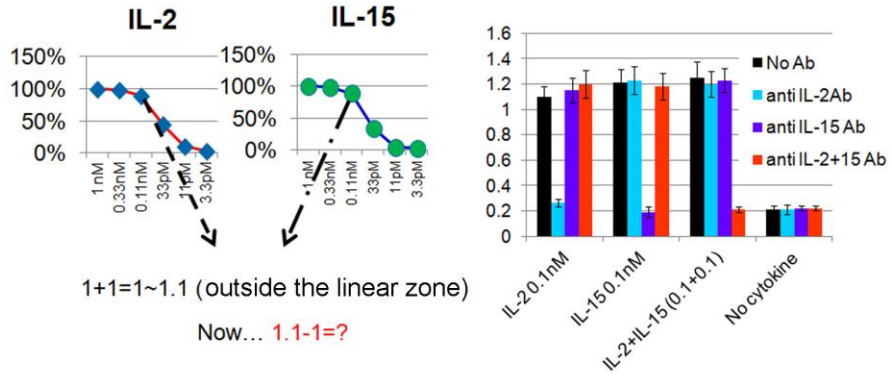
2-a. Human diseases caused by more than two cytokines (multi-cytokine diseases); Though cytokines belonging to the same family show functional redundancy, each cytokine has diverse unique functions (called the pleiotropic nature). Uncontrolled production or the lack of a cytokine leads to pathogenic conditions as demonstrated by the phenotypes of cytokine knock-out or transgenic mice. In humans, there are many examples of diseases caused by the abnormal functioning of cytokine (cytokine diseases). Anti-cytokine therapy has been long developed to treat cytokine diseases. However, we conducted a literature search and noted that quite a few cytokine diseases involve more than two cytokines (and very likely those belonging to the same family) (22) and that the current anti-cytokine strategy has limitations in dealing with cytokine diseases involving multiple cytokines (22), which prompted us to design and develop a unique group of cytokine inhibitors which can block more than two cytokines for treating such multi-cytokine diseases. One example of multi-cytokine diseases is a myelopathy associated with HTLV-1 (human T-cell leukemia virus 1) called HAM/TSP (HTLV-1 associated myelopathy/tropical spastic paraparesis) (23, 24). HAM/TSP is a progressive and chronic disease of the spinal cord seen in 0.3~0.5% of HTLV-1 infected individuals, manifesting painful stiffness and weakness of the legs. In HAM/TSP patients, T lymphocytes which cause pathogenesis cross the blood-brain barrier and damage the spinal cord by the production of proinflammatory factors and by the direct cytotoxic action. As we showed in our publications, T cells are constitutively activated in vivo in these patients and spontaneously proliferate when they were transferred to ex vivo culture without the addition of mitogens or cytokines (25, 26). We later discovered that two γc-cytokines, namely IL-2 and IL-15 are transcriptionally activated by the HTLV-1, and cause the autocrine proliferation and activation of CD8 T cells, a process likely leading to the tissue damage in HAM/TSP. We also demonstrated that both IL-2 and IL-15 need to be blocked in order to subside the pathogenic activation of T cells from HAM/TSP patients (22, 27-30). Examples of multi-cytokine disease are increasing in number. There are more examples of multi-cytokine diseases. To name a few,

rheumatoid arthritis which may involve IL-15, -21, -6, and -7 as pathogenic factors (31-33) and Celiac Disease which may involve IL-2, -10, -15 and -21 (34-41). Co-production of cytokines belonging to a family may not be too rare. It is reasonable to assume that cytokines belonging to a family evolved through gene duplication (of the cytokine and of the receptor subunits) and thus the regulatory mechanisms still share common elements. We use IL-2 and IL-15 as an example to look into this. IL-15 and IL-2 form a subfamily in the γc-cytokine family because they share another signaling subunit, IL-2R/IL-15Rβ (CD122) in addition to the γc (Figure 1). Despite the functional similarities between IL-2 and IL-15, their production seems strictly separated from each other under normal circumstances. IL-2 is almost exclusively produced by T cells as was demonstrated in an IL-2/GFP knock-in mouse (42) whereas IL-15 is produced by activated monocytes, dendritic cells, and stromal cells (43, 44). Yet, their promoter regions share a few motifs including the one for NFkB (45), accounting for the fact that Toll-like receptor stimulation in monocytes lead to the production of IL-15 by monocytes and T-cell activation results in production of IL-2 by T cells through the activation of the NFkB pathway. Moreover, the co-production of IL-2 and -15 in HAM/TSP seems to involve the transcriptional activation of these two genes by the trans-activator Tax of HTLV-1 (45) through the activation of the NFkB pathway. Therefore, it may not be surprising that IL-2 and IL-15 are simultaneously produced from different cells through an overlapping molecular mechanism, in particular under pathological conditions. This would apply to the co-production of multiple cytokines belonging to the same family.

2-b. A pitfall - neutralization of one of the factors in a multi-cytokine disease may not show visible effects even though the factor is pathogenically involved; After identifying that a human disease belongs to the category of cytokine disease, the logical strategy is to follow a reductionist approach; To gather information to identify the suspect cytokine and to test if neutralization of the target cytokine would effectively block the pathogenic signs associated with the disease. Even if the involvement of multiple factors was anticipated, the intuitive assumption is that the blockade of "a major factor" should have visible impacts. However this intuition may be misleading. We have shown this in our recent publication (22) but will discuss it here as it is a critical point of our argument; If two functionally redundant cytokines (IL-2 and IL-15 on T or NK cells is a perfect example) are stimulating the target cells as a 1:1 mixture, how much of the biological activity will be neutralized when only neutralizing anti-IL-2 or anti-IL-15 antibody (Ab) was added? The intuitive answer would be "a 50% inhibition". However, this is not true at some circumstances, because the biological response of a cell is not always linear to the dose of cytokine. The dose-response relationship follows a sigmoid-curve. At the near-saturating dose, additional increase of the stimulant no longer guarantees visible increase of the response. In reversal, anti-IL-2 antibody added to a mixture of 0.5 nM each of IL-2 and IL-15 to PHA-activated human T lymphocytes (which responds to both cytokines at high affinity; ~50 pM) would bring down the 100% response of the cells only to 90~95% (Figure 2). This was experimentally verified in our previous publication (22). The important take-home-message is that the "lack of a visible inhibition" using an antibody to one cytokine when more than two cytokines are cooperating to cause cellular activation does

not allow to eliminate the target cytokine from the equation. In other words, **no visible inhibition does not mean no participation of the target cytokine**. This may become more critical in vivo because it is difficult to know how many cytokines are in operation and how much is the contribution by each involved cytokine. For example, if cytokine A was highly suspected in the cause of a disease but antibody therapy targeting cytokine A failed. There might have been an example of this scenario in the actual clinical trials involving Asthma. Asthma has been connected to multiple cytokines including IL-4, IL-13, IL-9, and IL-5 in previous studies (16, 46-54). Accordingly, clinical trials involving anti-IL-4 and anti-IL-9 therapy have been approved but separately conducted (47, 48, 55-57). Unfortunately, neither gave rise to a satisfactory therapeutic result. This may simply mean that other cytokines are equally participating in the cause of the disease thus a co-inhibition of both factors could be a "simple" solution. However, the implementation of this strategy may not be as simple as it may look like.

Figure 2 Co-inhibition is the only way to effectivley block cytokine-cooperation



On the left shown is a typical proliferative response of mitogen-activated T cells to various doses of IL-2 or IL-15. They respond to low-concentrations of cytokines because they express high-affinity (Kd; ~50pM) IL-2 and IL-15 receptors. Importantly, when a cytokine doses pass over half-saturation concentration (which is equal to the Kd value), cellular responses are no longer linear to the increase of the dose of the cytokine. When the saturating doses of IL-2 and IL-15 were combined, they no longer create a response over that attained by either cytokine (1+1=1 in this non-linear world). Inversion of the equation (1-1=1) illustrates neutralization efforts on only one of the two cytokines (leaving the other cytokine at a saturation dose) which leads to almost zero inhibition of the cellular response. The other side of the coin is that an antibody to a cytokine may fail in vivo to show any visible inhibition even thought the target cytokine is relevantly involved in the activation of the target cell. This observation quite often misleads us into a conclusion that "the cytokine we studied was not involved in the pathogenic activation".

On the right shown is an actual experimental result that we conducted in support of our argument. Note that the same dose of anti-IL-2 (or anti-IL-15) mAb effectively neutralized the single target cytokine, but shows negligible effect when two cytokines are combined (the third group from left).

2-c. Technical limitations of the current anti-cytokine therapy; as demonstrated above, monotherapy may not only be rather ineffective but also the failure of it may mislead the researchers to a wrong conclusion when dealing with multiple-cytokine diseases. It is particularly true for cytokine diseases because of the unique nature of the cytokine that most of them belong to families and have multiple siblings/relatives sharing biological functions. So what about combining antibodies (the most preferred modality in anti-cytokine therapy) to neutralizing multiple cytokines? This is very simple with in vitro experiments, though we wish to point out that a random combination of antibodies may be a waste of resource, energy and time so that one has to build up a rational strategy before proceeding to the execution. However, a clinical application of combinatorial use of antibodies for therapeutic purposes seems infeasible, due to the expensive nature of the antibody therapy. The goal can still be attained by using other modalities than antibodies. We will discuss it below.

3. Rational approach to design multi-cytokine inhibitors

We will start from a general discussion on how to rationally design a cytokine intervention, and shift the emphasis to γc-cytokines which are of our interest.

3-a. Targeting the ligand (cytokine). As shown above in Figure 1, there are multiple-levels of targets when intercepting cytokine function. Neutralizing antibodies are available for most cytokines. However, as discussed above, a monoclonal anti-cytokine antibody (mAb) is not suited for treating multi-cytokine diseases, unless the antibody is bispecific (58). However, generating a bispecific antibody is a laborious work and is still in the early developmental stage for clinical applications.

3-b-1. Targeting a shared receptor component. The next level target is the cytokine receptor. More to the point, the aim is **targeting the shared receptor component.** Many cytokines share receptor components with other cytokines due to their evolution through gene duplication. This goal can be accomplished in general by using anti-cytokine receptor antibodies against the shared component. A soluble version of the shared receptor component can be used as well. Alternatively, a ligand analog that lacks agonistic function (antagonistic cytokine mimetics, (59)) can be a potent inhibitor. With the γc-cytokines, however, no neutralizing anti-γc antibody exists. This is perhaps because the binding surface of the γc is highly structurally conserved across species so the production of antibodies recognizing this portion may be blocked by the immune tolerance mechanism. As mentioned above, IL-2 and IL-15 share another signaling receptor subunit, IL-2R/IL-15Rβ (CD122). Hetero-dimerization of γc and β are needed for signal transduction by IL-2 or IL-15 (60) so inhibiting the β-cytokine interaction can block the signaling triggered by IL-2 or IL-15. There exist a few neutralizing anti-β antibodies including Mikβ1 (61). The humanized Mikβ1 has been tested in clinical trials involving T-cell large granular lymphocyte(LGL)-leukemia (62) and proposed for a diverse array of disorders including Celiac disease and other autoimmunity in which IL-2 and IL-15 might allegedly be pathogenic. Nevertheless, there is a setback for this antibody as well. However, we need to know a little more about the dual mode of IL-15 receptor configuration in order to understand this issue.

3-b-2. The *trans*- and *cis*-mode of IL-15 action. As mentioned above, the β/γc hetero-dimer is the functional core of the IL-2/IL-15 receptor, However, these two are not sufficient for the "in vivo" function of these cytokines because they only bind target cytokines at relatively low affinity (Kd; 0.7 nM) and thus unable to bind them at their physiological concentrations (10~100 pM). For the IL-2, a ligand-specific component IL-2Rα (CD25) joins and converts the β/γc receptor complex to a high-affinity IL-2 receptor (Kd; 10 pM). The in vivo functional IL-2R assumes an IL-2Rα -IL-2R/IL-15Rβ-γc hetero-trimeric configuration. However, IL-15R is structurally different. Like the IL-2Rα (CD25), there is an IL-15-specific binding partner called IL-15Rα. However, this molecule behaves as a component of a hetero-dimeric single cytokine (like IL-12 and its siblings) which consists of a complex of IL-15 and IL-15Rα (hence calling the latter molecule "a receptor" may not be entirely accurate though it is a binding protein to IL-15) (63-65). They can be formed as a membrane-bound form on activated dendritic cells (DCs) and monocytes and then be presented in *trans* to T or NK cells which only expresses the β-γc molecules (the *trans*-presentation mechanism, originally shown by us and validated by others (63-83). Several lines of studies indicated that this is the major physiologic form of IL-15 in vivo. In other words, the β-γc complex expressed on the surface of resting T or NK cells in the absence of IL-2Rα/CD25, is a natural receptor for IL-15 which can be brought to T/NK cells by monocytes or dendritic cells expressing the IL-15/IL-15Rα complexes on the surface. IL-15Rα can be induced on the surface of T/NK cells and converts the β-γc complex to respond to soluble IL-15 monomer (IL-15R in the *cis*-configuration), but we have observed that CD8 T cells constitutively expressing IL-15Rα are more prone to developing into leukemia (84), a plausible reason why the trans-configuration of the IL-15R might be preferred by nature. Now let us go back to the issue of cytokine-neutralizing anti-IL-2R/IL-15Rβ antibody. The setback is that the Mikβ1 mAb only effectively blocks the action of IL-2 or IL-15 when the private α is <u>not</u> present on the same cells that express the β/γ. This means IL-2 signaling in vivo cannot be efficiently blocked by this antibody (since the only physiological IL-2R consists of all 3 chains expressed by the same cell). Interestingly, the *trans*-action of IL-15 can be efficiently blocked by Mikβ1, but the *cis*-action is not (62). As we and others have indicated in the past, *trans*-IL-15 action is preferred upon normal immune responses, therefore Mikβ1 is effective in blocking such IL-15 actions in vivo. However, pathologic IL-15 action such as the one seen with CD8 T-cell leukemia seems to require the cis-IL-15R configuration by target cells (84) and the Mikβ1 mAb cannot effectively block this. Thus, Mikβ1 may have limitations for blocking IL-2, IL-15 or both under disease conditions.

3-b-3. Cytokine mimetics. The cytokine-mimetics are recent developments. These are fragments from a cytokine or mutated cytokines with altered functions. Small peptides containing the active site (one that is used for interacting with receptor subunit) of cytokines can mimic the cytokine action and trigger a signal (agnostic mimetics). Alternatively, they can block the active binding site on the cytokine receptor and inhibit the action of cytokines (antagonistic mimetics). For therapeutic purposes, antagonistic mimetics are perhaps more beneficial. Examples include IL-4 mimetics (85) which have been tested in clinical trials targeting Asthma with limited success (86, 87). Importantly, an IL-4 mutant (tyrosine at residue 124 mutated to

aspartic acid, Y124D) which has good binding affinity to IL-4Rα with no signaling capability can block both IL-4 and IL-13 actions due to the sharing of IL-4Rα between these two cytokines (88). In a way, this IL-4 mutant (hIL-4Y124D) is a prototype multi-cytokine inhibitor. Agonistic cytokine mimetics have been tested to substitute the cytokine, but they are not the major focus for this review. Examples include those for erythropoietin (89), thrombopoietin (90, 91), interferon (92), TGF-β (93), hepatocyte growth factor (94), and chemokines (95), which are being tested in clinical applications. Our BNZ peptides (BNZ-1~3) belong to antagonistic small-molecule γc-cytokine mimetics.

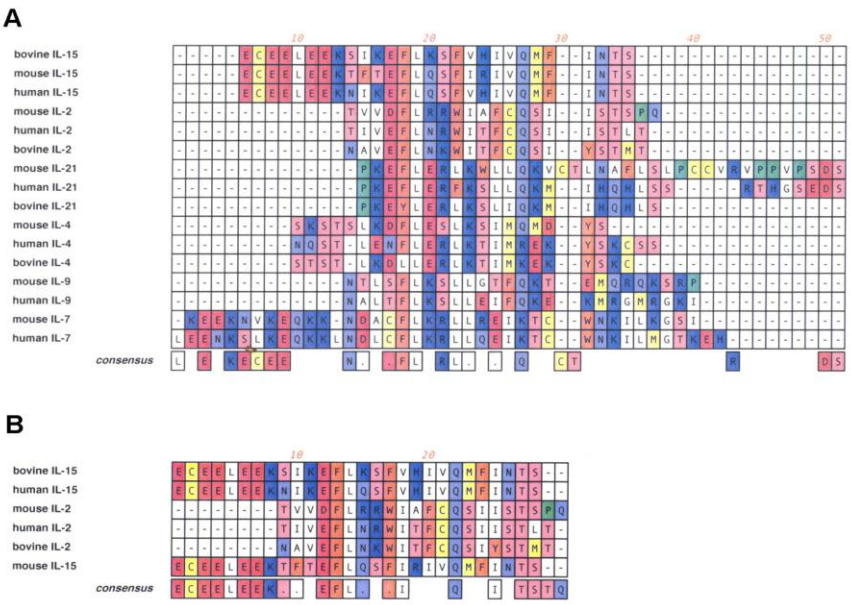
3-b-4 Soluble receptors. There have been attempts to generate soluble cytokine receptors for therapeutic purposes (96). Because soluble cytokine receptors lack the signal transducing elements, but still bind to the cytokine with similar affinity as do membrane-bound counterparts, they can compete with the membrane-bound receptors for the cytokine binding and function as inhibitors. Related to the γc-cytokine world, soluble IL-4R presents an intriguing example of multi-cytokine inhibition, as it blocks both IL-4 and IL-13 actions due to the IL-4Rα sharing between IL-4 and IL-13 as mentioned above (88, 97). Though IL-13 is not a γc-cytokine, IL-4 and IL-13 form a subfamily of their own, which has been implicated in Asthma. Thus, modalities (e.g., antibodies, mutant IL-4, and soluble IL-4R) neutralizing these cytokines have been tested in clinical trials with some success (47, 48, 98). However, only monotherapy targeting each cytokine has been tested so it is not yet clear if a co-inhibition of IL-4 and IL-13 has superior therapeutic effects to those attained by the single cytokine targeting.

3-c. Targeting Signaling molecules. Another class of targets for intervening cytokine action includes signaling molecules. Unlike many hormone receptors (e.g., those for insulin, EGF, and Growth Hormones), cytokine receptor molecules generally lack an intrinsic kinase activity. Instead, members of the Janus kinase family (Jak1, Jak2, Jak3, and Tyk2, reviewed in (99)) specifically interact with cytokine receptors and help transducing the signal. The membrane proximal events are probably easier to target because this is where the entire cascade of cytokine signaling becomes convergent. In particular, the γc-cytokine family presents a unique case because not only all γc-cytokines use Jak3, but also the Jak3 usage seems exclusively limited to γc-cytokines (100-107). This is underscored by the observation that Jak3 knockout mice (108) or humans with loss-of-function Jak3 mutations (105) show identical phenotypes to γc-knockout mice (20, 109) or humans with defective γc-molecule (X-linked SCID, (110)). A brilliant idea was proposed by John O'shea's group, based on their own discovery of Jak3 and of its exclusive use by γc-cytokines, to target Jak3 for blocking actions by a single or multiple γc-cytokines, and they developed a Jak3 inhibitor (CP690, 550 (111)) in collaboration with Pfizer for this purpose. The compound has been approved by the FDA as a clinical drug 'Tofacitinib' (29, 84, 112-120). Currently Tofacitinib is being tested in clinical trials for the treatment of rheumatoid arthritis (114), graft-versus-host diseases associated with organ transplantation (111, 115, 119), inflammatory bowel disease/celiac disease (117, 120) and T-cell leukemia/lymphoma (22, 29, 84). We will further evaluate the efficacy and shortcomings of Tofacitinib as a clinical drug below.

4. Can we selectively target multiple γc-cytokines?

4-a. Safety concerns for the Jak3 inhibitor. So a question may arise as to why we needed to develop a new technology to block multiple γc-cytokines? Why are we not satisfied with the Jak3 inhibitor(s)? Here is our answer: though a Jak3 inhibitor would comprehensively block γc-cytokines, it does not allow us to selectively choose the target cytokine(s). What we want was to specifically choose target cytokines within the γc-family and block only them, but not other cytokines. In addition, it became clear that the inhibition by Tofacitinib is not limited to Jak3 (121) because it inhibits the kinase activity of Jak2 and Jak1 at high concentrations (122). Since Jak2 is critically involved in erythropoiesis and thrombopoiesis, a continued use of Tofacitinib might induce anemia and bleeding tendency (118, 123-126). Other concerns also cropped up as represented by the action of the European Commission which initially declined to approve Tofacitinib citrate (Xeljanz) for treating rheumatoid arthritis due to potential carcinogenesis, mutagenesis and infertility observed in animal studies. Eventually, it was recently approved in Europe as well, and there are on-going efforts to develop purely-Jak3 specific inhibitors (127) which will undoubtedly improve the safety of this strategy. The use of Jak3 inhibitor as a clinical drug will thus keep advancing. Nevertheless, we saw in our own hands that its clinical application may require further sophistication of the technology. First, Tofacitinib is a very potent inhibitor to all γc-cytokine in vitro. However, it does not seem to show similarly potent inhibitory effects in vivo. In our mouse model of IL-15-induced leukemogenesis in which the continued production of IL-15 by transgenesis leads to the development of CD8 T cell leukemia, anti-IL-15 modalities such as neutralizing mAb to IL-15 or mAb to IL-2R/IL-15Rβ perfectly protected those mice from leukemic death (22, 29, 84). So did BNZ 132-1 (22). Tofacitinib was administered using subcutaneous pump, but failed to protect host mice from leukemic death, only prolonged their survival (22, 29, 84). This may be due to the hydrophobic chemical nature of Tofacitinib, which makes it difficult to maintain the compound at effective dose all the time in vivo. Furthermore, the strategy of blocking Jak3 kinase itself may present another setback, because it blocks IL-7. IL-7 is a uniquely important member of the γc-family because IL-7 broadly maintains the homeostasis and development of multiple lymphocyte lineages. Genetic perturbations or defects of the IL-7/IL-7R system lead to a profound loss of T and B cells in mice (9, 128), or a loss of T cells in humans (129). Normal numbers of B cells are present in the absence of the IL-7 signaling in human neonates, but a further study indicated that these individuals will develop B-cell dysfunction later in life (130). Thus, the impact of IL-7/IL-7R dysfunction is almost close to that of the loss of γc function itself in magnitude (SCID in mice (20, 109), and X-linked SCID in humans (110)). The only difference in IL-7- and γc-dysfunctions is the presence or absence of NK cells which are exclusively maintained by IL-15 (5, 131). These studies suggest that blocking IL-7 for a continued period of time may invite unfavorable outcomes as it could create severe immune suppression. We propose that a prolonged inhibition of IL-7 for therapeutic purposes should be avoided or kept at a minimum, unless IL-7 itself is the cause of the disease as reported in subgroups of T-cell or B-cell acute lymphocytic leukemia (T-ALL/B-ALL) (132, 133).

Figure 3, Alingment of D-helices of γc-cytokines from various species



Alignment was performed using the Clustal Omega algorism by the EMBL-EBI (151). The consensus sequence (A) contains many amino acids that participate in direct physical contact between the cytokine and γc-molecule, providing evidence that the sequence conservation is underscored by a structural reason.

Thus, we saw a need to develop a new technology to block IL-2 and IL-15 (or other combinations of multiple γc-cytokines, except IL-7) using a single agent. In a way, we tried to "shoot many γc-cytokines by a single stone with a precise target guidance system". We hypothesized that such blocking is possible by interrupting the interaction of γc-cytokines and the shared γc subunit. Though all γc-cytokines use a broad overlapping interface, there should be minor spots that are only occupied by select γc-cytokines, but not by others. By targeting such unique spots, we should block select γc-cytokine combinations.

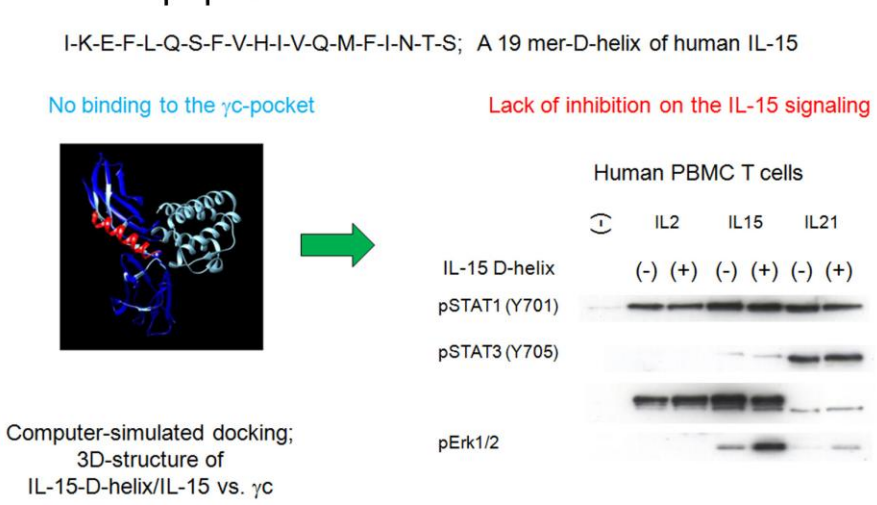
4-b. The BNZ technology. The past two decades have seen a tremendous advancement in our structural knowledge on the interaction between cytokines and receptors, owing to dedicated works by structural biologists. The IL-2/IL-15R system was no exception. The Garcia group (Stanford University) has conducted extensive studies on these receptor systems and mapped the amino acid residues by which various cytokines interact with their receptors. They have also delineated the binding interface utilized by IL-2 or IL-15 when they are binding to the γc-molecule (134-139). These studies taught us that the D-helix (the last of the four helical bundles, a structure shared by many cytokine molecules) of the γc-cytokines primarily interacts physically with the γc-subunit. Naturally, it makes sense that D-helix is the most conserved among the all four a-helices of a γc-cytokine across species (degree of similarities, D helix>B>A>C (22)). We noticed a moderately conserved motif

(which we named "the γc-box", (22)) in this region of 6-γc cytokines from many species (Figure 3A). We thus hypothesized that a peptide which mimics the sequence (and hence the 3D structure) of this region of γc-cytokines may have the capacity to inhibit multiple γc-cytokines. Since the amino acid sequences of the D-helices are moderately conserved, we hypothesized it would be possible to design a peptide which only resembles IL-2/IL-15, but not IL-4, -7, -9, or -21. However, this was not a simple task. We first synthesized a peptide corresponding to the D-helix of the human IL-15 protein, hoping this might be a specific inhibitor to IL-15, but not to other γc-cytokines. This peptide did not inhibit IL-15 (determined by the inhibition of the T/NK cell proliferation in vitro) (Figure 4). It later turned out that the IL-15's D-helix peptide has a very weak binding affinity to the γc so that it was easily competed off from the binding pocket by the natural ligand. We learned from this failure that we have to leverage the affinity of the peptide-γc interaction and specificity of the peptide in interfering with the binding of each cytokine to γc. So, we first carefully examined the published structural data concerning the binding of IL-15 to γc (138). This study identified several crucial amino acids of the D-helix of IL-2 and IL-15 which form the binding interface with the γc. We made these amino acids as the "backbone frame" of our peptides to ensure stable and tight binding of the peptides to the γc. We then compared the sequences of IL-2 and IL-15 across species and generated "a consensus sequence" which consists of "conserved" and "wobble (variable)" positions (Figure 3B, (22)). To the wobble positions, we assigned amino acids based on the usages by mammalian IL-2 and IL-15 molecules. We then ran rigorous computer docking simulation to rule out peptides that would not stably bind to the γc at the predicted binding surface. We then synthesized over 30 peptides which passed the computer testing and examined their inhibitory nature using a specific bioassay and came up with the BNZ 132-1 peptide (a 19-mer synthetic peptide with the sequence of "IKEFLQRFIHIVQSIINTS", (22, 30)).

5. Proof-of-concept of the BNZ technology

As shown in our recent publication, BNZ132-1 peptide efficiently blocks the in vitro function of IL-2 and IL-15 and to a lesser extent IL-9 as well (22, 30). We note that BNZ 132-1 has tight target specificity as it does not inhibit other γc-cytokines (e.g., IL-4, IL-7, IL-21) or non-γc cytokines (22). Our analysis indicated that the major signaling pathways downstream of the IL-2/IL-15/IL-9R are equally and completely blocked by BNZ 132-1 (22). To our knowledge this is the first example of a short peptide which blocks more than two cytokines from a family. We extended this technology to generate additional novel antagonistic cytokine mimetics which inhibit IL-15 and IL-21 (BNZ 132-2) and IL-4 and IL-9 (BNZ 132-3). It is also noteworthy that BNZ technology generates multi-cytokine inhibiting cytokine-mimetics without targeting IL-7, as we can exclude peptides which block IL-7 during the biological selection process. Though not yet published, we confirmed that BNZ 132-2, our second antagonistic mimetic peptide which blocks IL-15 and IL-21, does not block IL-7 function.

Figure 4 Failure of IL-15 inhibition by the D-helix peptide derived from human IL-15.



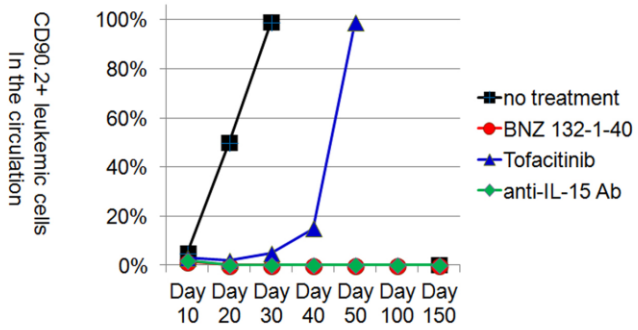
We first conducted a simple experiment to test our initial hypothesis that the D-helix peptide can be an antagonistic cytokine-mimetic agent. We ran a docking simulation (PatchDock algorism, (152, 153)) which predicted that this peptide does not form stable binding with the γc-molecule at the reported cytokine binding interface of this molecule (22). This highly suggests that the peptide may not work as an antagonist to γc-cytokines. We then conducted a biological assay in which we incubated mitogen-activated human T cells with low doses of γc-cytokines (IL-2; 50 pM, IL-15; 50 pM, IL-21; 10 pM) to induce tyrosine-phosphorylation of signaling molecules including STAT1, STAT3 and Erk1/2 as shown in the figure. We saw no inhibition of STAT1 and Erk1/2 which are signature signaling evens associated with IL-15 activation of T-lymphocytes. The signaling by IL-2 or IL-21 was not inhibited, either. This experiment taught us that the D-helix sequence needs to be optimized so that the peptide functions as an antagonist by competitively inhibiting the binding of target cytokines to the γc-molecule at the right interface.

6. Target diseases for the BNZ technology

When we chose IL-2 and IL-15 as the target and started designing the inhibitory peptide, we already had in mind that such a peptide can be used for treating HAM/TSP caused by human T-cell leukemia virus-1 (HTLV-1) (23, 24). The constitutive in vivo activation of T-lymphocytes in HAM/TSP patients is initiated by the Tax-1 protein (140) of this virus and maintained by the combination of IL-2 and IL-15 produced as a consequence of HTLV-1 activation of CD4 T cells as we and others have previously demonstrated (25-28, 45, 141). In addition to IL-2 and IL-15, a recent report suggests that the IL-9/IL-9R system is also playing a pertinent role in this disease (142). Incidentally, IL-9 is another target of BNZ 132-1 which was not originally anticipated by us when we designed BNZ132-1. As shown in our recent publication, BNZ132-1 alone can very efficiently block the spontaneous proliferation of T cells from multiple HAM/TSP patients (30). Furthermore, BNZ 132-1 also down-regulated various hallmark activation events associated with HAM/TSP T cells, including the tyrosine-phosphorylation of signaling molecule STAT5, and the survival of

HTLV-1 specific CD8 T cells (as determined by a Tax-tetramer staining) (30). Thus, BNZ 132-1 is a prime candidate by which to treat HAM/TSP patients. We expect that the PEGylated form of this peptide (see below) will stably circulate in the patients, subside the constitutive activation of T cells and revert the lymphokine-activated killer (LAK) cells and CTLs to resting state so that they will stop damaging neuronal tissues/cells in HAM/TSP patients. Subsequent studies made us convinced, however, that there are more diseases that can be efficiently treated by the BNZ 132-1 peptide. We will describe our recent progress below.

Figure 5 Expansion of IL-15 leukemic cells was completely suppresed by BNZ 132-1 in syngeneic mice



The IL-15 leukemic cells line was established from the IL-15 Tg mouse (C57B6, CD90.2 background) we generated (6, 84). These cells grow in syngeneic normal mice (C57B6 strains) and form secondary leukemia upon grafting. Without treatment, the recipient mice will die of CD8 T cell leukemia within 30 days of the injection of leukemic cells. The graph shows the preferential expansion of $CD90.2^+$ CD8 leukemic T cells in the $CD90.1^+$ congenic C57B6 mice as determined by flow cytometry (Black line). The only $CD90.2^+$ cells in these mice are from grafted IL-15 leukemic cells. Since these leukemic cells are dependent on IL-15 for their growth, injection of a neutralizing anti-IL-15 mAb (Mab247, R & D Systems, 150 µg /intraperitoneal injection, twice per week for two weeks) eradicates leukemic cells thus no CD90.2+ leukemic T cells are found in the blood or spleen of recipient mice (Green line). Treatment by BNZ132-1-40 (PEGylated form, 1 mg/kg per dose, intraperitoneal injections twice per week) shows similar protective effects (Red line) as anti-IL-15mAb treatment. Curiously, Tofacitinib, infused by a subcutaneous pump, only delayed the onset of explosive expansion of leukemic cells, but failed to show a complete remission (Blue line).

7. BNZ 132-1 as a therapeutic drug.

7-a. Stable Pharmacokinetic nature of BNZ 132-1. Since BNZ 132-1 is a short (19-mer) peptide, it will quickly be lost from the body as the result of rapid renal clearance. Thus, we tried a few modifications of the peptide to elongate the biological half life and PEGylation, a commonly used modifying technique for proteins and peptides, to enhance the pharmacokinetic (PK) properties of the peptide.

7-b. Pharmacodynamics of BNZ 132-1; The IL-15 leukemia model as the proving ground that BNZ 132-1 can be a therapeutic compound for LGL-type leukemia in humans.

After the confirmation that the PEGylated version (BNZ 132-1-40) has similar in vitro inhibiting activity on IL-2 or IL-15-geared cellular proliferation as the original peptide (22), Pharmacokinetics (PK) and pharmacodynamics (PD) of the drug were determined using mouse models of cytokine challenge. The over-expression of IL-2 leads to the preferential expansion of CD4 regulatory T cells (T-regs) ($CD4^+CD25^{hi}Foxp3^+$ T cells with suppressive function, reviewed in (143, 144)) as we have previously shown in the IL-2 transgenic (Tg) mouse that we generated (4). This can be imitated by administering recombinant human IL-2 into mice. Likewise, the injection of recombinant IL-15 into mice causes a temporal expansion of mouse NK cells (84). As reported (22), two bolus injections of recombinant human IL-2 or human IL-15 (150 μg/shot) led to a temporal expansion of T-regs or NK cells, respectively. This transient expansion of T-regs or NK cells were almost completely blocked by the concomitant administration of BNZ 132-1-40 (1 mg/kg, two injections over a week). These experiments convinced us that BNZ 132-1-40 is stable in vivo and can be a drug-candidate for humans. PK studies in the currently on-going phase I-a clinical trials validated that the $T_{1/2}$ of BNZ 132-1-40 is around 72 hr in normal individuals, thus we could maintain it above minimum effective dose (0.2~0.4 mg/kg) by injecting a single dose of 1~2 mg/kg BNZ 132-1-40 over the next 14 days, reaffirming that BNZ 132-1-40 is a stable compound suited for clinical use.

Next, we tested the in vivo efficacy of the PEGylated form of BNZ 132-1 in a clinically relevant model involving the over-production of IL-15. We and others had generated IL-15 transgenic (Tg) mice previously (6), and observed that they develop a fatal CD8 T cell leukemia (7, 8, 84). This experimental leukemia resembles human T-cell malignancies developing from CD8/LGL cells (7, 8). Using the IL-15 transgenic mouse they generated, the Caligiuri group (Ohio State University) demonstrated that this model recapitulates characteristics associated with human LGL leukemia and cutaneous T-cell leukemia (CTCL). In addition, they recently showed that the epigenetic modification which occurs at the *il15* locus in human LGL-leukemia patients can be observed in IL-15 Tg mice as well (145-147). Based on these observations, they proposed to treat patients with LGL leukemia and CTCL by anti-IL-15 therapies (147). Likewise, we explored our IL-15 Tg mouse model to test similar therapeutic possibility. As reported (84), we have established leukemic CD8 T cell clones from our IL-15 Tg mice. These cells can be expanded in culture without any addition of cytokines. These leukemic clones are still dependent on the IL-15 which they produce because the inclusion of anti-IL-15 antibody to the culture immediately stops cellular proliferation and induces apoptotic death (84). Importantly, these cells can be grafted into a syngeneic host mouse (the C57B6 strain) and kill the host within 4-6 weeks time by forming a secondary leukemia/lymphoma (29, 84). This model was used to test if BNZ 132-1 suppresses the leukemic growth of IL-15-producing CD8 T-cells and protects host mice from leukemic death. As control and comparison, anti-IL-15 neutralizing antibody and Tofacitinib were included in the experiment. Control mice without any treatment died within 30 days following the injection of IL-15 Tg leukemic cells (K2) as reported before (29, 84). Anti-IL-15, or anti-mouse IL-

2R/IL-15Rβ (anti-mouse CD122, TMβ1) mAb protected mice as expected. BNZ 132-1-40 (the PEGylated form), administered intravenously (IV)) 4 times (on days 1, 4, 8, and 11 after the injection of leukemic cells) at 1 mg/kg dose, prevented the mouse as efficiently as anti-cytokine/cytokine-receptor antibodies. Moreover, experiments were conducted to allow distinguishing grafted leukemic cells and host lymphocytes by congenic markers (i.e., leukemic cells are from CD90.2 C57B6 mice whereas the recipient mice are CD90.1 C57B6). A thorough flow-cytometric examination of the blood and secondary lymphoid organs revealed that there were no traceable $CD90.2^+$ leukemic cells remaining in host mice after the BNZ132-1-40 treatment. Autopsy and histological analyses validated the assessment. These data strongly suggest that BNZ132-1-40 has completely eradicated IL-15-dependent CD8 leukemic T cells from leukemic mice. Curiously, Tofacitinib, which was administered using a cutaneous pump showed only marginal therapeutic effects as described above (22, 29). These results suggest that BNZ132-1-40 may be a superior "in vivo blocker" of IL-2 or IL-15 than Tofacitinib.

7-c. IND approval and on-going clinical trials involving BNZ132-1. As described above, BNZ132-1 has received an IND approval from the FDA to conduct a phase I-a clinical trial targeting normal volunteers to evaluate its drug candidacy. In preclinical studies that we conducted before an IND submission, we saw reduction of NK and some other types of T cells in the mice and in the cynomologus macaques, as expected based on the known homeostatic roles of IL-15 to maintain these cells and those of IL-2 on T-regs, respectively. In the Phase I-a clinical trial that has just been completed, a significant decrease of NK cells and of other relevant lymphocyte subsets was observed following a single administration of BNZ 132-1-40 (0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/kg). The detailed results will be published elsewhere in the near future (manuscript in preparation). To apply BNZ132-1 to wider diseases, studies are in progress to test if BNZ132-1 effectively suppresses graft-versus-host (GvH) responses upon transplantation. Previous studies suggested that this process involves IL-15, but not IL-2 (148-150). However, in our experimental system we saw that only co-inhibition of IL-2 and IL-15 (by combining anti-IL-2 and anti-IL-15 antibodies, or by BNZ132-1-40), but not the blockade of a single cytokine, ameliorated the GvH symptoms (manuscript in preparation). This represents another compelling example in which monotherapy (targeting a single cytokine) fails to display therapeutic effects and multi-cytokine inhibition is needed for attaining the goal. Thus, we are continuing our efforts to obtain additional IND approvals on BNZ132-1 for treating other hematologic and immunologic diseases including GvH disease and autoimmunity.

Summary

Years ago, we observed that the ex vivo spontaneous proliferation of HAM/TSP T cells which is driven by a combination of almost equal amounts of IL-2 and IL-15 cannot be efficiently blocked by a single neutralizing mAb either to IL-2 or IL-15. This contrasted with our intuitive prediction in which we expected to see a near 50% neutralization by the individual antibody, which made us realize the fraud in our logic and a related gap in the existing anti-cytokine approach, and triggered subsequent research to develop a new tool (multi-cytokine inhibitor) that we have now. Our recent publications represent the proof-of-concept of our novel technology to rationally design multi-cytokine inhibitors.

Our research shows that an increasing number of human diseases may be efficiently treated by our multi-cytokine inhibitors (MCIs). Currently our compounds are still limited to blocking the γc-family cytokines, but we plan to develop new class of inhibitors that block cytokines belonging to different cytokine families..

Acknowledgements

The authors thank for the help received from members of the Tagaya lab (Marco Goicochea, Juan C. Zapata, Rick van Besien, Toshie Nata, Fiorenza Cocchi, Xiaorong Wu and Eugene Ateh) and the research lab at BIONIZ therapeutics (Asjad Basheer, and Nick Doerr) who have contributed, but not been included as authors of this article. We declare no financial conflicts of interests. This work was supported by internal funds from the Institute of Human Virology at the University of Maryland School of Medicine, and funds from BIONIZ therapeutics, Irvine, CA.

BNZ 132-1 and other BNZ peptides were protected by the US patent (08455449) and owned by the BIONIZ therapeutics.

Animal experiments were conducted at the BioQual Inc. (Rockville, MD) under protocols approved by the IACUC committee.

References

1. Bazan JF. Structural design and molecular evolution of a cytokine receptor superfamily. Proceedings of the National Academy of Sciences of the United States of America. 1990;87(18):6934-8. PubMed PMID: 2169613; PMCID: 54656.

2. Boulay JL, O'Shea JJ, Paul WE. Molecular phylogeny within type I cytokines and their cognate receptors. Immunity. 2003;19(2):159-63. PubMed PMID: 12932349.

3. Schorle H, Holtschke T, Hunig T, Schimpl A, Horak I. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. Nature. 1991;352(6336):621-4. doi: 10.1038/352621a0. PubMed PMID: 1830926.

4. Antony PA, Paulos CM, Ahmadzadeh M, Akpinarli A, Palmer DC, Sato N, Kaiser A, Hinrichs CS, Klebanoff CA, Tagaya Y, Restifo NP. IL-2-dependent mechanisms of tolerance and immunity in vivo. Journal of immunology. 2006;176(9):5255-66. PubMed PMID: 16621991; PMCID: 1473163.

5. Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, Matsuki N, Charrier K, Sedger L, Willis CR, Brasel K, Morrissey PJ, Stocking K, Schuh JC, Joyce S, Peschon JJ. Reversible defects in natural killer and memory CD8 T cell lineages in IL15-deficient mice. The Journal of experimental medicine. 2000;191(5):771-80. PubMed PMID: 10704459; PMCID: 2195858.

6. Marks-Konczalik J, Dubois S, Losi JM, Sabzevari H, Yamada N, Feigenbaum L, Waldmann TA, Tagaya Y. IL-2-induced activation-induced cell death is inhibited in IL-15 transgenic mice. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(21):11445-50. doi:10.1073/pnas.200363097. PubMed PMID: 11016962; PMCID: 17219.

7. Fehniger TA, Suzuki K, Ponnappan A, VanDeusen JB, Cooper MA, Florea SM, Freud AG, Robinson ML, Durbin J, Caligiuri MA. Fatal leukemia in IL15 transgenic mice follows early expansions in natural killer and memory phenotype CD8+ T cells. The Journal of experimental medicine. 2001;193(2):219-31. PubMed PMID: 11208862; PMCID: 2193336.

8. Fehniger TA, Suzuki K, Ponnappan A, VanDeusen JB, Cooper MA, Florea SM, Freud AG, Robinson ML, Durbin J, Caligiuri MA. Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8+ T cells. The Journal of experimental medicine. 2001;193(2):219-31. PubMed PMID: 11208862; PMCID: PMC2193336.

9. von Freeden-Jeffry U, Vieira P, Lucian LA, McNeil T, Burdach SE, Murray R. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. The Journal of experimental medicine. 1995;181(4):1519-26. PubMed PMID: 7699333; PMCID: PMC2191954.

10. Kuhn R, Rajewsky K, Muller W. Generation and analysis of interleukin-4 deficient mice. Science. 1991;254(5032):707-10. PubMed PMID: 1948049.

11. Noben-Trauth N, Kohler G, Burki K, Ledermann B. Efficient targeting of the IL-4 gene in a BALB/c embryonic stem cell line. Transgenic Res. 1996;5(6):487-91. PubMed PMID: 8840532.

12. Burstein HJ, Tepper RI, Leder P, Abbas AK. Humoral immune functions in IL-4 transgenic mice. Journal of immunology. 1991;147(9):2950-6. PubMed PMID: 1919000.

13. Tepper RI, Levinson DA, Stanger BZ, Campos-Torres J, Abbas AK, Leder P. IL-4 induces allergic-like inflammatory disease and alters T cell development in transgenic mice. Cell. 1990;62(3):457-67. PubMed PMID: 2116236.

14. Rich BE, Campos-Torres J, Tepper RI, Moreadith RW, Leder P. Cutaneous lymphoproliferation and lymphomas in interleukin 7 transgenic mice. The Journal of experimental medicine. 1993;177(2):305-16. PubMed PMID: 7678850; PMCID: PMC2190896.

15. Godfraind C, Louahed J, Faulkner H, Vink A, Warnier G, Grencis R, Renauld JC. Intraepithelial infiltration by mast cells with both connective tissue-type and mucosal-type characteristics in gut, trachea, and kidneys of IL-9 transgenic mice. Journal of immunology. 1998;160(8):3989-96. PubMed PMID: 9558107.

16. McLane MP, Haczku A, van de Rijn M, Weiss C, Ferrante V, MacDonald D, Renauld JC, Nicolaides NC, Holroyd KJ, Levitt RC. Interleukin-9 promotes allergen-induced eosinophilic inflammation and airway hyperresponsiveness in transgenic mice. American journal of respiratory cell and molecular biology. 1998;19(5):713-20. doi: 10.1165/ajrcmb.19.5.3457. PubMed PMID: 9806735.

17. Townsend JM, Fallon GP, Matthews JD, Smith P, Jolin EH, McKenzie NA. IL-9-deficient mice establish fundamental roles for IL-9 in pulmonary mastocytosis and goblet cell hyperplasia but not T cell development. Immunity. 2000;13(4):573-83. PubMed PMID: 11070175.

18. Brady J, Hayakawa Y, Smyth MJ, Nutt SL. IL-21 induces the functional maturation of murine NK cells. Journal of immunology. 2004;172(4):2048-58. PubMed PMID: 14764669.

19. Allard EL, Hardy MP, Leignadier J, Marquis M, Rooney J, Lehoux D, Labrecque N. Overexpression of IL-21 promotes massive CD8+ memory T cell accumulation. European journal of immunology. 2007;37(11):3069-77. doi: 10.1002/eji.200637017. PubMed PMID: 17918202.

20. Ohbo K, Suda T, Hashiyama M, Mantani A, Ikebe M, Miyakawa K, Moriyama M, Nakamura M, Katsuki M, Takahashi K, Yamamura K, Sugamura K. Modulation of hematopoiesis in mice with a truncated mutant of the interleukin-2 receptor gamma chain. Blood. 1996;87(3):956-67. PubMed PMID: 8562967.

21. Ikebe M, Miyakawa K, Takahashi K, Ohbo K, Nakamura M, Sugamura K, Suda T, Yamamura K, Tomita K. Lymphohaematopoietic abnormalities and systemic lymphoproliferative disorder in interleukin-2 receptor gamma chain-deficient mice. Int J Exp Pathol. 1997;78(3):133-48. PubMed PMID: 9306921; PMCID: PMC2694530.

22. Nata T, Basheer A, Cocchi F, van Besien R, Massoud R, Jacobson S, Azimi N, Tagaya Y. Targeting the binding interface on a shared receptor subunit of a cytokine family enables the inhibition of multiple member cytokines with selectable target spectrum. The Journal of biological chemistry. 2015;290(37):22338-51. doi: 10.1074/jbc.M115.661074. PubMed PMID: 26183780; PMCID: PMC4566211.

23. Osame M, Usuku K, Izumo S, Ijichi N, Amitani H, Igata A, Matsumoto M, Tara M.

HTLV-I associated myelopathy, a new clinical entity. Lancet. 1986;1(8488):1031-2. PubMed PMID: 2871307.

24. Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calender A, de The G. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet. 1985;2(8452):407-10. PubMed PMID: 2863442.

25. Tendler CL, Greenberg SJ, Blattner WA, Manns A, Murphy E, Fleisher T, Hanchard B, Morgan O, Burton JD, Nelson DL, et al. Transactivation of IL2 and its receptor induces immune activation in human T-cell lymphotropic virus type I-associated myelopathy: pathogenic implications and a rationale for immunotherapy. Proceedings of the National Academy of Sciences of the United States of America. 1990;87(13):5218-22. PubMed PMID: 2367534; PMCID: 54293.

26. Tendler CL, Greenberg SJ, Burton JD, Danielpour D, Kim SJ, Blattner WA, Manns A, Waldmann TA. Cytokine induction in HTLV-I associated myelopathy and adult T-cell leukemia: alternate molecular mechanisms underlying retroviral pathogenesis. J Cell Biochem. 1991;46(4):302-11. doi:10.1002/jcb.240460405. PubMed PMID: 1757474.

27. Azimi N, Jacobson S, Leist T, Waldmann TA. Involvement of IL-15 in the pathogenesis of human T lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis: implications for therapy with a monoclonal antibody directed to the IL-2/15R beta receptor. Journal of immunology. 1999;163(7):4064-72. PubMed PMID: 10491011.

28. Azimi N, Nagai M, Jacobson S, Waldmann TA. IL-15 plays a major role in the persistence of Tax-specific CD8 cells in HAM/TSP patients. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(25):14559-64. doi: 10.1073/pnas.251540598. PubMed PMID: 11717409; PMCID: 64721.

29. Ju W, Zhang M, Jiang JK, Thomas CJ, Oh U, Bryant BR, Chen J, Sato N, Tagaya Y, Morris JC, Janik JE, Jacobson S, Waldmann TA. CP-690,550, a therapeutic agent, inhibits cytokine-mediated Jak3 activation and proliferation of T cells from patients with ATL and HAM/TSP. Blood. 2011;117(6):1938-46. doi: 10.1182/blood-2010-09-305425. PubMed PMID: 21106989; PMCID: 3056641.

30. Massoud R, Enose-Akahata Y, Tagaya Y, Azimi N, Basheer A, Jacobson S. Common gamma-chain blocking peptide reduces in vitro immune activation markers in HTLV-1-associated myelopathy/tropical spastic paraparesis. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(35):11030-5. doi: 10.1073/pnas.1412626112. PubMed PMID: 26283355; PMCID: PMC4568234.

31. McInnes IB, Liew FY. Cytokine networks-towards new therapies for rheumatoid arthritis. Nature clinical practice Rheumatology. 2005;1(1):31-9. doi: 10.1038/ncprheum0020. PubMed PMID: 16932625.

32. Kishimoto T. IL-6: from its discovery to clinical applications. International immunology. 2010;22(5):347-52. doi: 10.1093/intimm/dxq030. PubMed PMID: 20410258.

33. Young DA, Hegen M, Ma HL, Whitters MJ, Albert LM, Lowe L, Senices M, Wu PW, Sibley B, Leathurby Y, Brown TP, Nickerson-Nutter C, Keith JC, Jr., Collins M. Blockade of the interleukin-21/interleukin-21 receptor pathway ameliorates disease in animal models of

rheumatoid arthritis. Arthritis and rheumatism. 2007;56(4):1152-63. doi: 10.1002/art.22452. PubMed PMID: 17393408.

34. Jabri B, de Serre NP, Cellier C, Evans K, Gache C, Carvalho C, Mougenot JF, Allez M, Jian R, Desreumaux P, Colombel JF, Matuchansky C, Cugnenc H, Lopez-Botet M, Vivier E, Moretta A, Roberts AI, Ebert EC, Guy-Grand D, Brousse N, Schmitz J, Cerf-Bensussan N. Selective expansion of intraepithelial lymphocytes expressing the HLA-E-specific natural killer receptor CD94 in celiac disease. Gastroenterology. 2000;118(5):867-79. PubMed PMID: 10784586.

35. Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T, Ebert EC, Green PH, Jabri B. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity. 2004;21(3):357-66. doi: 10.1016/j.immuni.2004.06.020. PubMed PMID: 15357947.

36. Meresse B, Curran SA, Ciszewski C, Orbelyan G, Setty M, Bhagat G, Lee L, Tretiakova M, Semrad C, Kistner E, Winchester RJ, Braud V, Lanier LL, Geraghty DE, Green PH, Guandalini S, Jabri B. Reprogramming of CTLs into natural killer-like cells in celiac disease. The Journal of experimental medicine. 2006;203(5):1343-55. doi: 10.1084/jem.20060028. PubMed PMID: 16682498; PMCID: 2121214.

37. Bhagat G, Naiyer AJ, Shah JG, Harper J, Jabri B, Wang TC, Green PH, Manavalan JS. Small intestinal CD8+TCRgammadelta+NKG2A+ intraepithelial lymphocytes have attributes of regulatory cells in patients with celiac disease. The Journal of clinical investigation. 2008;118(1):281-93. doi:10.1172/JCI30989. PubMed PMID: 18064301; PMCID: 2117760.

38. Tang F, Chen Z, Ciszewski C, Setty M, Solus J, Tretiakova M, Ebert E, Han J, Lin A, Guandalini S, Groh V, Spies T, Green P, Jabri B. Cytosolic PLA2 is required for CTL-mediated immunopathology of celiac disease via NKG2D and IL-15. The Journal of experimental medicine. 2009;206(3):707-19. doi:10.1084/jem.20071887. PubMed PMID: 19237603; PMCID: 2699120.

39. Abadie V, Discepolo V, Jabri B. Intraepithelial lymphocytes in celiac disease immunopathology. Seminars in immunopathology. 2012;34(4):551-66. doi: 10.1007/s00281-012-0316-x. PubMed PMID: 22660791.

40. Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. Nature. 2012;487(7405):104-8. doi: 10.1038/nature11225. PubMed PMID: 22722865; PMCID: 3393783.

41. Bodd M, Raki M, Tollefsen S, Fallang LE, Bergseng E, Lundin KE, Sollid LM. HLA-DQ2-restricted gluten-reactive T cells produce IL-21 but not IL-17 or IL-22. Mucosal immunology. 2010;3(6):594-601. doi: 10.1038/mi.2010.36. PubMed PMID: 20571486.

42. Naramura M, Hu RJ, Gu H. Mice with a fluorescent marker for interleukin 2 gene activation. Immunity. 1998;9(2):209-16. PubMed PMID: 9729041.

43. Bamford RN, DeFilippis AP, Azimi N, Kurys G, Waldmann TA. The 5' untranslated region, signal peptide, and the coding sequence of the carboxyl terminus of IL-15 participate in its multifaceted

translational control. Journal of immunology. 1998;160(9):4418-26. PubMed PMID: 9574546.

44. Waldmann TA, Tagaya Y. The multifaceted regulation of IL-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. Annual review of immunology. 1999;17:19-49. doi: 10.1146/annurev.immunol.17.1.19. PubMed PMID: 10358752.

45. Azimi N, Brown K, Bamford RN, Tagaya Y, Siebenlist U, Waldmann TA. Human T cell lymphotropic virus type I Tax protein trans-activates IL15 gene transcription through an NF-kappaB site. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(5):2452-7. PubMed PMID: 9482906; PMCID: 19372.

46. Nicolaides NC, Holroyd KJ, Ewart SL, Eleff SM, Kiser MB, Dragwa CR, Sullivan CD, Grasso L, Zhang LY, Messler CJ, Zhou T, Kleeberger SR, Buetow KH, Levitt RC. IL9: a candidate gene for asthma. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(24):13175-80. PubMed PMID: 9371819; PMCID: 24282.

47. Steinke JW. Anti-IL-4 therapy. Immunology and allergy clinics of North America. 2004;24(4):599-614, vi. doi: 10.1016/j.iac.2004.06.008. PubMed PMID: 15474861.

48. Steinke JW. Current prospective of anti-IL-4, -IL-9, and -IL-13 therapies in allergic disease. Recent patents on inflammation & allergy drug discovery. 2010;4(3):222-30. PubMed PMID: 20807191.

49. Huang SK, Xiao HQ, Kleine-Tebbe J, Paciotti G, Marsh DG, Lichtenstein LM, Liu MC. IL-13 expression at the sites of allergen challenge in patients with asthma. Journal of immunology. 1995;155(5):2688-94. PubMed PMID: 7650396.

50. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, Sheppard D, Mohrs M, Donaldson DD, Locksley RM, Corry DB. Requirement for IL-13 independently of IL-4 in experimental asthma. Science. 1998;282(5397):2261-3. PubMed PMID: 9856950; PMCID: PMC3897229.

51. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, Donaldson DD. Interleukin-13: central mediator of allergic asthma. Science. 1998;282(5397):2258-61. PubMed PMID: 9856949.

52. Takatsu K. Interleukin-5 and IL-5 receptor in health and diseases. Proc Jpn Acad Ser B Phys Biol Sci. 2011;87(8):463-85. PubMed PMID: 21986312; PMCID: PMC3313690.

53. Bischoff SC, Brunner T, De Weck AL, Dahinden CA. Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. The Journal of experimental medicine. 1990;172(6):1577-82. PubMed PMID: 1701820; PMCID: PMC2188769.

54. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, Pitman N, Mirchandani A, Rana B, van Rooijen N, Shepherd M, McSharry C, McInnes IB, Xu D, Liew FY. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. Journal of immunology. 2009;183(10):6469-77. doi: 10.4049/jimmunol.0901575. PubMed PMID: 19841166.

55. Antoniu SA. MEDI-528, an anti-IL-9 humanized antibody for the treatment of asthma. Current opinion in molecular therapeutics. 2010;12(2):233-9. PubMed PMID: 20373267.

56. Oh CK, Leigh R, McLaurin KK, Kim K, Hultquist M, Molfino NA. A randomized, controlled trial to evaluate the effect of an anti-IL-9 monoclonal antibody in adults with uncontrolled asthma. Respiratory research. 2013;14:93. doi: 10.1186/1465-9921-14-93. PubMed PMID: 24050312; PMCID: 3848834.

57. Oh CK, Raible D, Geba GP, Molfino NA. Biology of the IL-9 pathway and its therapeutic potential for the treatment of asthma. Inflammation & allergy drug targets. 2011;10(3):180-6. PubMed PMID: 21428906.

58. Kontermann RE, Brinkmann U. Bispecific antibodies. Drug Discov Today. 2015;20(7):838-47. doi: 10.1016/j.drudis.2015.02.008. PubMed PMID: 25728220.

59. Whitty A, Borysenko CW. Small molecule cytokine mimetics. Chem Biol. 1999;6(4):R107-18. doi: 10.1016/S1074-5521(99)80034-9. PubMed PMID: 10099129.

60. Nakamura Y, Russell SM, Mess SA, Friedmann M, Erdos M, Francois C, Jacques Y, Adelstein S, Leonard WJ. Heterodimerization of the IL-2 receptor beta- and gamma-chain cytoplasmic domains is required for signalling. Nature. 1994;369(6478):330-3. doi: 10.1038/369330a0. PubMed PMID: 8183373.

61. Tsudo M, Kitamura F, Miyasaka M. Characterization of the interleukin 2 receptor beta chain using three distinct monoclonal antibodies. Proceedings of the National Academy of Sciences of the United States of America. 1989;86(6):1982-6. PubMed PMID: 2467293; PMCID: PMC286829.

62. Waldmann TA, Conlon KC, Stewart DM, Worthy TA, Janik JE, Fleisher TA, Albert PS, Figg WD, Spencer SD, Raffeld M, Decker JR, Goldman CK, Bryant BR, Petrus MN, Creekmore SP, Morris JC. Phase 1 trial of IL-15 trans presentation blockade using humanized Mikbeta1 mAb in patients with T-cell large granular lymphocytic leukemia. Blood. 2013;121(3):476-84. doi: 10.1182/blood-2012-08-450585. PubMed PMID: 23212516; PMCID: 3548167.

63. Dubois S, Patel HJ, Zhang M, Waldmann TA, Muller JR. Preassociation of IL-15 with IL-15R alpha-IgG1-Fc enhances its activity on proliferation of NK and CD8+/CD44high T cells and its antitumor action. Journal of immunology. 2008;180(4):2099-106. PubMed PMID: 18250415.

64. Mortier E, Woo T, Advincula R, Gozalo S, Ma A. IL-15Ralpha chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation. The Journal of experimental medicine. 2008;205(5):1213-25. doi: 10.1084/jem.20071913. PubMed PMID: 18458113; PMCID: PMC2373851.

65. Bergamaschi C, Bear J, Rosati M, Beach RK, Alicea C, Sowder R, Chertova E, Rosenberg SA, Felber BK, Pavlakis GN. Circulating IL-15 exists as heterodimeric complex with soluble IL-15Ralpha in human and mouse serum. Blood. 2012;120(1):e1-8. doi: 10.1182/blood-2011-10-384362. PubMed PMID: 22496150; PMCID: 3390963.

66. Dubois S, Mariner J, Waldmann TA, Tagaya Y. IL-15Ralpha recycles and

presents IL-15 In trans to neighboring cells. Immunity. 2002;17(5):537-47. PubMed PMID: 12433361.

67. Chertova E, Bergamaschi C, Chertov O, Sowder R, Bear J, Roser JD, Beach RK, Lifson JD, Felber BK, Pavlakis GN. Characterization and favorable in vivo properties of heterodimeric soluble IL-15.IL-15Ralpha cytokine compared to IL-15 monomer. The Journal of biological chemistry. 2013;288(25):18093-103. doi: 10.1074/jbc.M113.461756. PubMed PMID: 23649624; PMCID: PMC3689953.

68. Li J, Valentin A, Ng S, Beach RK, Alicea C, Bergamaschi C, Felber BK, Pavlakis GN. Differential effects of IL-15 on the generation, maintenance and cytotoxic potential of adaptive cellular responses induced by DNA vaccination. Vaccine. 2015;33(9):1188-96. doi: 10.1016/j.vaccine.2014.12.046. PubMed PMID: 25559187.

69. Castillo EF, Acero LF, Stonier SW, Zhou D, Schluns KS. Thymic and peripheral microenvironments differentially mediate development and maturation of iNKT cells by IL-15 transpresentation. Blood. 2010;116(14):2494-503. doi: 10.1182/blood-2010-03-277103. PubMed PMID: 20581314; PMCID: PMC2953886.

70. Stonier SW, Ma LJ, Castillo EF, Schluns KS. Dendritic cells drive memory CD8 T-cell homeostasis via IL-15 transpresentation. Blood. 2008;112(12):4546-54. doi: 10.1182/blood-2008-05-156307. PubMed PMID: 18812469; PMCID: PMC2597127.

71. Huntington ND, Alves NL, Legrand N, Lim A, Strick-Marchand H, Mention JJ, Plet A, Weijer K, Jacques Y, Becker PD, Guzman C, Soussan P, Kremsdorf D, Spits H, Di Santo JP. IL-15 transpresentation promotes both human T-cell reconstitution and T-cell-dependent antibody responses in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(15):6217-22. doi: 10.1073/pnas.1019167108. PubMed PMID: 21444793; PMCID: PMC3076818.

72. Stoklasek TA, Colpitts SL, Smilowitz HM, Lefrancois L. MHC class I and TCR avidity control the CD8 T cell response to IL-15/IL-15Ralpha complex. Journal of immunology. 2010;185(11):6857-65. doi: 10.4049/jimmunol.1001601. PubMed PMID: 21041729; PMCID: PMC3150213.

73. Sandau MM, Schluns KS, Lefrancois L, Jameson SC. Cutting edge: transpresentation of IL-15 by bone marrow-derived cells necessitates expression of IL-15 and IL-15R alpha by the same cells. Journal of immunology. 2004;173(11):6537-41. PubMed PMID: 15557143.

74. Mortier E, Advincula R, Kim L, Chmura S, Barrera J, Reizis B, Malynn BA, Ma A. Macrophage- and dendritic-cell-derived interleukin-15 receptor alpha supports homeostasis of distinct CD8+ T cell subsets. Immunity. 2009;31(5):811-22. doi: 10.1016/j.immuni.2009.09.017. PubMed PMID: 19913445.

75. Terme M, Ullrich E, Delahaye NF, Chaput N, Zitvogel L. Natural killer cell-directed therapies: moving from unexpected results to successful strategies. Nature immunology. 2008;9(5):486-94. doi:10.1038/ni1580. PubMed PMID: 18425105.

76. Burkett PR, Koka R, Chien M, Chai S, Boone DL, Ma A. Coordinate expression and transpresentation of interleukin (IL)-15Ralpha and IL-15 supports natural killer cell and memory CD8+ T cell homeostasis. The Journal of experimental medicine. 2004;200(7):825-34. doi:

10.1084/jem.20041389. PubMed PMID: 15452177; PMCID: PMC2213280.

77. Burkett PR, Koka R, Chien M, Chai S, Chan F, Ma A, Boone DL. IL-15R alpha expression on CD8+ T cells is dispensable for T cell memory. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(8):4724-9. doi: 10.1073/pnas.0737048100. PubMed PMID: 12671073; PMCID: PMC153623.

78. Koka R, Burkett P, Chien M, Chai S, Boone DL, Ma A. Cutting edge: murine dendritic cells require IL-15R alpha to prime NK cells. Journal of immunology. 2004;173(6):3594-8. PubMed PMID: 15356102.

79. Koka R, Burkett PR, Chien M, Chai S, Chan F, Lodolce JP, Boone DL, Ma A. Interleukin (IL)-15R[alpha]-deficient natural killer cells survive in normal but not IL-15R[alpha]-deficient mice. The Journal of experimental medicine. 2003;197(8):977-84. doi: 10.1084/jem.20021836. PubMed PMID: 12695489; PMCID: 2193874.

80. Lodolce J, Burkett P, Koka R, Boone D, Chien M, Chan F, Madonia M, Chai S, Ma A. Interleukin-15 and the regulation of lymphoid homeostasis. Mol Immunol. 2002;39(9):537-44. PubMed PMID: 12431387.

81. Lodolce JP, Burkett PR, Boone DL, Chien M, Ma A. T cell-independent IL15Ralpha signals are required for bystander proliferation. The Journal of experimental medicine. 2001;194(8):1187-94. PubMed PMID: 11602647; PMCID: 2193508.

82. Lodolce JP, Burkett PR, Koka RM, Boone DL, Ma A. Regulation of lymphoid homeostasis by interleukin-15. Cytokine Growth Factor Rev. 2002;13(6):429-39. PubMed PMID: 12401478.

83. Kawamura T, Koka R, Ma A, Kumar V. Differential roles for IL-15R alpha-chain in NK cell development and Ly-49 induction. Journal of immunology. 2003;171(10):5085-90. PubMed PMID: 14607906.

84. Sato N, Sabzevari H, Fu S, Ju W, Petrus MN, Bamford RN, Waldmann TA, Tagaya Y. Development of an IL-15-autocrine CD8 T-cell leukemia in IL-15-transgenic mice requires the cis expression of IL-15Ralpha. Blood. 2011;117(15):4032-40. doi: 10.1182/blood-2010-09-307504. PubMed PMID: 21304101; PMCID: 3087530.

85. Domingues H, Cregut D, Sebald W, Oschkinat H, Serrano L. Rational design of a GCN4-derived mimetic of interleukin-4. Nat Struct Biol. 1999;6(7):652-6. doi: 10.1038/10706. PubMed PMID: 10404222.

86. Mueller TD, Zhang JL, Sebald W, Duschl A. Structure, binding, and antagonists in the IL-4/IL-13 receptor system. Biochim Biophys Acta. 2002;1592(3):237-50. PubMed PMID: 12421669.

87. Walker BL, Leigh R. Use of biologicals as immunotherapy in asthma and related diseases. Expert Rev Clin Immunol. 2008;4(6):743-56. doi: 10.1586/1744666X.4.6.743. PubMed PMID: 20477124.

88. Zurawski SM, Vega F, Jr., Huyghe B, Zurawski G. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. The EMBO journal. 1993;12(7):2663-70. PubMed PMID: 8101483; PMCID: PMC413514.

89. Perugini M, Varelias A, Sadlon T, D'Andrea RJ. Hematopoietic growth factor mimetics: from concept to clinic. Cytokine

Growth Factor Rev. 2009;20(1):87-94. doi: 10.1016/j.cytogfr.2009.01.002. PubMed PMID: 19223217.

90. Gutti U, Pasupuleti SR, Sahu I, Kotipalli A, Undi RB, Kandi R, Venakata Saladi RG, Gutti RK. Erythropoietin and thrombopoietin mimetics: Natural alternatives to erythrocyte and platelet disorders. Crit Rev Oncol Hematol. 2016;108:175-86. doi: 10.1016/j.critrevonc.2016.11.002. PubMed PMID: 27931836.

91. Spina P, Impera S, Todaro AM, Salerno M, Floridia PM, Castagna F, Consoli U. "On-demand" treatment with Thrombopoietin mimetics as "bridge to recovery" from chemotherapy in chronic immune thrombocytopenia patients with cancer. A single centre experience in nine patients. Br J Haematol. 2017. doi: 10.1111/bjh.14605. PubMed PMID: 28466471.

92. Ahmed CM, Johnson HM. Short peptide type I interferon mimetics: therapeutics for experimental allergic encephalomyelitis, melanoma, and viral infections. J Interferon Cytokine Res. 2014;34(10):802-9. doi: 10.1089/jir.2014.0041. PubMed PMID: 24811478; PMCID: PMC4186642.

93. Zhang H, Zou K, Tesseur I, Wyss-Coray T. Small molecule tgf-beta mimetics as potential neuroprotective factors. Curr Alzheimer Res. 2005;2(2):183-6. PubMed PMID: 15974916.

94. Ueki R, Ueki A, Kanda N, Sando S. Oligonucleotide-Based Mimetics of Hepatocyte Growth Factor. Angew Chem Int Ed Engl. 2016;55(2):579-82. doi: 10.1002/anie.201508572. PubMed PMID: 26592704.

95. Nedjai B, Li H, Stroke IL, Wise EL, Webb ML, Merritt JR, Henderson I, Klon AE, Cole AG, Horuk R, Vaidehi N, Pease JE. Small molecule chemokine mimetics suggest a molecular basis for the observation that CXCL10 and CXCL11 are allosteric ligands of CXCR3. Br J Pharmacol. 2012;166(3):912-23. doi:10.1111/j.1476-5381.2011.01660.x. PubMed PMID: 21895630; PMCID: PMC3417418.

96. Fernandez-Botran R, Crespo FA, Sun X. Soluble cytokine receptors in biological therapy. Expert Opin Biol Ther. 2002;2(6):585-605. doi: 10.1517/14712598.2.6.585. PubMed PMID: 12171504.

97. Smerz-Bertling C, Duschl A. Both interleukin 4 and interleukin 13 induce tyrosine phosphorylation of the 140-kDa subunit of the interleukin 4 receptor. The Journal of biological chemistry. 1995;270(2):966-70. PubMed PMID: 7822337.

98. Maliszewski CR, Sato TA, Vanden Bos T, Waugh S, Dower SK, Slack J, Beckmann MP, Grabstein KH. Cytokine receptors and B cell functions. I. Recombinant soluble receptors specifically inhibit IL-1-and IL-4-induced B cell activities in vitro. Journal of immunology. 1990;144(8):3028-33. PubMed PMID: 2139075.

99. Stark GR, Darnell JE, Jr. The JAK-STAT pathway at twenty. Immunity. 2012;36(4):503-14. doi:10.1016/j.immuni.2012.03.013. PubMed PMID: 22520844; PMCID: PMC3909993.

100. O'Shea JJ, Husa M, Li D, Hofmann SR, Watford W, Roberts JL, Buckley RH, Changelian P, Candotti F. Jak3 and the pathogenesis of severe combined immunodeficiency. Mol Immunol.

2004;41(6-7):727-37. doi: 10.1016/j.molimm.2004.04.014. PubMed PMID: 15220007.

101. Bozzi F, Lefranc G, Villa A, Badolato R, Schumacher RF, Khalil G, Loiselet J, Bresciani S, O'Shea JJ, Vezzoni P, Notarangelo LD, Candotti F. Molecular and biochemical characterization of JAK3 deficiency in a patient with severe combined immunodeficiency over 20 years after bone marrow transplantation: implications for treatment. Br J Haematol. 1998;102(5):1363-6. PubMed PMID: 9753072.

102. Cacalano NA, Migone TS, Bazan F, Hanson EP, Chen M, Candotti F, O'Shea JJ, Johnston JA. Autosomal SCID caused by a point mutation in the N-terminus of Jak3: mapping of the Jak3-receptor interaction domain. The EMBO journal. 1999;18(6):1549-58. doi: 10.1093/emboj/18.6.1549. PubMed PMID: 10075926; PMCID: PMC1171243.

103. Candotti F, Oakes SA, Johnston JA, Giliani S, Schumacher RF, Mella P, Fiorini M, Ugazio AG, Badolato R, Notarangelo LD, Bozzi F, Macchi P, Strina D, Vezzoni P, Blaese RM, O'Shea JJ, Villa A. Structural and functional basis for JAK3-deficient severe combined immunodeficiency. Blood. 1997;90(10):3996-4003. PubMed PMID: 9354668.

104. Candotti F, O'Shea JJ, Villa A. Severe combined immune deficiencies due to defects of the common gamma chain-JAK3 signaling pathway. Springer Semin Immunopathol. 1998;19(4):401-15. PubMed PMID: 9618765.

105. Macchi P, Villa A, Giliani S, Sacco MG, Frattini A, Porta F, Ugazio AG, Johnston JA, Candotti F, O'Shea JJ, et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature. 1995;377(6544):65-8. doi: 10.1038/377065a0. PubMed PMID: 7659163.

106. Russell SM, Tayebi N, Nakajima H, Riedy MC, Roberts JL, Aman MJ, Migone TS, Noguchi M, Markert ML, Buckley RH, O'Shea JJ, Leonard WJ. Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. Science. 1995;270(5237):797-800. PubMed PMID: 7481768.

107. Johnston JA, Kawamura M, Kirken RA, Chen YQ, Blake TB, Shibuya K, Ortaldo JR, McVicar DW, O'Shea JJ. Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. Nature. 1994;370(6485):151-3. doi: 10.1038/370151a0. PubMed PMID: 8022485.

108. Park SY, Saijo K, Takahashi T, Osawa M, Arase H, Hirayama N, Miyake K, Nakauchi H, Shirasawa T, Saito T. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. Immunity. 1995;3(6):771-PubMed PMID: 8777722.

109. Cao X, Shores EW, Hu-Li J, Anver MR, Kelsall BL, Russell SM, Drago J, Noguchi M, Grinberg A, Bloom ET, et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. Immunity. 1995;2(3):223-38. PubMed PMID: 7697543.

110. Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, McBride OW, Leonard WJ. IL-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell. 1993;73(1):147-57. PubMed PMID: 8462096.

111. Changelian PS, Flanagan ME, Ball DJ, Kent CR, Magnuson KS, Martin WH,

Rizzuti BJ, Sawyer PS, Perry BD, Brissette WH, McCurdy SP, Kudlacz EM, Conklyn MJ, Elliott EA, Koslov ER, Fisher MB, Strelevitz TJ, Yoon K, Whipple DA, Sun J, Munchhof MJ, Doty JL, Casavant JM, Blumenkopf TA, Hines M, Brown MF, Lillie BM, Subramanyam C, Shang-Poa C, Milici AJ, Beckius GE, Moyer JD, Su C, Woodworth TG, Gaweco AS, Beals CR, Littman BH, Fisher DA, Smith JF, Zagouras P, Magna HA, Saltarelli MJ, Johnson KS, Nelms LF, Des Etages SG, Hayes LS, Kawabata TT, Finco-Kent D, Baker DL, Larson M, Si MS, Paniagua R, Higgins J, Holm B, Reitz B, Zhou YJ, Morris RE, O'Shea JJ, Borie DC. Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor. Science. 2003;302(5646):875-8. doi: 10.1126/science.1087061. PubMed PMID: 14593182.

112. Furumoto Y, Smith CK, Blanco L, Zhao W, Brooks SR, Thacker SG, Zarzour A, Sciume G, Tsai WL, Trier AM, Nunez L, Mast L, Hoffmann V, Remaley AT, O'Shea JJ, Kaplan MJ, Gadina M. Tofacitinib Ameliorates Murine Lupus and Its Associated Vascular Dysfunction. Arthritis Rheumatol. 2017;69(1):148-60doi: 10.1002/art.39818. PubMed PMID: 27429362; PMCID: PMC5195893.

113. Ghoreschi K, Jesson MI, Li X, Lee JL, Ghosh S, Alsup JW, Warner JD, Tanaka M, Steward-Tharp SM, Gadina M, Thomas CJ, Minnerly JC, Storer CE, LaBranche TP, Radi ZA, Dowty ME, Head RD, Meyer DM, Kishore N, O'Shea JJ. Modulation of innate and adaptive immune responses by tofacitinib (CP-690,550). Journal of immunology. 2011;186(7):4234-43. doi: 10.4049/jimmunol.1003668. PubMed PMID: 21383241; PMCID: 3108067.

114. Kremer J, Li ZG, Hall S, Fleischmann R, Genovese M, Martin-Mola E, Isaacs JD, Gruben D, Wallenstein G, Krishnaswami S, Zwillich SH, Koncz T, Riese R, Bradley J. Tofacitinib in combination with nonbiologic disease-modifying antirheumatic drugs in patients with active rheumatoid arthritis: a randomized trial. Annals of internal medicine. 2013;159(4):253-61. doi: 10.7326/0003-4819-159-4-201308200-00006. PubMed PMID: 24026258.

115. Okiyama N, Furumoto Y, Villarroel VA, Linton JT, Tsai WL, Gutermuth J, Ghoreschi K, Gadina M, O'Shea JJ, Katz SI. Reversal of CD8 T-cell-mediated mucocutaneous graft-versus-host-like disease by the JAK inhibitor tofacitinib. J Invest Dermatol. 2014;134(4):992-1000. doi: 10.1038/jid.2013.476. PubMed PMID: 24213371; PMCID: PMC3961527.

116. Onda M, Ghoreschi K, Steward-Tharp S, Thomas C, O'Shea JJ, Pastan IH, FitzGerald DJ. Tofacitinib suppresses antibody responses to protein therapeutics in murine hosts. Journal of immunology. 2014;193(1):48-55. doi: 10.4049/jimmunol.1400063. PubMed PMID: 24890727; PMCID: PMC4106678.

117. Sandborn WJ, Ghosh S, Panes J, Vranic I, Su C, Rousell S, Niezychowski W, Study AI. Tofacitinib, an oral Janus kinase inhibitor, in active ulcerative colitis. The New England journal of medicine. 2012;367(7):616-24. doi: 10.1056/NEJMoa1112168. PubMed PMID: 22894574.

118. Strober B, Buonanno M, Clark JD, Kawabata T, Tan H, Wolk R, Valdez H, Langley RG, Harness J, Menter A, Papp K. Effect of tofacitinib, a Janus kinase inhibitor, on haematological parameters during 12 weeks of psoriasis treatment. The British journal of dermatology. 2013;169(5):992-9. doi:10.1111/bjd.12517. PubMed PMID: 23855761.

119. Wojciechowski D, Vincenti F. Tofacitinib in kidney transplantation. Expert opinion on investigational drugs. 2013;22(9):1193-9. doi: 10.1517/13543784.2013.811231. PubMed PMID: 23841583.

120. Yokoyama S, Perera PY, Waldmann TA, Hiroi T, Perera LP. Tofacitinib, a janus kinase inhibitor demonstrates efficacy in an IL-15 transgenic mouse model that recapitulates pathologic manifestations of celiac disease. Journal of clinical immunology. 2013;33(3):586-94. doi: 10.1007/s10875-012-9849-y. PubMed PMID: 23269601; PMCID: 3594487.

121. Kontzias A, Kotlyar A, Laurence A, Changelian P, O'Shea JJ. Jakinibs: a new class of kinase inhibitors in cancer and autoimmune disease. Curr Opin Pharmacol. 2012;12(4):464-70. doi:10.1016/j.coph.2012.06.008. PubMed PMID: 22819198; PMCID: PMC3419278.

122. Jiang JK, Ghoreschi K, Deflorian F, Chen Z, Perreira M, Pesu M, Smith J, Nguyen DT, Liu EH, Leister W, Costanzi S, O'Shea JJ, Thomas CJ. Examining the chirality, conformation and selective kinase inhibition of 3-((3R,4R)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-y l)-3-oxopropanenitrile (CP-690,550). J Med Chem. 2008;51(24):8012-8. doi: 10.1021/jm801142b. PubMed PMID: 19053756; PMCID: PMC2660606.

123. Fleischmann R, Cutolo M, Genovese MC, Lee EB, Kanik KS, Sadis S, Connell CA, Gruben D, Krishnaswami S, Wallenstein G, Wilkinson BE, Zwillich SH. Phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) or adalimumab monotherapy versus placebo in patients with active rheumatoid arthritis with an inadequate response to disease-modifying antirheumatic drugs. Arthritis and rheumatism. 2012;64(3):617-29. doi: 10.1002/art.33383. PubMed PMID: 21952978.

124. Kremer JM, Bloom BJ, Breedveld FC, Coombs JH, Fletcher MP, Gruben D, Krishnaswami S, Burgos-Vargas R, Wilkinson B, Zerbini CA, Zwillich SH. The safety and efficacy of a JAK inhibitor in patients with active rheumatoid arthritis: Results of a double-blind, placebo-controlled phase IIa trial of three dosage levels of CP-690,550 versus placebo. Arthritis and rheumatism. 2009;60(7):1895-905. doi: 10.1002/art.24567. PubMed PMID: 19565475.

125. Kremer JM, Cohen S, Wilkinson BE, Connell CA, French JL, Gomez-Reino J, Gruben D, Kanik KS, Krishnaswami S, Pascual-Ramos V, Wallenstein G, Zwillich SH. A phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) versus placebo in combination with background methotrexate in patients with active rheumatoid arthritis and an inadequate response to methotrexate alone. Arthritis and rheumatism. 2012;64(4):970-81. doi: 10.1002/art.33419. PubMed PMID: 22006202.

126. Tanaka Y, Suzuki M, Nakamura H, Toyoizumi S, Zwillich SH, Tofacitinib Study I. Phase II study of tofacitinib (CP-690,550) combined with methotrexate in patients with rheumatoid arthritis and an inadequate response to methotrexate. Arthritis Care Res (Hoboken). 2011;63(8):1150-8. doi:10.1002/acr.20494. PubMed PMID: 21584942.

127. Thoma G, Druckes P, Zerwes HG. Selective inhibitors of the Janus kinase Jak3--Are they effective? Bioorg Med Chem Lett. 2014;24(19):4617-21. doi: 10.1016/j.bmcl.2014.08.046. PubMed PMID: 25217444.

128. Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, Gliniak BC, Park LS, Ziegler SF, Williams DE, Ware CB, Meyer JD, Davison BL. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. The Journal of experimental medicine. 1994;180(5):1955-60. PubMed PMID: 7964471; PMCID: PMC2191751.

129. Puel A, Ziegler SF, Buckley RH, Leonard WJ. Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. Nat Genet. 1998;20(4):394-7. doi: 10.1038/3877. PubMed PMID: 9843216.

130. Parrish YK, Baez I, Milford TA, Benitez A, Galloway N, Rogerio JW, Sahakian E, Kagoda M, Huang G, Hao QL, Sevilla Y, Barsky LW, Zielinska E, Price MA, Wall NR, Dovat S, Payne KJ. IL-7 Dependence in human B lymphopoiesis increases during progression of ontogeny from cord blood to bone marrow. Journal of immunology. 2009;182(7):4255-66. doi: 10.4049/jimmunol.0800489. PubMed PMID: 19299724; PMCID: PMC2659466.

131. Ogasawara K, Hida S, Azimi N, Tagaya Y, Sato T, Yokochi-Fukuda T, Waldmann TA, Taniguchi T, Taki S. Requirement for IRF-1 in the microenvironment supporting development of natural killer cells. Nature. 1998;391(6668):700-3. doi: 10.1038/35636. PubMed PMID: 9490414.

132. Zenatti PP, Ribeiro D, Li W, Zuurbier L, Silva MC, Paganin M, Tritapoe J, Hixon JA, Silveira AB, Cardoso BA, Sarmento LM, Correia N, Toribio ML, Kobarg J, Horstmann M, Pieters R, Brandalise SR, Ferrando AA, Meijerink JP, Durum SK, Yunes JA, Barata JT. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet. 2011;43(10):932-9. doi: 10.1038/ng.924. PubMed PMID: 21892159.

133. Shochat C, Tal N, Bandapalli OR, Palmi C, Ganmore I, te Kronnie G, Cario G, Cazzaniga G, Kulozik AE, Stanulla M, Schrappe M, Biondi A, Basso G, Bercovich D, Muckenthaler MU, Izraeli S. Gain-of-function mutations in interleukin-7 receptor-alpha (IL7R) in childhood acute lymphoblastic leukemias. The Journal of experimental medicine. 2011;208(5):901-8. doi: 10.1084/jem.20110580. PubMed PMID: 21536738; PMCID: PMC3092356.

134. Hanick NA, Rickert M, Varani L, Bankovich AJ, Cochran JR, Kim DM, Surh CD, Garcia KC. Elucidation of the IL-15 binding site on its alpha receptor by NMR. Biochemistry. 2007;46(33):9453-61. doi: 10.1021/bi700652f. PubMed PMID: 17655329.

135. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, Keegan AD, Garcia KC. Molecular and structural basis of cytokine receptor pleiotropy in the IL-4/13 system. Cell. 2008;132(2):259-72. doi:10.1016/j.cell.2007.12.030. PubMed PMID: 18243101; PMCID: 2265076.

136. Marquez A, Orozco G, Martinez A, Palomino-Morales R, Fernandez-Arquero M, Mendoza JL, Taxonera C, Diaz-Rubio M, Gomez-Garcia M, Nieto A, Lopez-Nevot MA, de la Concha EG, Martin J, Urcelay E. Novel association of the IL2-IL21 region with inflammatory bowel disease. The American journal of gastroenterology. 2009;104(8):1968-75. doi: 10.1038/ajg.2009.224. PubMed PMID: 19471255.

137. Rickert M, Wang X, Boulanger MJ, Goriatcheva N, Garcia KC. The structure of IL-2 complexed with its alpha receptor. Science. 2005;308(5727):1477-80. doi:

10.1126/science.1109745. PubMed PMID: 15933202.

138. Ring AM, Lin JX, Feng D, Mitra S, Rickert M, Bowman GR, Pande VS, Li P, Moraga I, Spolski R, Ozkan E, Leonard WJ, Garcia KC. Mechanistic and structural insight into the functional dichotomy between IL-2 and IL-15. Nature immunology. 2012;13(12):1187-95. doi: 10.1038/ni.2449. PubMed PMID: 23104097; PMCID: 3501574.

139. Wang X, Rickert M, Garcia KC. Structure of the quaternary complex of IL-2 with its alpha, beta, and gammac receptors. Science. 2005;310(5751):1159-63. doi: 10.1126/science.1117893. PubMed PMID: 16293754.

140. Jacobson S, Shida H, McFarlin DE, Fauci AS, Koenig S. Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. Nature. 1990;348(6298):245-8. doi: 10.1038/348245a0. PubMed PMID: 2146511.

141. Azimi N, Shiramizu KM, Tagaya Y, Mariner J, Waldmann TA. Viral activation of IL-15: characterization of a virus-inducible element in the IL-15 promoter region. Journal of virology. 2000;74(16):7338-48. PubMed PMID: 10906187; PMCID: 112254.

142. Chen J, Petrus M, Bryant BR, Phuc Nguyen V, Stamer M, Goldman CK, Bamford R, Morris JC, Janik JE, Waldmann TA. Induction of the IL-9 gene by HTLV-I Tax stimulates the spontaneous proliferation of primary adult T-cell leukemia cells by a paracrine mechanism. Blood. 2008;111(10):5163-72doi: 10.1182/blood-2007-09-113654. PubMed PMID: 18339896; PMCID: 2384140.

143. Sakaguchi S. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annual review of immunology. 2004;22:531-62. doi:10.1146/annurev.immunol.21.120601.141122. PubMed PMID: 15032588.

144. Sakaguchi S, Vignali DA, Rudensky AY, Niec RE, Waldmann H. The plasticity and stability of regulatory T cells. Nature reviews Immunology. 2013;13(6):461-7. doi: 10.1038/nri3464. PubMed PMID: 23681097.

145. Yokohama A, Mishra A, Mitsui T, Becknell B, Johns J, Curphey D, Blaser BW, Vandeusen JB, Mao H, Yu J, Caligiuri MA. A novel mouse model for the aggressive variant of NK cell and T cell large granular lymphocyte leukemia. Leuk Res. 2010;34(2):203-9. doi: 10.1016/j.leukres.2009.06.031. PubMed PMID: 19660811; PMCID: PMC2814907.

146. Mishra A, Liu S, Sams GH, Curphey DP, Santhanam R, Rush LJ, Schaefer D, Falkenberg LG, Sullivan L, Jaroncyk L, Yang X, Fisk H, Wu LC, Hickey C, Chandler JC, Wu YZ, Heerema NA, Chan KK, Perrotti D, Zhang J, Porcu P, Racke FK, Garzon R, Lee RJ, Marcucci G, Caligiuri MA. Aberrant overexpression of IL-15 initiates large granular lymphocyte leukemia through chromosomal instability and DNA hypermethylation. Cancer Cell. 2012;22(5):645-55. doi: 10.1016/j.ccr.2012.09.009. PubMed PMID: 23153537; PMCID: PMC3627362.

147. Mishra A, Sullivan L, Caligiuri MA. Molecular pathways: interleukin-15 signaling in health and in cancer. Clin Cancer Res. 2014;20(8):2044-50. doi: 10.1158/1078-0432.CCR-12-3603. PubMed PMID: 24737791; PMCID: PMC3989546.

148. Blaser BW, Roychowdhury S, Kim DJ, Schwind NR, Bhatt D, Yuan W, Kusewitt DF, Ferketich AK, Caligiuri MA, Guimond M. Donor-derived IL-15 is critical for acute allogeneic graft-versus-host disease. Blood. 2005;105(2):894-901. doi: 10.1182/blood-2004-05-1687. PubMed PMID: 15374888.

149. Blaser BW, Schwind NR, Karol S, Chang D, Shin S, Roychowdhury S, Becknell B, Ferketich AK, Kusewitt DF, Blazar BR, Caligiuri MA. Trans-presentation of donor-derived interleukin 15 is necessary for the rapid onset of acute graft-versus-host disease but not for graft-versus-tumor activity. Blood. 2006;108(7):2463-9. doi: 10.1182/blood-2006-04-019059. PubMed PMID: 16757683; PMCID: PMC1895554.

150. Roychowdhury S, Blaser BW, Freud AG, Katz K, Bhatt D, Ferketich AK, Bergdall V, Kusewitt D, Baiocchi RA, Caligiuri MA. IL-15 but not IL-2 rapidly induces lethal xenogeneic graft-versus-host disease. Blood. 2005;106(7):2433-5. doi: 10.1182/blood-2005-04-1597. PubMed PMID: 15976176; PMCID: PMC1895258.

151. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 2011;7:539. doi: 10.1038/msb.2011.75. PubMed PMID: 21988835; PMCID: PMC3261699.

152. Andrusier N, Nussinov R, Wolfson HJ. FireDock: fast interaction refinement in molecular docking. Proteins. 2007;69(1):139-59. doi: 10.1002/prot.21495. PubMed PMID: 17598144.

153. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res. 2005;33 (Web Server issue): W363-7. doi:10.1093/nar/gki481. PubMed PMID: 15980490; PMCID: PMC1160241.

