Title: Targeting the binding interface on a shared receptor subunit of a cytokine family enables the inhibition of multiple member-cytokines with selectable target spectrum.

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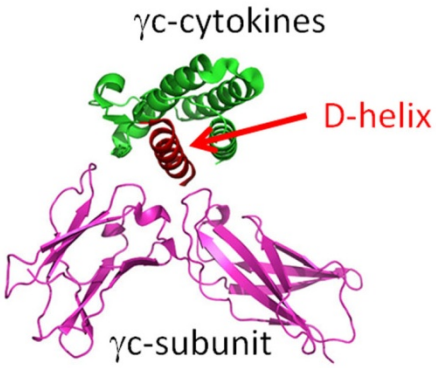
Supplementary Figure 1

A



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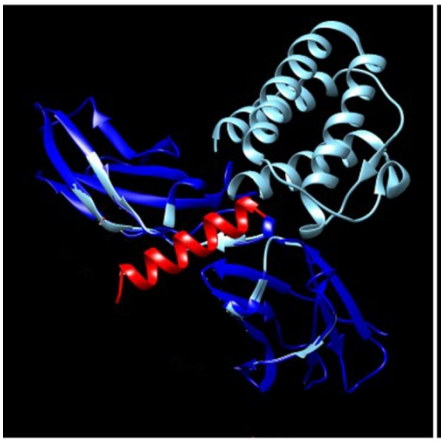

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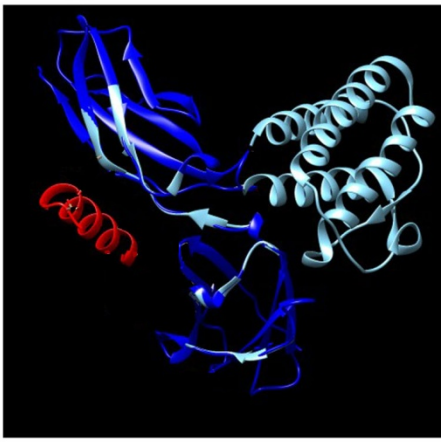


D

E

F

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
I		E	F	L	Q			I	H/T	I		Q	M/S		I	N/S	T	S

Figure S1 Computer-assisted docking simulation of candidate peptides.
The 120 candidate peptides designed by the criteria shown below (Table S2) were then analyzed by a series of computer-assisted docking algorithm whether their binding to γc show overlap with the binding interfere between IL-2 or IL-15 vs. γc (deduced from PDB 2ERJ or 4GS7).
A: binding of IL-15 (blue) to γc (red) depicting the geometry and location of the D-helix of IL-15 with respect to the γc molecule.
B: An example of inclusion to the second-round screening ; Peptides that show interfering geometry to the IL-15/γc interaction (deduced from the IL-15 vs. α–β–γ ternary receptor-complex , PDB; 4GS7).
C: An example excluded from the second-round screening; Peptides that show non-interfering geometry to the cytokine-receptor binding.

D. Simulated binding geometry of the isolated Helix D of IL-2
E. Simulated binding geometry of the isolated Helix D of IL-2
To demonstrate that the isolated Helix D from either cytokine could potentially bind to the γc in a different geometry than does the whole original cytokine, binding of the D-helix (of IL-2 or IL-15) and that of the cytokine to γc-were overlaid in the same picture. This could be explained by the interaction of A-C helices of these cytokines with private chains. Obviously, all these speculations need experimental confirmation which is in progress.

F. Construction logic behind the design of BNZ132-1.
Based on previous reports on the interaction of IL-2/IL-15 and their hetero-trimeric receptor complexes including the γc molecule ((1,2), the following logic was adopted for the design of BNZ132-1 peptide. Amino acids shown in Bold Red above indicate "Fixed" residues. Positions at 10, 14, and 17 allow some "flexibility" but participate in the physical interaction of cytokines vs γc therefore denoted "high priority wobble" below.

[Algorithm of Screening]; We mutated the "wobble" residues *in silico* (total 120 possible peptides, shown in Table S2) and ran docking simulation to the γc-molecule for each one of them. This screening was used to "eliminate" those that do not show binding to the γc at the correct position (the binding pocket for IL-2 or IL-15). Nearly 80% of the candidates were dropped by the *in silico* screening, after which the remaining peptides were synthesized in small scale (Bachem, Torrance CA) and tested by biological assays involving recombinant IL-2/IL-15 and CTLL-2 cell line (see Experimental Procedures in the main text). Finally we chose the BNZ132-1 which showed the most potent inhibition on both cytokines.

Fixed positions from binding chemistry (emphasized with yellow boxes):

Gln13, Ile16 –identical binding residues between human IL-2 and IL-15.

Gln6 - this position participates in the binding only in IL-2, but not with IL-15. Fixing it with Asn (IL-2 specific residue) may add IL-2-biased characteristic to the peptide. Therefore, Gln was adopted from the IL-15 sequence, but this should have neutral effect on the binding of the peptide to γc.

Fixed positions from shared amino acid usages (emphasized by magenta boxes):

Ile1, Glu3, Phe 4, Leu5, Thr18 – these residues do not participate directly in the binding between cytokines' D-helices and the γc-molecule. However, these amino acids were conserved between human IL-2 and IL-15.

Fixed positions from favored amino acid usages across mammalian species:

Ile9 – Although human IL-15 has Val at this position, murine IL-15 and most mammalian IL-2 has I at this position.

Ile11 – similar to Ile9, except that mouse IL-2 and mammalian IL-15 use Ile at this position.

Ser19 – Many mammalian IL-15 and rat IL-2 use Ser at this position.

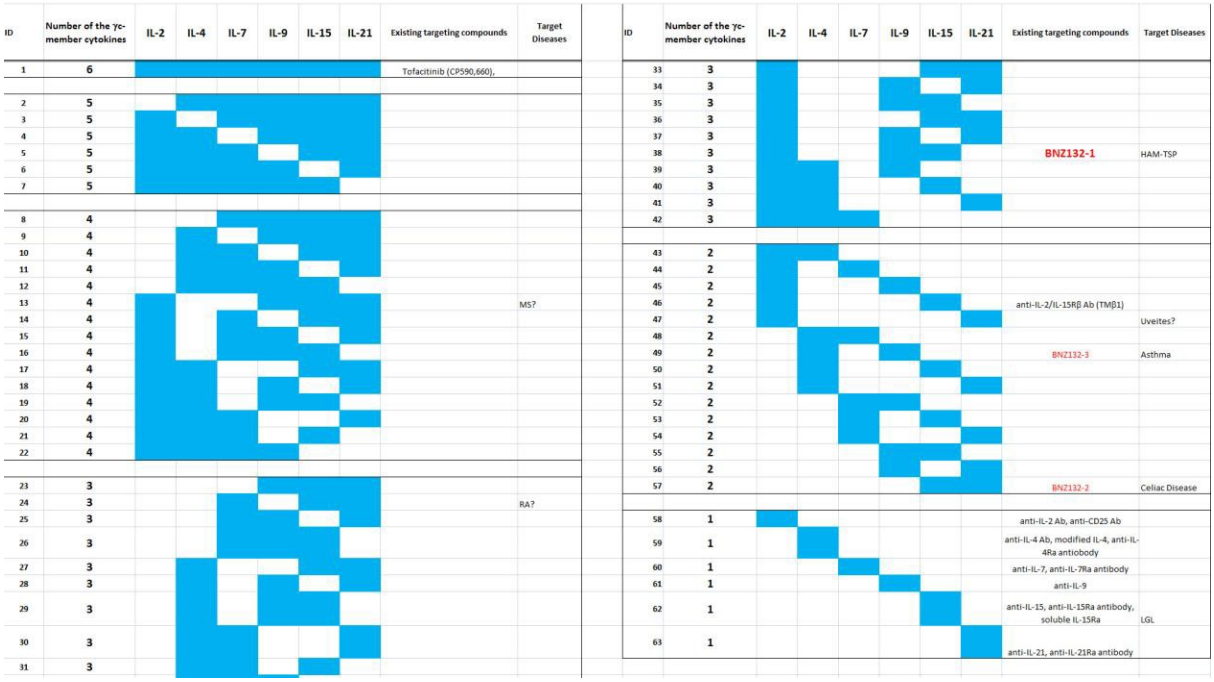
High priority "Wobble" positions from binding chemistry:

His10 or Thr10, Met14 or Ser14, Asn17 or Ser17 – these amino acids in either cytokine are in contact with the surface of the γc-molecule. We have tested combining two from one cytokine and the last from the other cytokine. In other words, these three positions were designed with a linkage to each other. Three out of IL-2 or three out of IL-15 for these positions might cause structural bias and thus have been eliminated.

Low priority "Wobble" positions: 2,7,8,12,15

As the positions that have been fixed based on the logic shown above could introduce bias either to IL-2 or IL-15 sequence, these low-priority positions could provide leverage. However, these five positions have weaker impact on the binding chemistry, and thus we did not seek for a perfect counter-balance from these positions. For example, if His10, Met14 have been chosen from IL-15 and Ser17 from IL-2, then we allowed either three from IL-2+two from IL-15, or two from IL-2+three from IL-15 at these five positions.

Supplementary Table 1



ID	Number of the γc-member cytokines	IL-2	IL-4	IL-7	IL-9	IL-15	IL-21	Existing targeting compounds	Target Diseases
1	6	■	■	■	■	■	■	Tofacitinib (CP590,660),	
2	5		■	■	■	■	■		
3	5	■		■	■	■	■		
4	5	■	■		■	■	■		
5	5	■	■	■		■	■		
6	5	■	■	■	■		■		
7	5	■	■	■	■	■			
8	4			■	■	■	■		
9	4		■		■	■	■		
10	4		■	■		■	■		
11	4		■	■	■		■		
12	4		■	■	■	■			
13	4	■		■	■	■	■		MS?
14	4	■			■	■	■		
15	4	■		■		■	■		
16	4	■		■	■		■		
17	4	■	■		■	■			
18	4	■	■		■		■		
19	4	■	■			■	■		
20	4	■	■	■			■		
21	4	■	■	■		■			
22	4	■	■	■	■				
23	3				■	■	■		
24	3			■		■	■		RA?
25	3			■	■		■		
26	3			■	■	■			
27	3		■			■	■		
28	3		■		■		■		
29	3		■		■	■			
30	3		■	■			■		
31	3		■	■	■				
33	3	■			■	■	■		
34	3	■			■	■			
35	3	■			■		■		
36	3	■				■	■		
37	3	■			■	■			
38	3	■			■		■	BNZ132-1	HAM-TSP
39	3	■		■		■			
40	3	■		■			■		
41	3	■		■	■				
42	3	■		■					
43	2	■				■	■		
44	2	■			■	■			
45	2	■			■		■		
46	2	■			■			anti-IL-2/IL-15Rβ Ab (TMβ1)	
47	2	■					■		Uveites?
48	2		■	■		■			
49	2		■	■	■			BNZ132-3	Asthma
50	2		■	■		■			
51	2		■	■			■		
52	2			■	■				
53	2			■		■			
54	2			■			■		
55	2				■	■			
56	2				■		■		
57	2					■	■	BNZ132-2	Celiac Disease
58	1	■						anti-IL-2 Ab, anti-CD25 Ab	
59	1		■					anti-IL-4 Ab, modified IL-4, anti-IL-4Rα antiobody	
60	1			■				anti-IL-7, anti-IL-7Rα antibody	
61	1				■			anti-IL-9	
62	1					■		anti-IL-15, anti-IL-15Rα antibody, soluble IL-15Rα	LGL
63	1						■	anti-IL-21, anti-IL-21Rα antibody	

Table S1 Provisional library of compounds that comprehensively inhibit any possible combinations of the 6 γc-cytokines that are potentially represented in human diseases.

The expansion of our current concept may have useful clinical ramifications. The γc-family is a mathematical group of 6 members. There exist 63 subsets ($_6C_{6+6}C_{5+6}C_{4+6}C_{3+6}C_{2+6}C_1$=63) that consist of differential combinations of all 6 members (Supplementary Table 2). It may be possible to eventually

complete a library of compounds (peptides, antibodies and small chemicals) to target all the subsets. Such library would enable to treat any human diseases which pathogenically involve combinations of γc-cytokines and is our long-term goal.

Our three peptides, BNZ132-1, -2 and -3 (the sequences of BNZ132-2 and -3 are not disclosed in this paper) have distinct target cytokine spectrums. BNZ132-1, as shown in the text, specifically inhibits IL-2, IL-15 and IL-9. BNZ132-2 inhibits IL-15 and IL-21 (data not shown) and can be a candidate for treating Celiac disease (3-6) in which the combined effects of IL-15 and IL-21 are implicated (7).
BNZ132-3, which inhibits IL-4 and IL-9 (data not shown), can be a novel treatment compound for Asthma (8).

Supplementary Table 2

Rational design of BNZ132-1 peptide

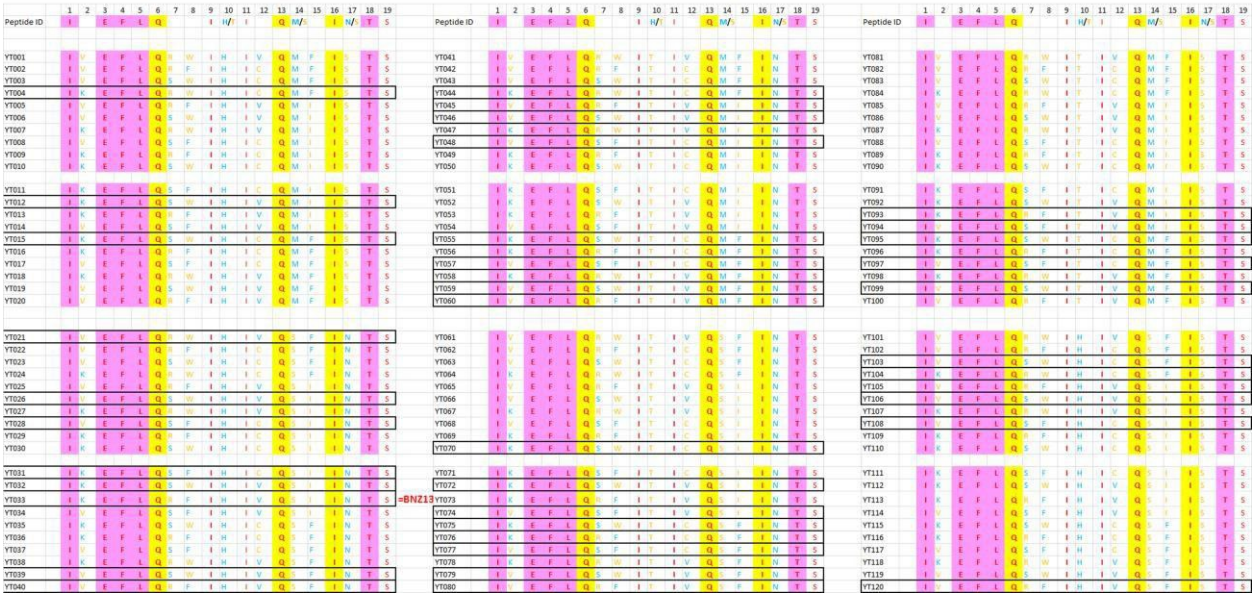


Table S2. Rational Design of BNZ132-1

The 19-aa BNZ132-1 was designed based on a logic described in legend for Figure S1. The selection logic gave rise to 120 peptides to test using a simulation program (Pep-Fold) if the candidate sequence would form thermodynamically stable complex with the γc-molecule in geometry similar to that used by the binding of γc-cytokines and γc. Finally, 39 candidate peptides were chosen for peptide synthesis, each of which was biologically tested using CTLL-2 cells if it equally blocks cellular proliferation triggered by IL-2 and IL-15. Peptide YT033 corresponds to the final BNZ132-1 peptide. Table S1 depicts a possible expansion of our strategy.

Supplemental Experimental Procedures;

Protein docking simulation
3D structure of each peptide was constructed using the Pep-fold algorithm (9-11). Binding of each peptide and the γc-molecule was calculated using PatchDock (12) and FireDock (13,14). Binding solutions with Gibbs free energy less than -15k J/mol were considered non-significant.

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