

Letter to the Editor

The diversity of Bet v 1-specific IgG₄ antibodies remains mostly constant during the course of birch pollen immunotherapy

To the Editor:

It is well documented that allergen-specific immunotherapy (AIT) induces high titers of allergen-specific IgG antibodies, which may block IgE-mediated effector mechanisms by preventing IgE binding to allergens. Both IgG₁ and IgG₄ antibodies have been considered as blocking antibodies; however, the functional role of allergen-specific IgG₄ antibodies has been most intensely studied so far. Still, only limited data on the epitope specificity and diversity of AIT-induced allergen-specific IgG₄ antibodies exist. Recently, the antibody repertoire after a median time period of 41 months of oral AIT has been studied in a high-throughput peptide microarray by using linear peptides of major peanut allergens.¹ The authors concluded that the peanut-specific IgG₄ repertoire broadly expanded in a polyclonal fashion and included novel specificities that recognized epitopes different from IgE antibodies.¹ In a different type of AIT, namely, subcutaneous treatment with birch pollen extract (BP-AIT) containing 5% to 10% of the sole major allergen Bet v 1, we have reported that most Bet v 1-specific IgG₄ antibodies induced after 36 months recognized IgE epitopes and only a minority represented *de novo* specificities.² In the present longitudinal study, we sought to investigate whether the diversity of Bet v 1-specific IgG₄ antibodies expands, decreases, or remains similar in the course of BP-AIT.

Because antibody epitopes on Bet v 1 are conformational,³⁻⁵ we performed competitive immunoscreening of phage-displayed peptides to predict IgG₄-binding areas^{2,6} using sera collected after 6 and 36 months of BP-AIT from 5 BP-allergic patients with significantly improved respiratory symptoms to natural BP exposure and reduced skin prick test reactivity to BP extract.⁷ Briefly, each serum sample was incubated with antihuman IgG₄-coated magnetic beads that were then used to screen a phage-displayed peptide library (Ph.D.-12 Phage Display Peptide Library, New England BioLabs, Beverly, Mass). Bound phages were specifically eluted by competition with Bet v 1. The amino acid (aa) sequences of the phage-displayed 12-mer peptides were determined. This procedure was repeated until at least 4 peptides for each serum sample were obtained (see Table E1 in this article's Online Repository at www.jacionline.org). Each peptide sequence was mapped onto the molecular surface of Bet v 1 (PDB code 1BV1) with the "EpiSearch" algorithm to predict conformational regions.⁸ In total, 36 and 40 IgG₄ epitopes, each comprising 10 to 23 aa, were predicted at month 6 and 36 of BP-AIT, respectively (see Table E1). Next, we assessed the percentage of identical aa in the same IgG₄-binding Bet v 1-surface areas predicted at both time points. For patient 1, 44.7% aa were identical; for patient 2, 61.5%; for patient 3, 62.5%; for patient 4, 53.8%; and for patient 5, 36.8%. Fig 1 illustrates the entire surface area predicted to be recognized by IgG₄ antibodies on Bet v 1 at each time point and their overlap for each individual. The remarkable overlap of predicted IgG₄ epitopes at 6 and 36 months of BP-AIT in 4 of the 5 patients (80%)

indicated that the early established IgG₄ repertoire did not expand further with prolonged treatment.

To complement the *in silico* data, we assessed the IgE-blocking activity by facilitated antibody-binding assays as described.² Briefly, each serum sample was preincubated with Bet v 1 and the same indicator serum containing more than 100 kU/L of Bet v 1-specific IgE with a presumed highly diverse IgE repertoire. Thereafter, the activity of each serum to reduce the binding of Bet v 1-IgE complexes to CD23⁺ B cells was determined. Bet v 1-specific IgG₁₋₄ levels in the sera were assessed by ELISA. None of the patients developed Bet v 1-specific IgG₂ and IgG₃ antibodies (data not shown), and only patients 1, 3, and 4 developed Bet v 1-specific IgG₁ antibodies (see Fig E1 in this article's Online Repository at www.jacionline.org). Bet v 1-specific IgG₄ antibody levels were also quantified by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). Table I shows that the IgE-blocking activity of sera from patient 2 had reached 89% already after 6 months of BP-AIT and was not markedly enhanced after 36 months despite further increase in Bet v 1-specific IgG₄ antibody levels. The finding that the IgE-blocking activity did not rise in parallel to the quantity of specific IgG₄ antibodies suggested that the sera at both time points contained comparable IgG₄ specificities able to block most IgE epitopes recognized by the indicator serum. These data accorded with the large overlap of the predicted IgG₄-binding regions at both time points (Fig 1). However, patient 4 displayed very high levels of Bet v 1-specific IgG₄ antibodies at month 6 and 3.5-fold lower levels at month 36 (Table I). At this time point, Bet v 1-specific IgG₁ antibodies were almost reduced to the level before AIT (see Fig E1). In parallel, the IgE-blocking activity decreased only around 2-fold (Table I). The limited reduction in IgE-blocking activity together with the high overlap of predicted IgG₄ epitopes at both time points (Fig 1) suggested a comparable IgG₄ repertoire in the early and late phases of BP-AIT also in this patient. In contrast, patient 5 showed a parallel increase in IgE-blocking activity and IgG₄ levels but no IgG₁ induction during the course of BP-AIT (see Table I and Fig E1). In this individual, the increased IgE-blocking activity may result not only from the enhanced IgG₄ concentration but also from a broader IgG₄ diversity. The latter is strongly suggested by the occurrence of prominent IgG₄-binding areas predicted at month 36 but not at month 6 (Fig 1). We conclude that the Bet v 1-specific IgG₄ repertoire of patient 5 expanded in the course of BP-AIT.

In summary, this study provides first evidence that Bet v 1-specific IgG₄ repertoires induced by BP-AIT do not broadly expand in the course of treatment in most of the patients. Still, 1 of 5 individuals (20%) showed an increase in the IgG₄ diversity with prolonged therapy. We are aware that these conclusions are based on a limited number of patients, in particular in view of the heterogeneous and individual immune response to allergens. However, competitive immunoscreening is laborious and cannot be performed in a high-throughput set-up. Therefore, we sought an additional approach to support our conclusions, namely, the use of 4 chimeric proteins that had been created by grafting surface areas of Bet v 1 onto the scaffold of Api g 1, the Bet v 1-homologue in celery.⁹ The IgG₄ reactivity to these chimeras

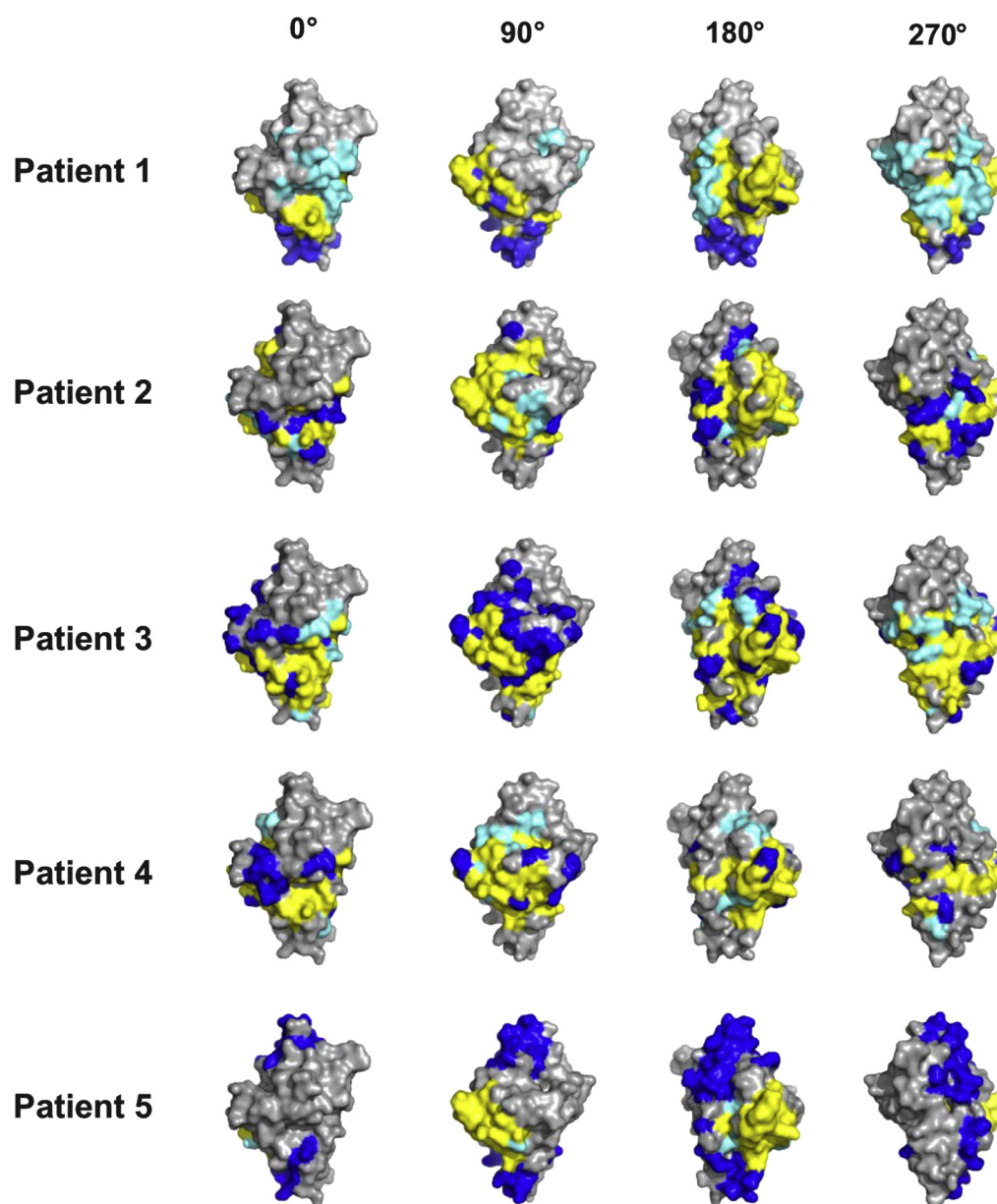


FIG 1. Bet v 1-specific IgG₄-binding areas at 6 and 36 months of BP-AIT largely overlap. The sum of predicted IgG₄ epitopes on Bet v 1 (pdb 1BV1) after 6 (*turquoise*) and 36 (*blue*) months of BP-AIT and their overlap (*yellow*) are shown.

TABLE I. Bet v 1-specific IgG₄ levels and IgE-blocking activity at month 6 and 36 of BP-AIT

Patient	IgE-blocking activity (%) [*]			IgG ₄ levels (μg/L) [†]		
	Before	Month 6	Month 36	Before	Month 6	Month 36
1	1	98	98	0.00	0.72	2.49
2	0	89	92	0.03	0.58	1.12
3	0	96	99	0.28	6.34	26.8
4	0	86	45	0.55	18.5	5.29
5	0	36	62	1.22	1.67	11.8

^{*}The IgE-blocking activity of sera was assessed by facilitated antibody-binding assays.

[†]Bet v 1-specific IgG₄ levels were determined by ImmunoCAP.

was tested by ELISA. So far, 8 of 9 patients (89%) displayed IgG₄ reactivity to the same chimera after 6 and 36 months of BP-AIT, whereas 1 of 9 individuals (11%) showed a diverse response at both time points (unpublished observations). Identical reactivity patterns at early and late time points of the therapy indicate that the IgG₄ repertoire remained constant for most of the patients during BP-AIT. These preliminary data obtained by a second, entirely different experimental approach to analyze the IgG₄ diversity in the course of BP-AIT strongly underline our conclusions drawn from competitive immunoscreening of phage-displayed peptides and facilitated antibody-binding assays.

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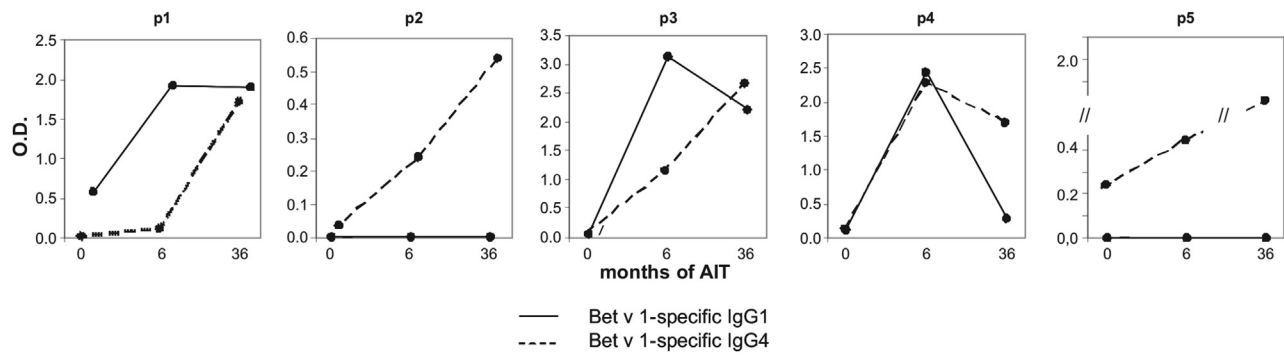


FIG E1. Bet v 1-specific IgG₁ and IgG₄ levels before (0) and after 6 and 36 months of BP-AIT. Antibody levels were determined with ELISA as described previously.² OD, Optical density.

TABLE E1. Phage-displayed peptides and predicted IgG₄-binding regions on Bet v 1

Patient	BP-SIT (mo)	Peptide sequence	aa residues of predicted IgG ₄ -binding regions on Bet v 1	% Of identical aa at 6 and 36 mo
1	6	QSVRIMTGADSN	1. S39, I56, S57, G61, V67, V85, G88, G89, I91, G92, Q132, A135, S136, M139	44.7
		KCSLPECVVARE	2. S39, K55, S57, P59, E60, L62, P63, K65, V67, K68, V85, E87, P90, A135, S136	
		SPGLPLQLGSMK	3. S39, K55, S57, P59, G61, L62, P63, K65, K68, G88, G89, P90, G92, Q132, S136, M139	
		SKMAHMERSWEV	4. V2, E6, V85, K97, S99, E101, K115, S117, K119, H121, V128, V133, K134, A135, S136, K137, M139	
		SKMAHMERSWEV	5. E6, V67, V85, S117, K119, V128, K129, A130, E131, V133, K134, A135, S136, K137, E138, M139, E141	
	36	VQHNTKYSVVIR	6. N4, Y5, T7, T9, I23, K80, Y81, N82, Y83, S99, I102, K103, V105, I113, K115, I116, S117, N118, K119, V133, S136, K137	
		VQHNTKYSVVIR	7. V2, N4, Y5, T7, N82, Y83, V85, K97, S99, I102, K115, I116, S117, N118, K119, H121, V128, Q132, V133, K134, S136, K137	
		YLDLPTNLSHRI	8. T7, T9, I23, L24, D72, D75, N78, Y81, N82, Y83, S99, I102, I113, I116, S117, N118, S136, L143	
		TLRVPPNPNNMV	9. T7, T9, L62, P63, V67, V85, P90, N118, V133, M139, T142, L143, R145	
		GHVTESMTKRSD	10. G1, V2, V67, V85, E87, G88, G89, G92, D93, T94, E96, K97, S99, K119, H121, T122, K123, G124, H126, E127, V128, K129, V133	
2	6	GLTKPSLYTMEK	11. S39, K55, S57, P59, E60, G61, L62, P63, K65, Y66, K68, E87, G88, G89, P90, G92, S136, M139	61.5
		VKLNPSMNYLSG	12. S39, K55, S57, P59, G61, L62, P63, K65, Y66, V67, K68, V85, G88, G89, P90, G92, S136, M139	
		SVTESPNRMGLV	13. S39, S57, P59, E60, G61, L62, P63, V67, V85, E87, G88, G89, P90, G92, S136, M139	
		GMSLAQVTAKQN	14. S39, K55, S57, G61, L62, K65, V67, K68, V85, G88, G89, G92, Q132, A135, S136, M139	
		SLTNPWPVSFSP	15. T7, T9, F30, F58, L62, P63, F64, V67, V85, P90, N118, V133, S136, T142, L143	
	36	YIPPLPQMLTAI	16. I56, P59, L62, P63, Y66, P90, I91, Q132, A135, M139	
		KTASDWMTAQYP	17. S39, K55, S57, P59, P63, K65, Y66, K68, P90, Q132, A135, S136, M139	
		LMTKYHIPEISD	18. S39, K55, I56, S57, P59, E60, L62, P63, K65, Y66, K68, E87, P90, I91, S136, M139	
		SNPLNMLRITSL	19. S39, S40, I44, I53, I56, S57, L62, P63, R70, I86, P90, I91, L95, S136, M139	
		YSFTPHVDFISP	20. D27, F30, P31, S39, S40, V41, I44, I53, I56, S57, F58, P59, F64, Y66, V67, D69, Y83, V85, I86	
3	6	AHNLLYNLKYHP	21. N4, Y5, N82, Y83, P90, L95, K97, K115, N118, K119, H121, K134, A135, K137	62.5
		TTDLVLNMLPLS	22. D25, D27, N28, P31, V33, P35, S39, S40, V41, S57, P59, D69, M139, T142, L143	
		HPSAENPLTMPP	23. E6, T7, P63, P90, S117, N118, A130, E131, A135, S136, E138, M139, E141, T142, L143	
		HPSAENPLTMPP	24. S39, S57, P59, E60, L62, P63, E87, P90, A135, S136, M139	
		VSTPANTKYNNR	25. V2, N4, Y5, T7, N82, Y83, V85, P90, K97, S99, K115, S117, N118, K119, V128, V133, K134, A135, S136, K137	
	36	TSSAPDNWTYWL	26. D25, D27, N28, P31, P35, S39, S40, S57, P59, D69, T142, L143, A146, Y150	
		IAAPNSLRFSPL	27. S39, I56, S57, F58, P59, L62, P63, F64, P90, I91, A135, S136	
		TLTSAPLKWTPR	28. S39, S40, K54, K55, S57, L62, P63, K65, K68, R70, P90, L95, K97, A135, S136	
		HLISSNKPQHTQ	61. N4, T7, N82, P90, L95, K97, S99, I102, K115, I116, S117, N118, K119, H121, Q132, K134, S136, K137	
		WNISSAKPMLPY	62. S39, S40, I44, I53, K54, K55, I56, S57, L62, P63, K65, Y66, K68, Y83, I86, P90, I91, L95, K97, A135, S136, M139	
		WNISSAKPMLPY	63. S39, K55, I56, S57, P59, L62, P63, K65, Y66, K68, P90, I91, A135, S136, M139	
		GMLSSLKPPIAH	64. S39, K55, I56, S57, P59, G61, L62, P63, K65, K68, G88, G89, P90, I91, G92, A135, S136, M139	
		SINAWVPWLIDS	65. I23, D25, D27, N28, P31, V33, P35, S39, S40, V41, I56, S57, P59, D69, L143, A146	
		SWMTSFKHSANS	66. F3, N4, T7, N82, K97, S99, K115, S117, N118, K119, H121, K134, A135, S136, K137, M139	
		AFMSSAKLPDSR	67. S39, K55, S57, F58, P59, L62, P63, F64, K65, K68, P90, A135, S136, M139	
	36	SSAAMLHSTKSL	68. T7, L95, K97, S99, K115, S117, K119, H121, K134, A135, S136, K137, M139	
		ESIHASGKMGRH	69. S39, K55, I56, S57, E60, G61, K65, K68, E87, G88, G89, I91, G92, A135, S136, M139	
		ANHLSGNNYGIS	70. S39, S40, I44, I53, I56, S57, L62, Y66, Y83, I86, G88, G89, I91, G92, L95, A135, S136	
		ANHLSGNNYGIS	71. N43, I44, G48, G49, G51, I53, I56, S57, Y66, Y81, N82, Y83, I86, G88, G89, I91, S99	
		GTTTLNHNYSAS	72. N4, Y5, T7, N82, Y83, L95, K97, S99, K115, S117, N118, K119, H121, K134, A135, S136, K137, G140	
		GTTTLNHNYSAS	73. N4, Y5, T7, T9, K80, Y81, N82, Y83, S99, K103, K115, S117, N118, K119, S136, K137, G140, L143	
		YHPNGMNPYTKA	74. K55, P59, G61, P63, K65, Y66, K68, G88, G89, P90, G92, A135, M139	
		NDNYPATIKHSN	75. I23, D27, P50, I53, K54, I56, K68, D69, D72, K80, Y81, N82, Y83, I86, K97, S99, I102, I116, S117, N118, K119	
		SVDMPHYGQRP	76. S39, S40, V41, S57, P63, Y66, V67, D69, R70, Y83, V85, G88, G89, P90, G92, S136, M139	

(Continued)

TABLE E1. (Continued)

Patient	BP-SIT (mo)	Peptide sequence	aa residues of predicted IgG ₄ -binding regions on Bet v 1	% Of identical aa at 6 and 36 mo
4	6	WNQGSPLNRYTS	40. T7, T9, Q36, L62, P63, G89, P90, N118, Q132, S136, G140, T142, L143, R145	53.8
		WNQGSPLNRYTS	41. S39, S40, S57, L62, P63, Y66, R70, Y83, G88, G89, P90, G92, L95, S136	
		VSTAKYETLNEQ	42. N4, Y5, E6, T7, E8, T9, K80, Y81, N82, Y83, S99, E101, K103, V105, K115, S117, N118, K119, V133, S136, K137, E141, L143	
		VSTAKYETLNEQ	43. V2, N4, Y5, E6, T7, N82, Y83, V85, L95, K97, S99, E101, K115, S117, N118, K119, V128, Q132, V133, K134, A135, S136, K137	
		VPTECSGRSTCT	44. G1, V2, V67, V85, E87, G88, G89, P90, G92, T94, E96, S99, T122, G124, E127, V128, V133	
		DPLDQGKMLRTP	45. K55, P59, G61, L62, P63, K65, K68, G88, G89, P90, G92, Q132, M139	
		SIPRYYHTENSP	46. S39, S40, E42, I44, I53, I56, S57, P63, Y66, R70, Y83, I86, E87, P90, I91, S136	
		SIPRYYHTENSP	47. I23, P50, I53, I56, R70, E73, Y81, N82, Y83, I86, S99, E101, I102, I116, S117, N118	
		SIPRYYHTENSP	48. N28, P31, S40, E42, N43, I44, E45, N47, I53, I56, S57, Y66, R70, Y83, I86, E87	
		SSPSAAQWLNIG	49. S39, I56, S57, P59, G61, L62, P63, G88, G89, P90, I91, G92, Q132, A135, S136	
	36	LPGRAHDPWKVP	50. V41, K54, K55, L62, P63, K65, V67, K68, D69, R70, V85, G88, G89, P90, G92, L95, K97, A135	
		HPATSNTKYWIN	51. S39, S40, I44, I53, K54, K55, I56, S57, P63, K65, Y66, K68, Y83, I86, P90, I91, K97, A135, S136	
		HPATSNTKYWIN	52. N4, Y5, T7, N82, Y83, P90, K97, S99, I102, K115, I116, S117, N118, K119, H121, K134, A135, S136, K137	
		FTTESGGLNPHA	53. S39, S57, F58, P59, E60, G61, L62, P63, F64, E87, G88, G89, P90, G92, A135, S136	
		VEAHNQWTGLVA	54. G1, V2, N4, G92, T94, L95, E96, N118, H121, T122, H126, E127, V128, A130, E131, Q132, V133, A135	
		<i>YHPNGMNPYTKA</i>	55. K55, P59, G61, P63, K65, Y66, K68, G88, G89, P90, G92, A135, M139	
		APTIAYNTFMPM	56. I44, I53, I56, F58, P63, F64, Y66, Y83, I86, P90, I91, A135, M139	
		APTIAYNTFMPM	57. T9, F22, I23, F30, P31, P35, I56, I102, I116, M139, T142, A146, Y150	
		APTIAYNTFMPM	58. F22, I23, N28, F30, P31, P35, I56, F58, P59, M139, T142, A146, Y150	
		APTIAYNTFMPM	59. I56, F58, P59, P63, F64, Y66, P90, I91, A135, M139	
		APTIAYNTFMPM	60. F30, P31, N43, I44, I53, I56, F58, P59, F64, Y66, Y83, I86, M139	
		IYQVLSTVQYR	29. S39, I56, S57, G61, L62, Y66, V67, V85, G88, G89, I91, G92, Q132, S136	
		DLTFTVNPLSKA	30. S39, K55, S57, F58, P59, L62, P63, F64, K65, V67, K68, V85, P90, A135, S136	
5	6	STIAERSIHPFN	31. S39, I56, S57, F58, P59, E60, P63, F64, E87, P90, I91, A135, S136	36.8
		NLSTGEKPEVTK	32. S39, K55, S57, P59, E60, G61, L62, P63, K65, V67, K68, V85, E87, G88, G89, P90, G92, S136	
		SLSSMNTIRVPG	33. S39, I56, S57, P59, G61, L62, P63, V67, V85, G88, G89, P90, I91, G92, S136, M139	
		HGVMTVSTTEKF	34. S39, K55, S57, F58, E60, G61, F64, K65, V67, K68, V85, E87, G88, G89, G92, S136, M139	
		HMGMTKINYSAL	35. S39, K55, I56, S57, G61, L62, K65, Y66, K68, G88, G89, I91, G92, A135, S136, M139	
		LSSHKATSTRAD	36. D93, T94, L95, K119, H121, T122, K123, H126, K129, A130, K134, A135, S136	
		LSSHKATSTRAD	37. R17, D25, K32, L143, R145, A146, S149, L152, A153, H154, S155, D156, A157	
		SLPFGPNTTIRP	38. S39, I56, S57, F58, P59, G61, L62, P63, F64, G88, G89, P90, I91, G92, S136	
		LESHYTQASYTQ	39. T9, T10, E141, T142, L143, A146, E148, S149, Y150, L152, A153, H154, A157, Y158	

Identical peptides in the same individual (boldface) or in different patients (italics) are indicated.