Modulation of Beta-Adrenergic Autoantibodies Over Time in Post-Viral ME/CFS is Related to Fatigue and Pain Symptoms

Lena Busch PhD[1,2*], Carsten Schriek MD[1,2*], Matthias Paul MD FESC FHFA[1,3], and Harald Heidecke PhD[4]

[1]Academy for Diagnostics and Prevention Münster, Münster, Germany
[2]University of Münster, Münster, Germany
[3]Department of Cardiovascular Medicine, University Hospital Münster, Steinfurt, Germany
[4]CellTrend GmbH, Luckenwalde, Germany

ABSTRACT **Background:** Myalgic encephalomyelits/chronic fatigue syndrome (ME/CFS) is an acquired disease with symptoms of fatigue and pain. In pathogenesis, the induction of autoantibodies (AAB) against G-protein coupled receptors (GPCR), such as β-adrenergic receptors (β-AdR), has been suspected. GPCR-AAB correlate with symptom severity and autonomic dysfunction in ME/CFS.

Objectives: To describe symptoms and treatment of a patient presenting with infection-triggered ME/CFS demonstrating that levels of β-AdR-AAB underlie modulation over time, correlating with the severity of symptoms.

Methods: At T1 and T2, GPCR-AAB were measured and questionnaires assessing symptom severity were completed. TSHDS-IgM-AAB were tested, and SF density was analyzed via skin probe.

Results: At T2, elevated levels of β-AdR-AAB were found, corresponding with an aggravation of fatigue and pain symptoms. Elevated TSHDS-IgM-AAB were found, which corresponded with reduced fiber density from the skin probe.

Conclusions: The levels of β-AdR-AAB in post-infectious ME/CFS can be modulated. Future studies might target interventions to reduce these AAB.

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KEY WORDS: autoantibodies, autoimmunity, chronic fatigue syndrome, myalgic encephalomyelitis, small fiber neuropathy

*These authors contributed equally to this study

Myalgic encephalomyelits/chronic fatigue syndrome (ME/CFS) is an acquired disease including immune, autonomic, and metabolic dysregulation as core aspects [1-3]. Specifically, ME/CFS is characterized by fatigue, pain, post-exertional malaise (PEM), sleep dysregulation, and immune and cognitive dysfunctions [3]. ME/CFS affects 0.3% of the population [2]. It has been suspected that ME/CFS is infection-triggered in most patients [4]. However, the pathogenesis of ME/CFS has not fully been understood [1].

ME/CFS is associated with hypoperfusion and vascular dysfunction that is aggravated during exertion and resulting in muscle pain and mental and physical fatigue [5]. In this context, natural regulatory autoantibodies (AAB) against vasoregulative G-protein coupled receptors (GPCR) for vasoactive hormones have been suspected to play a fundamental role in pathogenesis [2,6]. Specifically, β-adrenergic (AdR), muscarinic acetylcholine receptors (M-AChR), angiotensin II type 1 receptors (AT1-R), and endothelin-1 type A and B receptors (ETA/B-R) have been identified as relevant vasoactive receptors in this context [6]. Accordingly, elevated levels of AAB against GPCR have been associated with ME/CFS [2,7,8]. Recently, it has also been found that AAB against GPCR correlate with symptom severity, autonomic dysfunction, and disability in ME/CFS [9].

AAB responses are frequently activated by infections [7], not only against GPCR but also against small fiber. Specifically, AAB have been found against FGF Receptor-3 (FGF R-3-AAB) and TSHDS-IgM in patients with acute onset of small fiber neuropathy (SFN) [10,11]. SFN is the result of damage to peripheral nerves, including myelinated (Aδ) and unmyelinated (C) fibers [11]. Symptoms of SFN can include a wide range of symptoms, including muscle pain, fatigue, and cognitive dysfunction in addition to cardiac symptoms such as orthostatic hypotension [11,12].

Following evidence that levels of AAB against GPCR correlate with symptom severity and autonomic dysfunction in ME/CFS [6] between individuals, we present the first evidence that the levels of such AAB might also underlie modulation over time within an individual, correlating with the severity of symptoms. This case report has been prepared following the case report (CARE) writing guidelines [12].

PATIENTS AND METHODS

A woman presenting with a post-infection-triggered onset ME/CFS arrived at our clinic. We observed levels of AAB against GPCR correlating with symptom severity and autonomic dys-

function over time. In addition, we observed elevated AAB against small fiber such as TSHDS-IgM. These findings were in accordance with reduced fiber density identified in a skin probe.

PATIENT INFORMATION AND TIMELINE

In July 2021 (T1), a 27-year-old woman presented to our clinic with a 4-year history of fatigue disease following an Epstein-Barr-Virus (EBV) infection in 2017 [Figure 1]. Specifically, she reported mental and physical stress intolerance, neuromuscular pain, and fatigue. A post-viral fatigue disease was suspected. Based on the Canadian Consensus Criteria [3,13], ME/CFS was diagnosed. Other medical and neurological diseases that might have caused similar symptoms (i.e., hypo- or hyperthyroidism, diabetes mellitus, rheumatological disorders, immune disorders, Addison's disease, Cushing's syndrome, treatable forms of anemia such as iron deficiency, primary psychiatric disorders, multiple sclerosis) [3] were excluded by laboratory and clinical evaluation.

Specific questionnaires for symptom scoring were completed by the patient. According to published recommendation [6], we tested levels of β1-AdR-AAB, β2-AdR-AAB, M3-AChR-AAB, and M4-AChR-AAB. No significant elevations of AAB were found. The day the patient presented in our clinic, her mental and physical conditions were relatively stable according to her earlier history of physical and mental complaints.

In the following weeks, the patient reported significant aggravation of her symptoms. Because of her strong and persisting neuromuscular symptoms with nerval and muscular pain in her neck and extremities, we took a skin probe to conduct a nerval fiber density test. The results indicated SFN.

When the woman presented in our clinic in September 2021 (T2), her condition had become worse concerning her fatigue and pain status. We then suspected an increase of AAB in relation to the aggravation of her symptoms. Thus, we conducted a second test of β1-AdR-AAB, β2-AdR-AAB, M3-AChR-AAB, and M4-AChR-AAB. For more elaborative analysis, we also assessed the levels of AT1R-AAB, ETAR-AAB, α1-AdR-AAB, α2-AdR-AAB, M1-AChR-AAB, M2-AChR-AAB, and M5-AChR-AAB. According to the reduced density of nerval fibers in the sample of her subcutaneous tissue, we also initiated an analysis of AAB to small fiber, including FGF R-3-AAB and TSHDS-IgM-AAB. As in T1, the patient completed the specific questionnaires for symptom scoring.

DIAGNOSTIC ASSESSMENT

AT1R-AAB, ETAR-AAB, α1-AdR-AAB, α2-AdR-AAB, β1-AdR-AAB, β2-AdR-AAB, M1-AChR-AAB, M2-AChR-AAB, M3-AChR-AAB, M4-AChR-AAB, M5-AChR-AAB, FGF Receptor-3-AAB, and TSHDS-IgM-AAB were measured at the laboratory at CellTrend GmbH, Germany. A sandwich ELISA kit was used. The participant's whole blood sample was centrifugated and the purified serum was stored at -35°C. After washing steps, the plates were incubated with a 1:20,000 dilution of horseradish-peroxidase-labeled goat anti-human IgG used for detection. The ELISAs were validated according to the U.S. Food and Drug Administration's Guidance for Industry: Bioanalytical Method Validation. The

Figure 1. Case report timeline
EBV = Epstein-Barr-Virus

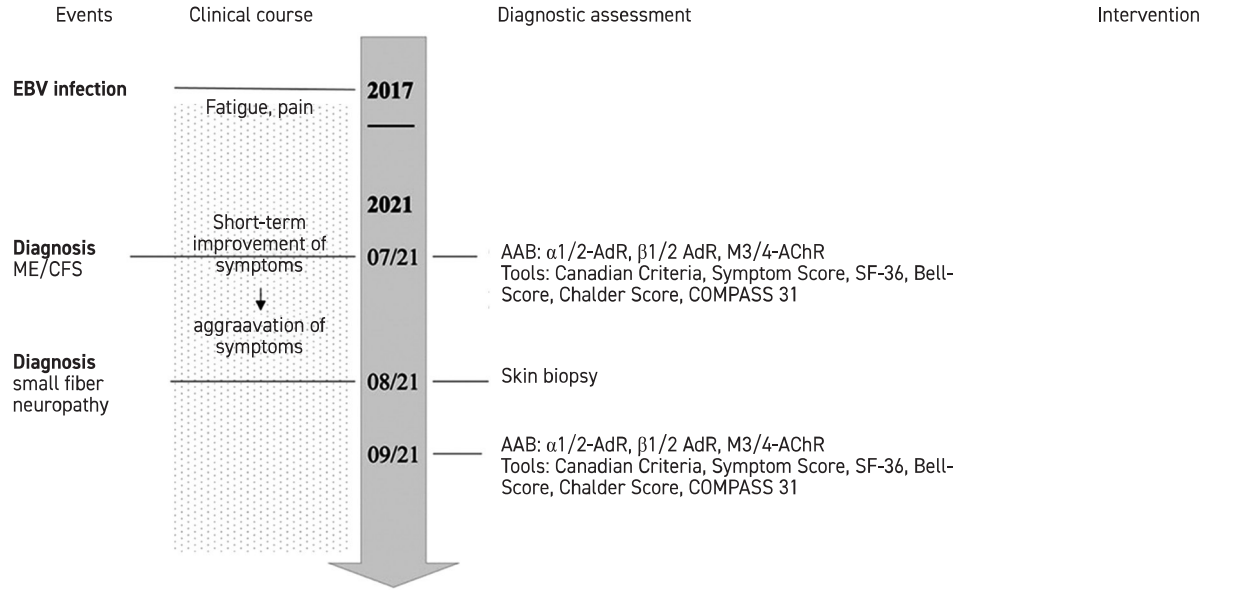


Table 1. Results of the questionnaires for symptom scoring

Assessment tool	T1 Score	T2 Score	Reference‡‡	Change T1-T2
Fatigue‡‡	**9.00** (0.71)	**9.80** (0.71)	8.00	0.035*
Muscle pain‡‡	**7.33** (1.53)	**9.69** (0.58)	7.00	0.045*
Cognitive‡‡	**7.67** (1.15)	**9.00** (1.00)	7.21	0.102
Cardiovascular‡‡	5.33 (0.58)	7.00 (1.73)	–	0.127
Immune‡‡	**7.33** (2.52)	**7.33** (2.52)	5.66	0.500
Chalder-Fatigue Score	27	**33**	28	0.003**
Bell Score	**20**	**20**	30	–
SF-36 Physical Function	**21**	**18**	45	0.296
COMPASS 31 TOTAL score	44	**70**	45.47	–
COMPASS 31 Orthostatic	10	10	28.00	–
COMPASS 31 Vasomotor	**2**	**6**	0.00	–
COMPASS 31 Secretomotor	3	**7**	6.42	–
COMPASS 31 Gastrointestinal	**14**	**24**	8.90	–
COMPASS 31 Bladder	**2**	**9**	1.10	–
COMPASS 31 Pupillomotor	**13**	**14**	2.40	–

Bold signifies values exceeding reference values
Significant differences between T1 and T2 (*$P < 0.05$, **$P < 0.01$)
‡Reference values are taken from the literature [8]
‡‡Values are presented as means (standard deviation). For all other values, sum scores are presented.

Table 2. AAB levels at T1 and T2. Cut-off values are taken from the literature

	T1 AAB	T2 AAB	Cut-off
AT1R-AAB		13.40 U/ml	17.00 U/ml
ETAR-AAB		12.30 U/ml	17.00 U/ml
α1-AdR-AAB		10.30 U/ml	11.00 U/ml
α2-AdR-AAB		7.80 U/ml	15.00 U/ml
β1-AdR-AAB	9.50 U/ml	**20.50 U/ml**	15.00 U/ml
β2-AdR-AAB	9.70 U/ml	**18.40 U/ml**	14.00 U/ml
M1-AChR-AAB		5.40 U/ml	9.00 U/ml
M2-AChR-AAB		7.70 U/ml	9.00 U/ml
M3-AChR-AAB	5.60 U/ml	7.70 U/ml	10.00 U/ml
M4-AChR-AAB	7.80 U/ml	10.60 U/ml	10.70 U/ml
M5-AChR-AAB		10.80 U/ml	14.20 U/ml
FGF-R3-AAB		5.20 U/ml	12.00 U/ml
TSHDS-IgM-AAB		**17.60 U/ml**	17.00 U/ml

Values presented in bold exceed cut-off values taken from the literature [8]

optimal cut-off level for each anti-GPCR autoantibody test was analyzed using the receiver operating characteristic (ROC) analysis.

The diagnosis of ME/CFS was based on the Canadian Consensus Criteria [3,13]. To assess these criteria, a referring assessment tool was applied that has been used in previous research [6]. Four scales representing the main symptoms fatigue (5 items) muscle pain (3 items), cognitive impairment (3 items), and immune symptoms (3 items) were administered. In addition, the scale assessing cardiovascular symptoms was applied. A high mean score indicates severe symptoms. To assess symptoms of autonomic dysfunction, the Composite Autonomic Symptom Score 31 (COMPASS 31) [14] was implemented. A high total score indicates more severe symptoms. The Bell score [15] was used to assess disability, specifically focusing on the level of restriction in daily functioning on a scale ranging from 0–100. A low score indicates high disability. To assess the level of fatigue, the Chalder Fatigue Score [16] was applied. High total scores indicate high levels of fatigue. Total physical functioning (i.e., daily physical activities) was tested via the Short Form Health Survey 36 (SF-36) [17]. High total scores indicate high physical functioning.

The statistical analysis was performed using Microsoft Excel™ 2022, Version 16.69 (Microsoft® Corporation, Redmond, WA, USA). According to the referring test, results were presented as sum scores or as means and standard deviations. The values were compared to mean scores of patients presenting with infec-

tion-triggered ME/CFS identified in the literature [6]. If mean values and standard deviations could be calculated, data assessed at T1 and T2 was compared. Therefore, a non-parametric *t*-test (i.e., Mann-Whitney U-Test) was applied as unequal variances were identified. Significance level was defined $P < 0.05$.

RESULTS

At T1, the scales fatigue, muscle pain, cognitive impairment, and immune symptoms exceeded the mean values identified in a population of infection-triggered ME/CFS [6] [Table 1]. Regarding the scale measuring cardiovascular symptoms, no reference values exist. The patient's results on the Chalder Fatigue Scale Score were similar compared to the reference value. The patient's result from the Bell Score was lower than the reference value, indicating more severe disability. The SF-36 value was also lower than the reference value, indicating lower physical activity than reported by the mean population of infection-triggered ME/CFS. Regarding the COMPASS 31 scores, the vasomotor symptoms, gastrointestinal symptoms, and bladder symptoms exceeded the reference values, whereas the total score and the scales orthostatic and secretomotor symptoms did not exceed the reference values.

The results of the AAB tests showed a slight elevation of β2-AdR-AAB; however, the β1-AdR-AAB, M3-AChR-AAB, and M4-AChR-AAB were not elevated [Table 2]. Regarding the skin biopsy testing the small fiber density, a reduced nerval fiber density of 6.97 fiber/mm compared to the reference (women 18–39 years of age 11.65 fiber/mm) was found.

At T2, all symptoms were rated as similar or more severe than at T1. Specifically, the values for fatigue, muscle pain, cognitive impairment, and cardiovascular symptoms exceeded the reference values and the values measured at T1 [Table 1]. The change from T1 to T2 was statistically significant for the fatigue scale, $t(7) = -2.138$, $P = 0.035$, and the muscle pain scale, $t(3) = -2.474$, $P = 0.045$, indicating more severe symptoms. The Chalder Fatigue Scale Score value was significantly higher than the value measured at T1, $t(10) = -3.464$, $P = 0.003$, indicating higher rated fatigue. The SF-36 value was also lower than the value measured at T1, indicating lower physical activity measured at T1. Regarding the COMPASS 31 scores, the scales vasomotor symptoms, gastrointestinal symptoms, and bladder symptoms again exceeded the reference values and the values measured at T1. In addition, the total score and the secretomotor scale exceeded the reference values.

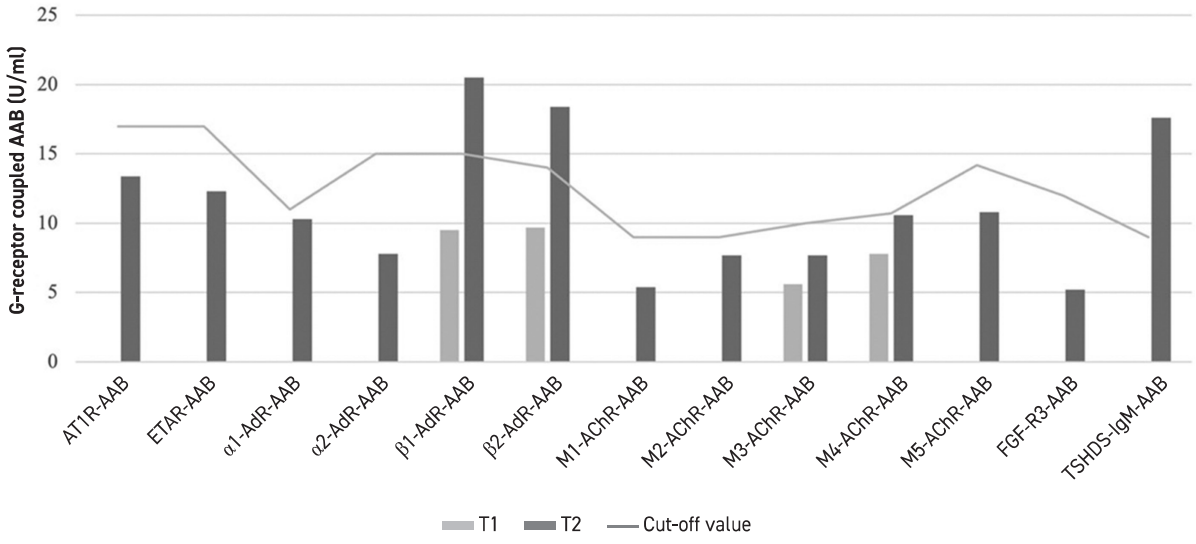
The results of the AAB tests revealed an elevation of AAB at T2 [Table 2, Figure 2]. Specifically, significant increases in the β1-AdR-AAB and the β2-AdR-AAB were found, exceeding the cut-off values. Furthermore, elevated TSHDS-IgM-AAB were found.

DISCUSSION

We found that modulation of β1/2-AdR-AAB over time was associated with severity of the patient's pain and fatigue symptoms. Specifically, at T1, the patient reported symptoms of fatigue, muscle pain, cognitive impairment, and immune dysfunction. These symptoms were comparable to or more severe than the symptoms reported in a sample of patients with infection-triggered ME/CFS [6]. GPCR-AAB were not or marginally elevated.

At T2, the patient reported aggravation of symptoms that

Figure 2. G-receptor coupled autoantibodies measured at T1 and T2 and referring cut-off values



were represented by significant changes in the questionnaires measuring fatigue and muscle pain. These findings correlated significantly with elevated β1/2-AdR-AAB measured at T2. GPCR for vasoactive hormones, such as catecholamines and acetylcholine regulate blood flow [18]. In this context, hypoperfusion and reduced muscular oxygen supply during exercise have been found in patients presenting with ME/CFS [5]. Also, deterioration of exercise variables, such as peak workload, has been found in ME/CFS on day 2 testing [19]. Thus, aggravation of hypoperfusion and the resulting fatigue and muscle pain are recognized as key symptoms of ME/CFS [2,6]. AAB against GPCR have also been associated with dysregulated processes in immune related diseases, including rheumatic diseases [20,21]. AAB responses are frequently activated by infections [6]. In this context, symptoms of ME/CFS and elevated AAB against GPCR have been found in post-coronavirus disease 2019 (COVID-19) patients [22].

Specifically, β2-AdR-AAB were the first to be identified in ME/CFS patients. In this context, elevated levels of β2-AdR-AAB and M-AChR-AAB are connected with amyosthenia and neurocognitive symptoms [7]. Also, β1/2-AdR-AAB have been associated with altered structural brain networks and pain modulation [23] and with reduced stimulation of immune cells under ME/CFS [24]. It has been demonstrated that higher levels of GPCR, including β1/2-AdR-AAB, are associated with the severity of symptoms (i.e., specifically muscle pain and fatigue), autonomic dysfunction, and disability in ME/CFS [6]. In our patient, symptom severity was also associated with elevated β1/2-AdR-AAB and significant changes in fatigue and pain symptoms. To the best of our knowledge, this case report is the first to demonstrate this association within a patient. Thus, the results also indicate that modulation of AAB against GPCR is possible.

We found elevated AAB to small fiber (i.e., TSHDS-IgM-AAB, but not FGF-R3-AAB) corresponded to the reduced fiber density found with the skin probe. As AAB responses are frequently activated by infections, our findings indicate that post-infectious pain syndromes might be caused by a variety of AAB directed against nerval structures, some of which have been identified [9,10]. Thus, not only GPCR-AAB but also AAB against small fiber might be induced in patients presenting with post-viral ME/CFS, causing the characteristic neuromuscular pain syndrome [11,12]. Like these assumptions, reduced small fiber density has also been found in post-COVID-19 syndrome with ME/CFS-related symptoms (i.e., pain and fatigue) [25].

A limitation to this study is that we could not identify potential influencing factors that could explain the amelioration or aggravation of symptoms within the patient. Also, only a sub-selection of AAB was tested at T1 compared to T2. Thus, a less comprehensive and less evaluative analysis was conducted of modulation of AAB, especially regarding the connection between FGF R-3-AAB, TSHDS-IgM-AAB, and small fiber density.

Treatment of ME/CFS is difficult [2]. Extracorporeal apheresis might be a potential yet expensive treatment strategy in patients with ME/CFS and post-COVID-19 [22]. Mixed evidence has been found for treatment with high dose intravenous IgG, the monoclonal anti-CD20 antibody rituximab, and immunoadsorption [1]. In this case report, we demonstrated that modulation of AAB against GPCR can also occur without the application of invasive methods. These findings might lead to new pathways in the treatment of post-infectious ME/CFS via modulation of autonomous regulation. Thus, future studies should identify influencing factors or therapeutic interventions that might contribute to additional and efficient treatment of ME/CFS.

CONCLUSIONS

Amodulation of β1/2-AdR-AAB over time was associated with the severity of a patient's pain and fatigue symptoms. Our results correspond to recent findings postulating that the level of active natural regulatory AAB is associated with the severity of symptoms in patients with ME/CFS [6]. Furthermore, we found TSHDS-IgM-AAB in the patient presenting with both ME/CFS and reduced fiber density. These findings show that the levels of AAB in patients presenting with post-infectious ME/CFS can be modulated. Thus, future studies might target specific interventions to reduce these AAB.

DECLARATION OF INTEREST

Harald Heidecke, managing director of CellTrend GmbH, holds a patent for the use of beta-adrenergic receptor antibodies in diagnosis of CFS.

Correspondence

Dr. L. Busch
Academy for Diagnostics and Prevention Münster, 48149 Münster, Germany
Fax: (49-251) 200-6211
Email: lena.busch@uni-muenster.de

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Poetry is the shadow cast by our streetlight imaginations.

Lawrence Ferlinghetti (1919–2021), poet and painter

Hail to the man who went through life always helping others, knowing no fear, and to whom aggressiveness and resentment are alien.

Albert Einstein (1879–1955), German-born theoretical physicist who developed the theory of relativity, one of the two pillars of modern physics (alongside quantum mechanics)

Capsule

Non-viral precision T cell receptor replacement for personalized cell therapy

T cell receptors (TCRs) enable T cells to specifically recognize mutations in cancer cells. **Foy** et al. developed a clinical-grade approach based on CRISPR-Cas9 non-viral precision genome-editing to simultaneously knockout the two endogenous TCR genes *TRAC* (which encodes TCRα) and *TRBC* (which encodes TCRβ). They also inserted into the *TRAC* locus two chains of a neoantigen-specific TCR (neoTCR) isolated from circulating T cells of patients. The neoTCRs were isolated using a personalized library of soluble predicted neoantigen-LA capture reagents. Sixteen patients with different refractory solid cancers received up to three distinct neoTCR transgenic cell products. Each product expressed a patient-specific neoTCR and was administered in a cell-dose-escalation, first-in-human phase I clinical trial. One patient had grade 1 cytokine release syndrome and one patient had grade 3 encephalitis. All participants had the expected side effects from the lymphodepleting chemotherapy. Five patients had stable disease and the other eleven had disease progression as the best response on the therapy. neoTCR transgenic T cells were detected in tumor biopsy samples after infusion at frequencies higher than the native TCRs before infusion. The authors demonstrated the feasibility of isolating and cloning multiple TCRs that recognize mutational neoantigens. Moreover, simultaneous knockout of the endogenous TCR and knock-in of neoTCRs using single-step, non-viral precision genome-editing are achieved. The manufacture of neoTCR engineered T cells at clinical grade, the safety of infusing up to three gene-edited neoTCR T cell products and the ability of the transgenic T cells to traffic to the tumors of patients are also demonstrated.

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Eitan Israeli