A novel therapeutic drug for *Myasthenia Gravis* : －ＴＫ２１－

"TK21" is a novel fusion protein, which consist of the extracellular region of human nicotinic acetylcholine receptor (nAChR) alpha1 subunit and the Fc region of human immunoglobulin IgG1. TK21 is expected a sustained symptom improvement effect against *Myasthenia Gravis* (MG) by following dual actions.

1. Neutralization against the anti-acetylcholine receptor autologous antibodies , which is the main cause of MG.

2. Selective killing of the specific B cells producing the anti-acetylcholine receptor autologous antibodies.

About MG

MG is one of the autoimmune diseases, caused by autologous antibodies binding to the membrane protein, nAChR, on the muscle end-plate at neuro-muscular junction (NMJ), and then nAChR become dysfunctional. The main symptoms of MG are systemic loss of muscle strength and easy fatigability, in the serious MG condition, a paralysis of a respiratory muscle. In Japan, MG is officially designated as an intractable disease.

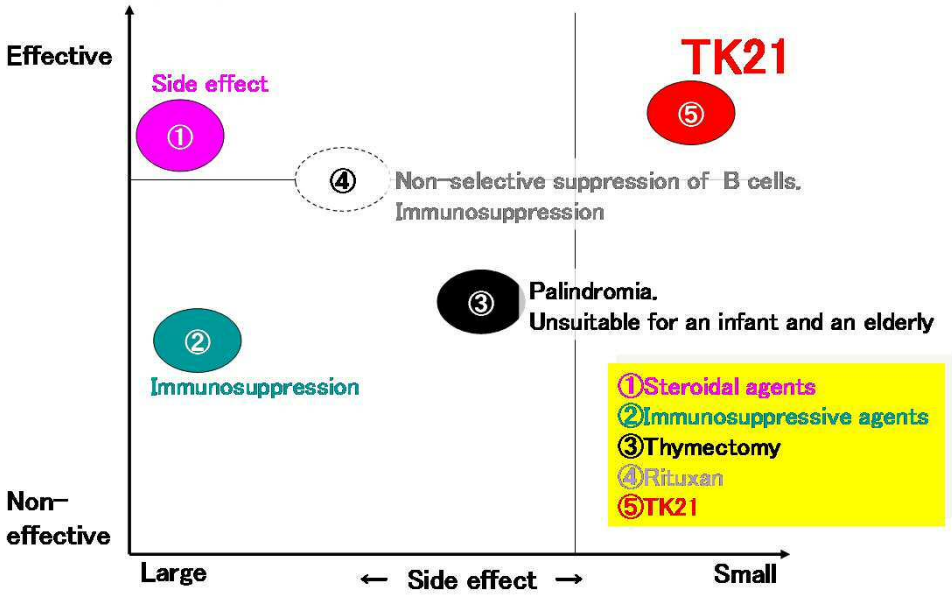
The number of MG patients in Japan are thought to be 15,100 (2006 epidemiological survey), and the total number of MG patients in the seven major countries is 106,000, increasing year by year.

As for the present therapies, anti-cholinesterase drugs as a symptomatic treatment, steroidal drugs, immunosuppressive drugs, plasmapheresis (plasma exchange), and the IVIG treatment are performed to the patients.

However, above therapies have some problems, such as necessity for continuation therapy and their side effects. Therefore, it is thought that the medical needs to new MG therapeutic drug are high.

Superiority for present therapies

Aiming fundamental treatment



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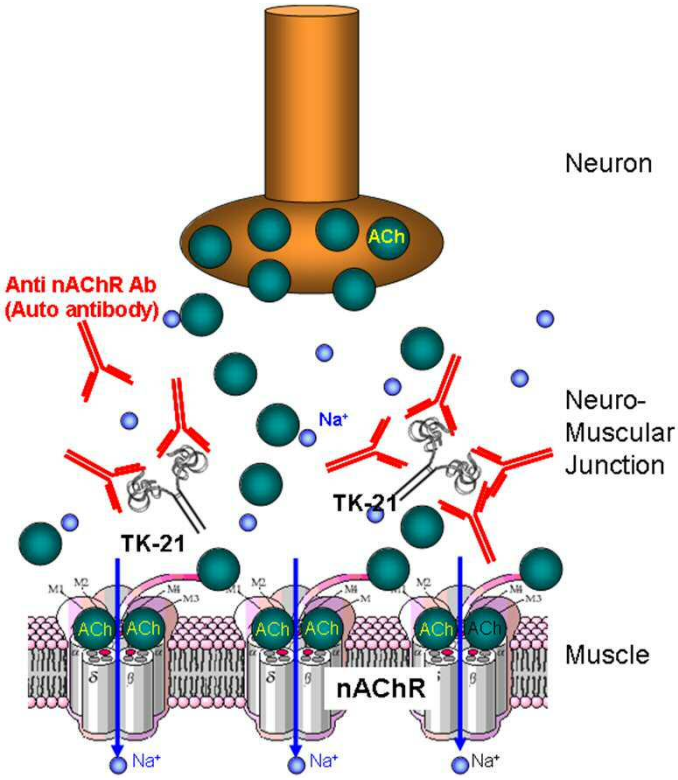
Dual Actions of “TK21”

1． Blocking effect / Decoy effect

TK21 directly block the autologous antibodies bind to nAChR, and then reduce the symptom of MG for shortly after.

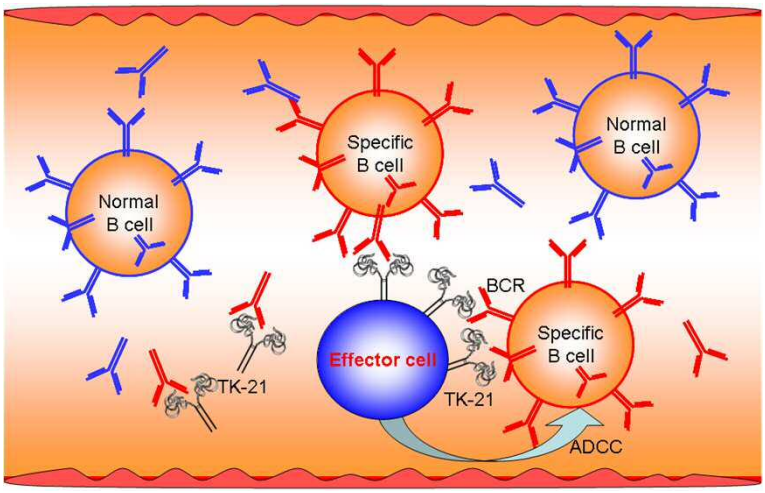


2． Antibody-Dependent Cell Cytotoxicity （ADCC) effect

TK21 binds to autoantibody-producing B cells in blood by Fc structure of TK21, then, TK21 kills only B cells producing autoantibody through an effector cell selectively.

Therefore, unlike immunosuppressants, TK21 does not suppress the whole of immune system, and there are no side effects such as immunocompromised.

And, by reducing the number of specific B cells, sustained clinical effect is expected.



About TK21

Profile of substance

・Recombinant protein （MW: about 104kDa)

・Fusion protein (extracellular region of human nAChR α1 subunit-①
and the Fc region of human IgG1-②)

・Glycosylation protein

Production

・Expression system with CHO cell line

・Fed-batch culture process

・Expression level of about 2 g/L(commercially-available media)

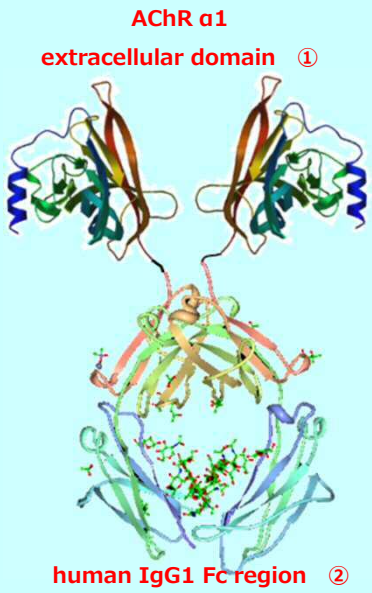
・Assuming 3 to 4 steps including Protein A column chromatography

Drug substance

・Monomer (homodimer) ratio of about 92%

Patents

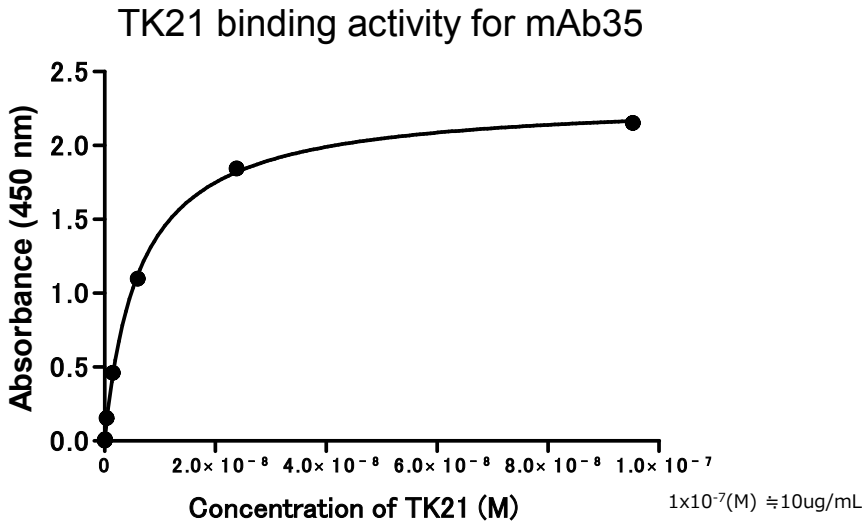
・JP 4495776
・JP 4857396 (PCT/JP2012/058912)



Pharmacological actions(*in vitro*)

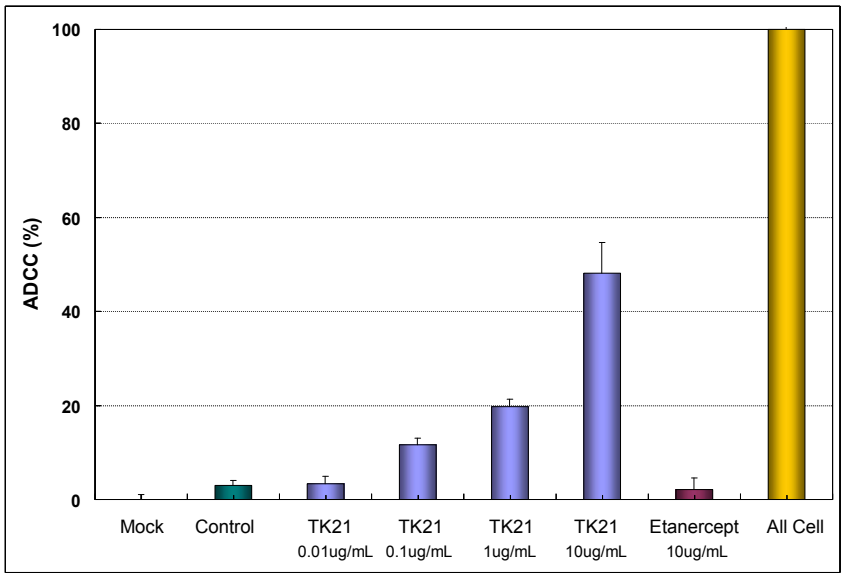
1． Autoantibody-neutralizing Activity (rat antibodies)

TK21 was confirmed to bind to mAb35, a rat anti-AChR α1 antibody, in a concentration-dependent manner.



2. ADCC activity to B cell producing autoantibodies (rat hybridoma)

It is shown that TK21 has ADCC activity against hybridoma producing autoantibodies to nAChR.



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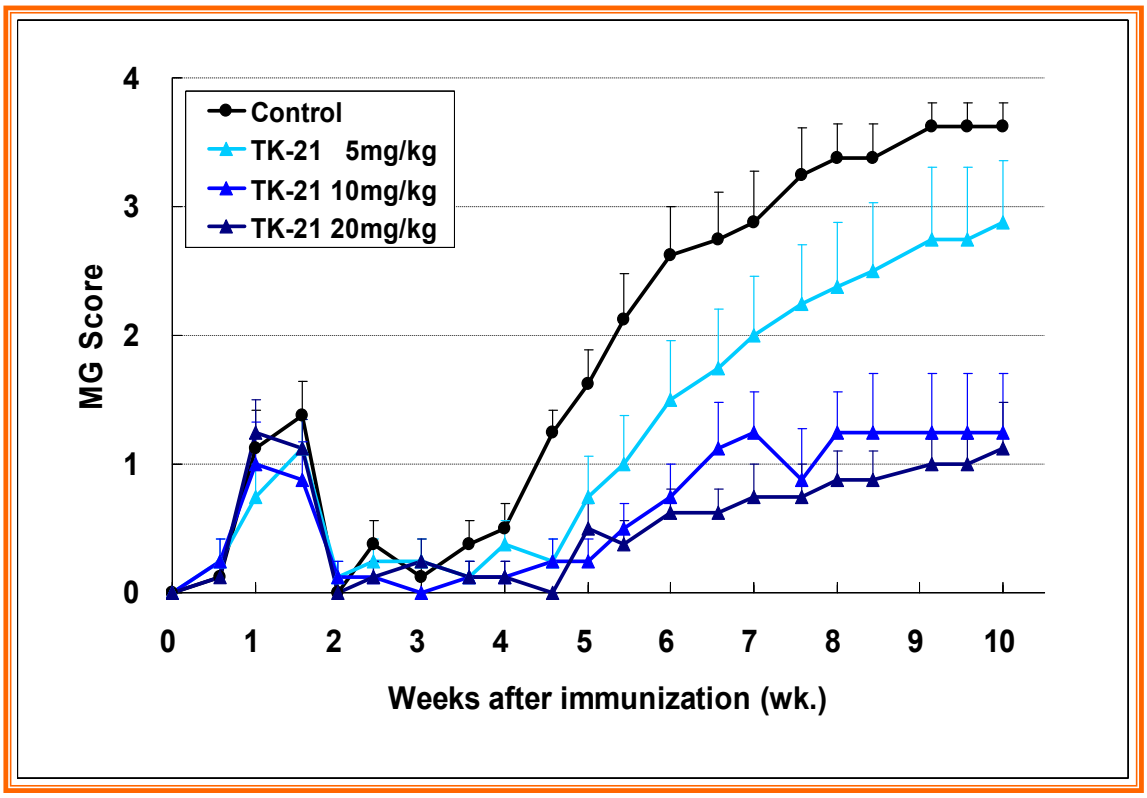
5. Effect of TK21 on the rat EAMG model (*in vivo*)

Rats were immunized with nAChR derived from electric ray fish to induce MG symptoms (rat immune EAMG model). TK21 was intravenously administered once daily 7-11 days, 21-25 days, and 35-39 days after immunization.
Dose-dependent suppressive effect was confirmed in EAMG model



MG score
0:normal
1:mildly decreased activity,weak grip,with fatigability
2:weakness, hunched posture at rest, loss of body weight and shaking
3:severe generalized weakness, obvious loss of body weight,moribund
4:dead

Safety assessment

Toxicity : Single dose toxicity test（pre test） : LD50＞1,500mg/kg
Antigenecity : It is confirmed that TK21 has lower antigenecity than marketed human antibody products in *in sillico* test.

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