[4/18/2008(Fri) 16:55~17:30/1st FL)]

Development of a Novel Anti-TNFα Monoclonal Antibody for Rheumatoid arthritis treatment

Moo-Young Song, Sang-Koo Park, Tae Hyoung Yoo, Seung Hoon Kim, Kyung Mi Park, Hyung Jin Moon, Hyun-ho Choi, Bongtae Kim, Kyoung Kyu Ahn, Won Jae Kwag, Byung-Kyu Lee

Yuhan Research Institute, 416-1, Gongse-dong, Giheung-gu, Yongin-si, Gyeunggi-do, Korea. Fax: 031-275-6146, e-mail: LBKS@yuhan.co.kr

TNFα is a potent pro-inflammatory cytokine playing both paracrine and endocrine roles in the immune response. Since TNFα plays such a key role in mediating pathological inflammation, and its excess production has been directly implicated in a wide variety of inflammatory diseases such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriatic arthritis, and psoriasis.

We produced and characterized a murine monoclonal antibody (mAb) TSK114 against human TNFα, and further developed the humanized mAb YHB1411-2 by complementary-determining region (CDR) grafting procedure. The neutralizing activity of YHB1411-2 to inhibit human TNFα-induced cytotoxicity *in vitro* was tested using WEHI cell based assay system. The neutralizing activity of YHB1411-2 was 2~3 fold higher than that of infliximab and adalimumab. *In vivo* TNFα neutralizing activity of YHB1411-2 was evaluated using galactosamine-sensitized mouse model. YHB1411-2 showed dose-dependent inhibition of mortality and was more potent than infliximab at the same dosage. Epitope mapping was conducted by yeast display technology and site directed mutagenesis. YHB1411-2 has two predominant binding regions located on the apical bottom and top of the subunit surfaces of trimeric TNFα. The therapeutic efficacy of YHB1411-2 was assessed by using polyarthritis model (human TNFα transgenic mouse, Tg 1006-T). YHB1411-2 showed dose-dependent prevention of polyarthritis. Especially, the severity of arthritis in Tg mice treated with YHB1411-2 was significantly reduced in comparison with infliximab treated Tg mice at the same dosage. The results showed that YHB1411-2 has higher TNFα-inhibitory activity *in vitro* and *in vivo* than other TNFα blockers. Preclinical studies for YHB1411-2 are scheduled late in this year

Development of a Novel Anti-TNFα Monoclonal Antibody for Rheumatoid Arthritis Treatment

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Byung-Kyu Lee

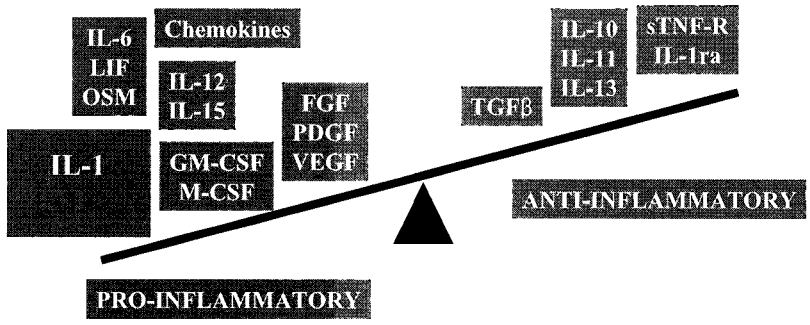
Yuhan Research Institute

Rheumatoid Arthritis

- A common human autoimmune disease with a prevalence of about 1% in adult population
- Chronic inflammation of the synovial joints and infiltration by blood-derived cells, macrophages, and plasma cells.

TNF-α as a therapeutic target in rheumatoid arthritis

<Cytokine disequilibrium in rheumatoid arthritis>



Marc Feldmann et al 2002

Anti-TNFα Drugs & Candidates

Product	Target	Developer	Characteristics	Stage
Remicade®	TNFα	Centocor	Chimeric	Launched
Enbrel®	TNFα	Immunex (Amgen)	Fusion protein	Launched
Humira®	TNFα	Abbott	Fully Human	Launched
Golimumab	TNFα	J&J(Centocor)	Fully Human	Phase 2
Cimzia	TNFα	Celltech	Fab	Phase 3

TSK114 (murine anti-TNFα antibody)

Balb/c

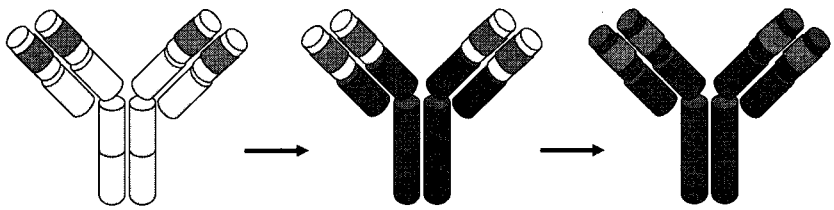
Immunization with Recombinant human TNF-α

Spleen → Hybridomas (Sp2/0) → 4T46

	TSK11	TSK13	TSK43	TSK66	TSK73	TSK161	TSK179
Media selection (% positive)	173	155	129	151	174	151	172
OD_{405} (150 ng Ab)	0.968	1.002					0.949
Affinity (Kd, M^{-1})	5×10^{-11}	5×10^{-10}	1.4×10^{-10}				1.3×10^{-10}
Selected	O						

	TSK11-4	TSK11-19
Affinity (IC_{50}, M)	1.42×10^{-9}	1.73×10^{-9}
Selected	O	

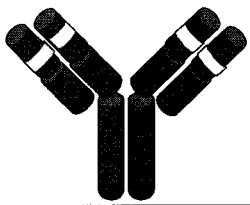
Development of Humanized Antibody



Murine mAb
- Immunogenicity
- Short half life

Chimeric mAb
- 75% human IgG
- Immunogenicity

Humanized mAb
- 90% human IgG
- CDR grafting



Yuhan's humanized mAb
- 95.4% human IgG
- CDR walking

YHB1411-2

- Developed from murine lead antibody (TSK114)
- Very high affinity against human TNFα
- Immunoglobulin (Ig) G_1 type monoclonal antibody
- Human framework selected based on homology: Hh1, Hk1
- Humanization through recombinant DNA technology (by CDR grafting)
- Specificity for human TNFα

  YHB1411-2 does not react with human TNFβ and TNF superfamily.

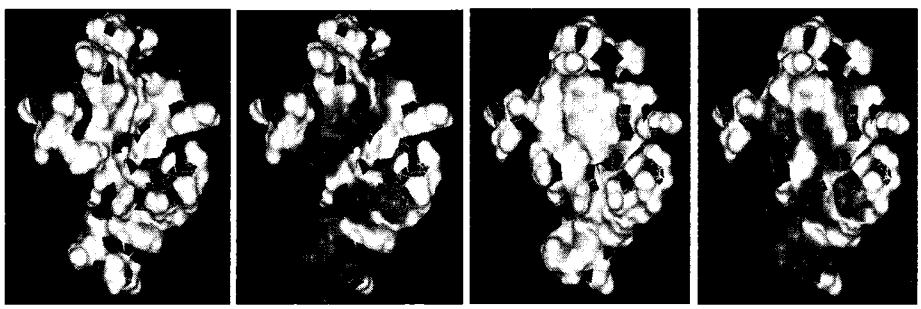
Yuhan's humanized mAb, YHB1411-2
- IgG1k
- Humanized by CDR grafting and walking

[1]; EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 40, No. 1, 35-42, February 2008

3-D Modelling

Lead TSK114 **Humanized Ab YHB1411-2**

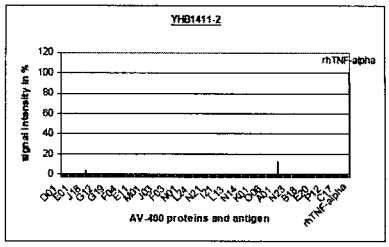
A B C D



A, C: Surface of CDR region B, D: Charge distribution of CDR region
Red : negative charge Blue : positive charge

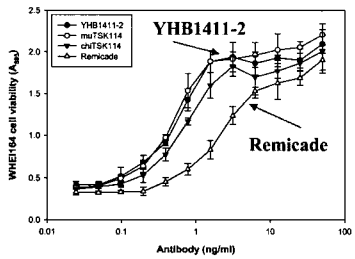
Cross-reactivity

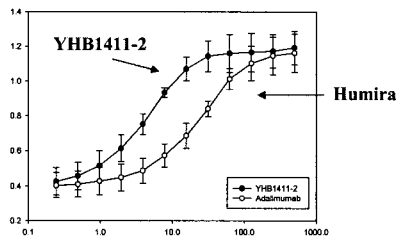
- Cross-reactivity was conducted with the UNIchip AV-400 of Protagen Inc. (Dortmund, Germany)
- YHB1411-2 showed no cross reactivity except two proteins with low signal intensity



	YHB1411-2	Remicade	Humira	Enbrel
UNIchip AV-400	▪ 2 /384 proteins ▪ Weak response (4, 13%)	▪ 1 /384 ▪ Weak response (8%)	▪ 22 /384 ▪ 12~23%	▪ 11 /384 ▪ 20~33%

In vitro cell assay



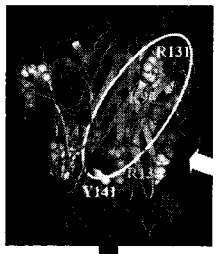


Cell line	YHB1411-2 EC_{50} (ng/ml)	Remicade EC_{50} (ng/ml)	Humira EC_{50} (ng/ml)
WEHI 164	**5.90±0.79**	**16.37±2.16**	**23.33±5.43**

* WEHI 164 is extremely sensitive cell-lines to TNFα

Epitope analysis

- Epitope was analyzed by yeast display technology and site directed mutagenesis
- Binding residues of YHB1411-2 are K90, R131



YHB1411-2

Enbrel Remicade

	YHB1411-2	Remicade	Enbrel
Binding residues	**K90, R131** Y141, L142	**R138, D140, Y141** L142	**Y141** R138, L142

Analysis of Complex formation and Binding pattern

Analyzed by SEC & DLS

J. Mol. Biol. (2007) 374, 1374–1388

Comparative analyses of complex formation and binding site between anti-TNFα blockers

↓

Elucidate different neutralizing mechanism & PK profiles

Table 1. Hydrodynamic parameters of TNFα, its antagonists, and their complexes formed at the optimal binding ratios as measured by SEC and DLS

			DLS		
		SEC (kDa)	Diffusion coefficient ($\mu m^2/s$)	Stokes radius (nm)	M_r (kDa)
TNFα		56 ± 6	49 ± 2	3.66 ± 0.12	69 ± 7
Etanercept	r=0	407 ± 35	37 ± 3 [31 ± 3]*	6.76 ± 0.22 [6.99 ± 0.25]*	294 ± 15 [318 ± 19]*
	r=1	414 ± 24	34 ± 3	7.54 ± 0.24	381 ± 18
Infliximab	r=0	153 ± 8	42 ± 2	5.49 ± 0.14	180 ± 14
	r=0.5	1251 ± 64	23 ± 1	11.24 ± 0.21	1033 ± 124
YHB1411-2	r=0	165 ± 16	34 ± 2	5.34 ± 0.16	169 ± 9
	r=0.5	590 ± 19[b]	27 ± 1	9.27 ± 0.15	616 ± 15
		n.a.[c]	7.8 ± 0.9	31.93 ± 0.47	11,140 ± 1169

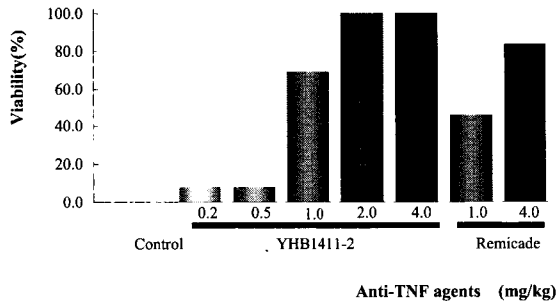
All of the values are represented as mean values ± SD (standard deviation) for at least duplicate experiments. The r value designates the molar ratio of antagonist to trimeric TNFα, i.e. r = [trimeric TNFα]/ [antagonist].

Humira: 598~4560, 598 (stable form)

(*Analytical Biochemistry* 299, 119-129(2001))

In vivo neutralization

Prevention of human TNFα-induced lethality by anti-TNFα Ab



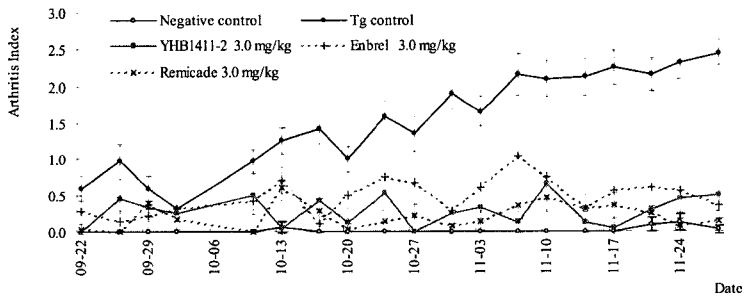
▪**Test model:** Galactosamine-sensitized Balb/c mouse model

YHB1411-2 has more effective *in vivo* neutralizing ability against human TNFα than Remicade®.

Efficacy study

Clinical arthritis Score

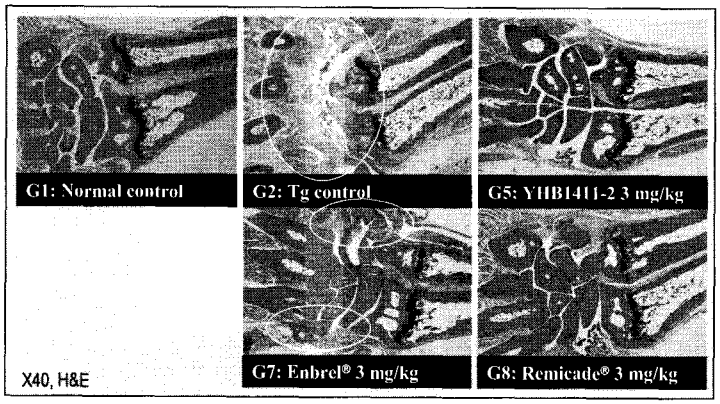
Tg mice: Tg T-1006, C57BL/6NTac-TgN mice (Taconic, USA)



The clinical arthritis score was markedly decreased in the groups treated with anti-TNFα agents

Efficacy study

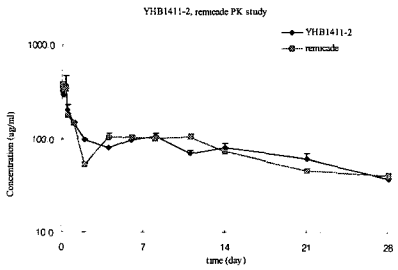
Histopathological study



Histopathological study shows significant therapeutic effect of YHB1411-2 treatment on arthritic knee joint of hTNF-α transgenic mice.

(The circle indicates severe articular cartilage degeneration, including cartilage loss and fibrillation, and bone resorption)

Pharmacokinetics

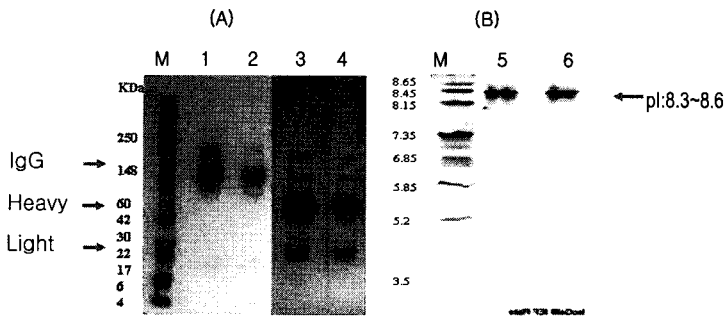


Serum concentration-time profiles of YHB1411-2 and Remicade after intravenous administration of YHB1411-2 and Remicade to rats at a dose of 4 mg/kg (n=5 ~ 6).

PK parameters of YHB1411-2 and Remicade after intravenous administration at a dose of 4 mg/kg

Parameter	AUC_{0-t} (mg.day/ml)	$T_{1/2}$ (day)	Clearance (ml/day/kg)
YHB1411-2	2199.8 ± 107.4	15.3 ± 1.7	1.3 ± 0.1
Remicade	2205.0 ± 45.4	13.3 ± 0.8	1.3 ± 0.0

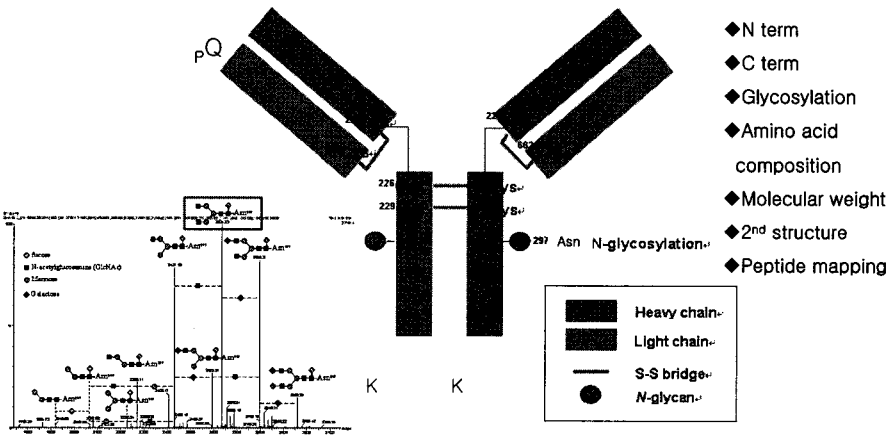
Electrophoresis of YHB1411-2



(A) SDS-PAGE, (B) Iso-electric focusing

1~2: non-reducing condition, 3~4: Reducing condition

Predicted structure of YHB1411-2



- ◆N term
- ◆C term
- ◆Glycosylation
- ◆Amino acid composition
- ◆Molecular weight
- ◆2nd structure
- ◆Peptide mapping

Formulation

- Current formulation (pre-formulation)
  - Developed by Integrity Biosolution (USA)
  - Liquid, non-preserved
  - 50 mg/mL
  - SC

	YHB1411-2	Remicade	Humira	Enbrel
Formulation	Liquid	Lyophilized powder	Liquid, Pen & Prefilled syringe	Lyophilized powder & Prefilled syringe
Route	SC	IV	SC	SC
Dosage & Frequency	?	3mg/kg Repeat at 2 and 6 wks, 3mg/kg every 8wks	40mg/every other week	50mg/week

Summary

- We successfully developed humanized anti-TNFα antibody YHB1411-2 from lead antibody TSK114
- YHB1411-2 has more effective *in vitro* neutralizing activity than both Remicade and Humira.
- Analysis of YHB1411-2 with the UNIchip AV-400 of Protagen AG showed no cross reactivity except only two proteins with low signal intensity among 384 human proteins.
- Binding residues of YHB1411-2 are K90, R131. Stable complex size of YHB1411-2-Ag is about 500kDa.
- Efficacy study (Arthritic score & histological study) showed the excellent therapeutic effect of YHB1411-2 treatment on arthritic fore ankle of human TNFa transgenic mice (C57BL/6NTac-TgN).
- Pre-clinical studies are scheduled in late 2008.