A bispecific antibody that targets IL-6 receptor and IL-17A for the potential therapy of patients with autoimmune and inflammatory diseases.

Michael Lyman, Vincent Lieuw, Robyn Richardson, Anjuli Timmer, Christine Stewart, Steve Granger, Richard Woods, Michela Silacci, Dragun Grabulovski and Roland Newman*

From Tanabe Research Labs U.S.A. Inc. 4540 Towne Centre Court, San Diego, CA USA and Covagen AG, Wagistrasse 25, 8952 Schlieren, Switzerland

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To whom correspondence should be addressed: Roland Newman, Tanabe Research Labs U.S.A. Inc. 4540 Towne Centre Court, San Diego, CA USA. Tel (858)-622-7033; email rnewman@trlusa.com

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Despite the success of current biological therapeutics for rheumatoid arthritis, these therapies, targeting individual cytokines or pathways, produce beneficial responses in only about half of patients. Therefore, better therapeutics are needed. IL-6 and IL-17A are proinflammatory cytokines in many autoimmune and inflammatory diseases, and several therapeutics have been developed to specifically inhibit them. However, targeting both of these cytokines with a bispecific therapeutic agent could account for their nonoverlapping proinflammatory functions and for the fact that IL-6 and IL-17A act in a positive feedback loop. Here, we present the development of MT-6194, a bispecific antibody targeting both IL-6R and IL-17A that was developed with the FynomAb technology. We also present data from mouse inflammatory disease experiments, indicating that simultaneous inhibition of both IL-6 and IL-17A yields enhanced efficacy compared with inhibition of each cytokine alone.

*To whom all correspondence should be addressed.

Conventional non-biologic disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate are still the mainstay and primary first-line treatment option for rheumatoid arthritis (RA) and other inflammatory diseases. However, biologic agents, such as the tumor necrosis factor (TNF) inhibitors, have proven to be efficacious in patients not responding to the small molecule DMARDs. Despite the relative success of biologics, many patients do not have substantial or durable responses, for example, it is estimated that 20–30% of RA patients are non-responsive to anti-TNF therapy (i.e. do not reach ACR20), and more than 50% of RA
patients treated with current biologics do not achieve a robust response, defined as 50% improvement according to American College of Rheumatology criteria (ACR50). Furthermore, many patients that initially respond to TNF blockers will eventually lose their responsiveness over time with a need to switch to other agents. Despite the enormous progress that has been seen over the last two to three decades in understanding the inflammatory mechanisms of disease, prognosis and drug responses, the current treatment modalities are still insufficient for many patients, and the mechanisms of non-responsiveness in many patients remains unknown. Consequently, biologic agents are still used on a ‘trial-and-error’ sequential basis rather than on rational patient stratification. Therefore, a high unmet medical need still remains, and alternative strategies are needed to further improve patients’ quality of life.

We have explored the use of FynomAbs, which are fusion proteins of an antibody and a Fynomer. Fynomers are small 7-kDa globular proteins derived from the SH3 domain of the human Fyn kinase that can be engineered to bind with high affinity to virtually any target of choice through random mutation of 2 different binding loops (RT- and src-loop). Fynomers binding to variety of targets have been previously described, as well as bispecific FynomAbs. Here we describe MT-6194, a bi-specific FynomAb that binds and inhibits two clinically validated targets - human IL-6R and IL-17A.

Results

Biophysical characterization and binding affinity of MT-6194.

MT-6194, a bi-specific FynomAb targeting both human IL-17A and IL-6R was constructed by genetically fusing the anti-IL-17A Fynomer 11L9C09 to the C-terminus of the light chain of the anti-IL-6R antibody tocilizumab (Figure 1A). The amino acids 356-358 were mutated from DEL to EEM in the antibody heavy chain, which changes the allotype from G1m1 to nG1m1. This was done to reduce any potential immunogenicity. MT-6194 produced in stably transfected CHOK1SV cells was assessed by SDS-PAGE and analytical HPLC-SEC. SDS-PAGE analysis of MT-6194 showed bands of the expected size with a light chain larger than that of the parental Ab tocilizumab (Figure 1B). The HPLC-SEC profile of MT-6194 showed that the FynomAb eluted as a single peak confirming its monomeric nature and high purity with no measurable aggregation or degradation (Figure 1C).

To determine the affinity and kinetic parameters of MT-6194 binding to human and cynomolgus IL-17A and IL-6R, SPR analysis (BIAcore) was performed with MT-6194 captured as ligand and either IL-17A or IL-6R flowed as the analyte. Secukinumab was used as a positive control for IL-17A binding and as a negative control for IL-6R binding. Tocilizumab was used as a positive control for IL-6R binding and as a negative control for IL-17-A binding. The kinetic binding data for each FynomAb and secukinumab is summarized in Table 1 (individual sensorgrams not shown). MT-6194 consistently had a similar affinity as tocilizumab for both human and cynomolgus monkey IL-6R and a higher affinity for human and monkey IL-17A than secukinumab over 4 separate experiments. These data suggest that the presence of the Fynomer on the C-terminus of the light chain of tocilizumab did not affect binding of IL-6R to the antibody moiety. Simultaneous binding of MT-6194 to both IL-17A and IL-6R was also assessed by capturing MT-6194 on an IL-17A coated surface (0 s) and then injecting 20 nM sIL-6R after 300 s. Figure 2 clearly shows that MT-6194 is able to bind IL17A and IL6R simultaneously. The dual binding of IL-17A and sIL-6R was confirmed using a sandwich ELISA and dual binding of IL-17A and cell surface expressed IL-6R was demonstrated by FACS using an IL-6R+ cell line and biotinylated IL-17A (data not shown).

MT-6194 blocks IL-17A and IL-6 functional activity.

Stimulation of HT-29 cells with human IL-17A results in the production of the chemokine GRO-α. To assess the activity of MT-6194 against human IL-17A, HT-29 cells were
stimulated with IL-17A (1.9 nM) in the presence of various concentrations of the FynomAb. Secukinumab was used as a positive control for blockade of IL-17A function, and tocilizumab was used as a negative control. As shown in Figure 3A, MT-6194 inhibited the IL-17A-induced production of GRO-α in a dose dependent fashion and consistently showed a more potent blockade of IL-17A than secukinumab.

To assess the bioactivity of MT-6194 against IL-6, the HEK Blue™ IL-6 reporter cell line (Invivogen) was stimulated with IL-6 (15 pM) in the presence of various concentrations of the FynomAb. Commercial grade Actemra was used as a positive control for IL-6R blockade. Addition of IL-6 to the HEK Blue™ IL-6 cells resulted in stimulation of the IL-6R signaling pathway, which in turn activated a STAT3-inducible reporter gene leading to the expression of a secreted embryonic alkaline phosphatase (SEAP). Representative data from multiple experiments are shown in Figure 3B. MT-6194 showed dose dependent blockade of IL-6R similar to commercial grade Actemra. These data demonstrated that the presence of the IL-17A binding FynomAb on the tocilizumab mAb backbone did not affect the IL-6R blocking activity of the mAb portion of the FynomAb.

MT-6194 exhibits favorable PK in cynomolgus monkeys.

The PK parameters of MT-6194 and Tocilizumab are shown in Figure 4. Although both the IL-6R and IL-17A ELISAs were used to calculate the serum concentrations and PK parameters, only the IL-6R data is shown here. However, similar serum concentrations and PK parameters were determined from the IL-17A ELISA data. Thus the serum concentration versus time curves for the two different ELISA methods looks very similar for both MT-6194 and Tocilizumab. This again suggests that MT-6194 is stable in vivo, and its capacity to bind both IL-6R and IL-17A is retained. In general, MT-6194 showed a favorable PK profile and comparable to Tocilizumab, as none of it was cleared rapidly from circulation. Data could not be extrapolated for the last time-point for monkey #4 (Figure 4) as it fell out of the standard curve range and was considered unreliable.

Dual targeting of IL-17A and IL-6 shows a synergistic therapeutic effect in a mouse DTH model of inflammation.

Previous mouse and human studies have shown that IL-17A and IL-6 both play important roles in inflammation and autoimmunity. Due to the non-redundant functions of these cytokines on different cell types in vivo and due to the positive feedback loop that exists between these inflammatory cytokines, there is a strong rationale for a dual targeting strategy to inhibit both cytokines. However, to our knowledge, there have been no reports demonstrating whether dual targeting of IL-17A and IL-6 would have any therapeutic benefit over targeting either cytokine alone. Since there are no known synergistic effects of these two cytokines acting on a particular cell type, in vitro synergy assays are not feasible. Therefore, we sought to determine whether dual targeting of IL-17A and IL-6 would have any therapeutic effect in a mouse model of inflammation where both cytokines are known to be involved.

It has been previously reported that DTH responses in some mouse models can be partially suppressed by agents that inhibit IL-17A or IL-6. In order to determine whether dual targeting of IL-17A and IL-6R would have any therapeutic benefit, we tested whether a combination of mAbs against murine IL-17A and murine IL-6R was more efficacious than monotherapies alone. As shown in Figure 5A, treatment with a combination of suboptimal doses of anti-IL-17A and anti-IL-6R had a very dramatic and statistically significant (p < 0.001) effect on DTH responses to the model antigen ovalbumin (OVA). Each antibody given alone (at twice the dose given in the combination therapy) had no measurable effect on OVA-specific DTH responses. Thus, simultaneous blockade of both IL-17A and IL-6R demonstrated a clear synergistic effect in modulating this in vivo inflammatory response. Figure 5B shows histochemical staining of ear tissue form the DTH experiment. Reduced ear
Discussion

Using biologics for the treatment of RA was initially explored using TNF inhibitors and patients had a number of choices including infliximab, etanercept and adalimumab. More recently these have included golimumab and certolizumab-ppegol. However other reagents targeting other cell surface molecules have also been employed, notably rituximab targeting B cells, and abatacept targeting T cells. In addition, cytokine pathways (ex: IL-1, IL-17, IL-23 and IL-6) have emerged in recent years as important intervention points for blocking pathways instrumental in the onset or the perpetuation of RA and other inflammatory diseases. Most notably by tocilizumab, a humanized anti-IL-6R mAb that binds both membrane-bound and soluble IL-6R forms and thus inhibits IL-6 signal transduction. A great deal of clinical evidence on the efficacy and safety of tocilizumab in RA has been established. Tocilizumab has been approved for treatment of RA in more than 100 countries and is currently being explored in other disease indications. Tocilizumab has also been shown to be effective in patients that do not respond to the combination of methotrexate and TNF blockers. Although tocilizumab and the TNF blockers are most commonly given in combination with methotrexate, a recent report suggested that tocilizumab was more efficacious than adalimumab (an anti-TNF) in RA patients when given as a monotherapy. Furthermore, tocilizumab is only marginally more effective when given in combination with methotrexate as compared to tocilizumab monotherapy. This may represent a significant advantage of tocilizumab treatment since many patients cannot tolerate side effects caused by methotrexate therapy. Another potential advantage of tocilizumab is its apparent lack of immunogenicity or induction of anti-drug antibodies (ADA). ADAs may lead to the withdrawal of these drugs due to adverse events or diminished efficacy over time.

While the TNF blockers and tocilizumab are the most efficacious and widely used biologics for RA, these mAbs recognize only a single target. Because of the multifactorial nature of RA and other inflammatory diseases, it is likely that the concomitant disruption of two or more targets, or disease pathways, would be more efficacious than the current monotherapies, provided that this could be done without further increasing the toxicity. In order to target multiple pro-inflammatory factors more than 30 antibody-based bi-specific therapeutics are currently in development. IL-6 and IL-17A are pleiotropic cytokines with a variety of non-overlapping pro-inflammatory functions that act on a variety of different cell types during an inflammatory response. IL-6 is an important mediator in rheumatoid disease. For example, in arthritis models the known functions of IL-6 include recruitment of neutrophils and other inflammatory cells, differentiation of pathogenic Th17 cells and autoantibody-producing B cells and plasma cells, and differentiation of osteoclasts.

IL-6 also promotes VEGF-dependent angiogenesis and contributes to pannus formation, induces acute-phase protein production and induces matrix metalloproteases which mediate tissue destruction. The pro-inflammatory cytokine interleukin (IL)-17 is secreted by a variety of immune and non-immune cells including CD4+ Th17 T cells, and it has been shown to be an important contributor to the pathogenesis of several autoimmune diseases including RA and psoriasis. IL-17A acts synergistically with IL-1β, TNF-α, and IFN-γ to enhance activation of synovial fibroblasts, chondrocytes, and osteoclasts. It amplifies immune responses by induction of IL-6 production and recruits monocytes and neutrophils by increasing local chemokine production. Secukinumab (Cosentyx), is a human monoclonal antibody that binds to IL-17A and is approved for the treatment of psoriasis. This drug is also being investigated as a treatment for uveitis, rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis.
The data reported here show that we can independently target two separate pathways with a single reagent. The IL6 and IL17 pathways and cell types overlap significantly and often inhibition of only one of these pathways can result in compensation by the redundancy in other pathways. Thus a drug that can neutralize both pathogenic pathways has the potential to ameliorate the immune response in disease where it has become unregulated. The most common disease indication for example, is RA although several other diseases involve these two cytokines.

Experimental procedures

Isolation of IL-17 binding Fynomers.
IL-17A binding Fynomers were selected from Fynomer phage display libraries using recombinant IL-17A as previously described\textsuperscript{14}. Four individual Fynomer sequences were selected to be used for the construction of FynomAbs, all of which showed similar binding affinities to IL17A.

Cloning and expression of MT-6194
The heavy and light chain amino acid sequences for tocilizumab, obtained from the CAS database, were used. The amino acids 356-358 were mutated from DEL to EEM. This changed the allotype from G1m1 to nG1m1 which was carried out to reduce any potential immunogenicity. DNA sequences were codon optimized for CHO cell expression.

The four Fynomer sequences were cloned onto either the C-terminus of the light chain or the C-terminus of the heavy chain using the 15 amino acid linker (GGGGS x 3). The resulting 8 construct combinations were cloned into the expression vector pEE 6.4 (heavy chain) or pEE 12.4 (light chain) and then combined to form a single expression vector.

The eight constructs were linearized and nucleofected (Amaxa) into CHO-K1-SV cells (Lonza). The cells were grown under methionine sulfoximine (MSX) selection in static flasks until cultures had recovered, whereupon they were transferred to shake flasks.

Supernatants for all FynomAb cultures were purified under aseptic conditions using HiTrap MabSelect SuRe columns (1 or 5mL) using a flow rate of 5-20 mL/min. Columns were washed with PBS until a baseline A280 reading was reached followed by elution with pH3.0 glycine solution.

Analytical Chemistry.

SPR Affinity measurements
SPR measurements were all performed using the BioRab ProteOn XPR36. An anti-human Ig was coupled to a GLM chip by standard amine coupling. Either MT-6194 or a control antibody was initially captured as the ligand and then either IL-17A or sIL-6R was flowed over as the analyte. PBST was used as the mobile phase and regeneration achieved with two 15 second injections on 100mM HCl. Secukinumab was used as a positive control for IL-17A binding and as a negative control for sIL-6R binding. Conversely, tocilizumab was used as a positive control for sIL-6R binding and as a negative control of IL-17A binding.

SEC
Three separate lots of MT-6194 were assessed by HPLC-SEC. 10 µg of each FynomAb protein were injected onto a Zenix-C SEC-300 column (Sepax Technologies) using PBS as the mobile phase with a flow rate of 1mL/min. Absorbance at 280nM was monitored.

SDS-PAGE
SDS-PAGE analysis was performed under both reducing and non-reducing conditions on 4-20% Tris-glycine gradient gels. 5-10 µg of protein were run per well and gels stained with Coomassie brilliant blue (SimplyBlue SafeStain, Invitrogen).

HT29 Groα IL-17 assay
To assess the bioactivity of MT-6194 in neutralizing the effects of IL-17A, HT29 cells (ATCC #HTB-38) were stimulated with IL-17A (1.9nM) in the presence of various concentrations of MT-6194. Concentrations from 100nM to 6pM were used. 20,000 viable...
cells per flat bottomed well of a 96 well plate were added and incubated for 48 hours at 37°C, 5% CO₂. Secukinumab was used as a positive control for blockade of IL-17A function.

Stimulation of HT29 with IL-17A resulted in the production and release of Groα into cell supernatant. Groα levels in the supernatants were measured by ELISA using the DuoSet ELISA kit from R&D systems (DY275). The ELISA test was performed according to the manufacturer’s manual except that all antibodies were used at a 1:200 dilution. Means of duplicate wells were plotted and standard deviations calculated using a four parameter dose response inhibition function in GraphPad Prism®

**HEK Blue IL6R blockade assay**

HEK Blue IL-6 cells (Invivogen, hkb-il6) were stimulated with IL-6 (15pM) in the presence of various concentrations of MT-6194 from 500nM to 2nM. MT-6194 was added to the cells 30 minutes prior to adding IL-6. For each sample well 50,000 viable cells were added to the wells and incubated for 20-24 hours at 37°C and 5% CO₂ in triplicate. IL-6 stimulation activates the STAT3-inducible SEAP reporter gene which releases SEAP into the supernatant. SEAP was measured using the HEK-BlueTM detection reagent (Invivogen, hb-det2) according to the manufacturer’s manual.

IC₅₀ values were calculated using a four parameter dose response inhibition function in GraphPad Prism®

**Dual binding to IL-17A and IL-6R**

HEK Blue IL-6 cells (Invivogen, hkb-il6) were used to assess the ability of MT-6194 to simultaneously bind IL17A and IL6R. MT-6194 was incubated for 60 min with HEK-Blue IL-6 cells (using HEK-293 cells as a negative control) from concentrations between 300nM and 5pM. After the incubation with MT6194 a secondary incubation using biotinylated IL-17A (R&D 317-ILB) was carried out followed by incubation with streptavidin-allophycocyanin (APC: eBioscience 17-4317).

Dual binding was also demonstrated by “sandwich” ELISA where IL-17A (Cell Signaling Technologies, 8928BF) was immobilized on plastic and IL-6R-HIS was used for detection. Maxisorp 96 MicroWell plates were coated with 5 µg/ml of IL-17A, at 4 °C, in PBS overnight. Plates were washed in PBS + 0.05% Tween (PBST) and blocked for 2 hours with BSA (Fischer Scientific, 37525) diluted to 1% in PBST. After blocking, plates were washed 6 times with PBST. MT-6194 diluted 5 fold in 1% BSA/PBST from a starting concentration of 50 nM to 0.003nM was used for the standard curve for the plate. A final concentration of 1 µM IL-6R-HIS is added to all samples and standards and incubated for 1 hour at room temperature. Plates were washed again 6 times in PBST and an anti-HIS HRP (Sigma, A7058) diluted to 1:5,000 in 1% BSA/PBST was added and allowed to incubate at room temperature for 30 minutes. After another round of washing in PBST, 50 µl of TMB liquid substrate (Sigma-Aldrich Inc., T0440-1L) were added to all wells. Plates were incubated for 2-3 min and stopped with 50 µl stop solution. Absorbance values were recorded at 405 nM (PHERAstar Plus).

**Pharmacokinetic studies in cynomolgus monkeys**

The determination of the pharmacokinetic properties of MT-6194 and tocilizumab, experiments were performed by measuring the concentration of MT-6194 in cynomolgus monkey serum with an ELISA assay. Samples were taken at different time points after a single i.v. injection. Charles River Laboratories carried out the injections and serum sample collections. Male and female monkeys were intravenously injected with a 5 mg/kg dose based on individual weight. Each group consisted of 4 monkeys. Blood was withdrawn at 5 min, 30 min, 2 hr, 6 hr, 24 hr, day 3, day 4, day 5, day 7, day 10, day 13, day 17, day 22, and day 27 after i.v. injection. Blood was collected in tubes without any anti-coagulants and serum prepared by centrifugation for 1-2 min at 10,000 rpm. Sera were stored at -80 °C until analysis.

**PK Data Analysis**

Serum concentrations for each monkey were calculated using the standard curve and 4-parameter fit analysis (X value logarithmic, Y
value linear). For every time-point and monkey, the average nM serum concentration of duplicate were multiplied by the corresponding dilution factor and then converted to µg/ml concentration. If data could not be extrapolated at any time point or dilution factor because it fell above or below the standard curve range, it was considered unreliable and not used. STDEV and %CV were calculated for each time point and for the 3 dilution factors. One value with lowest %CV and best represented the linear portion of the standard curve was chosen as the value for each time point. These values were subjected to pharmacokinetic parameter analysis (Phoenix 64, WinNonlin 6.3). Half-life (h), area under the curve (AUC; h*µg/ml), volume of distribution (Vz; ml) and clearance (Cl; ml/h) were calculated using Non-compartmental PK analysis, Model Type: Plasma (200-202), Calculation Method: Linear Trapezoidal Linear Interpolation. Standard curves for each monkey were based on uniform weighting and best fit lambda Z calculation. This required at least 3 data points in the regression, and did not include the Cmax.

**IL-6R and IL-17A ELISA**
The serum concentrations of MT-6194 and Tocilizumab were determined by ELISA using immobilized human IL-17 alpha (Cell Signaling Technologies, 8928BF) and recombinant human IL-6R alpha (R&D Systems, Inc. 227-SR/CF) on high binding Nunc ELISA plates (Thermo Scientific, 456537), respectively. Both were coated at 5 µg/ml at 4 °C in PBS overnight. Plates were washed in PBS + 0.05% Tween (PBST) and blocked for 2 hours with BSA (Fischer Scientific, 37525) diluted to 1% in PBST. For each monkey and time point, 800, 4,000 and 20,000-fold dilutions of serum were prepared in blocker diluent. The corresponding standard for each was prepared making serial dilutions in blocker diluent starting at 4 nM with 2 fold titrations (4 nM-0.03125 nM). Fifty µl of standards (in duplicate) and serum dilutions (in triplicate) were added to plates and incubated for 1 hour at RT. The plates were washed 3 times with PBST and incubated with 50 µl of Peroxidase-AffiniPure Goat Anti-Human IgG (H+L) (Jackson ImmunoResearch, 109-035-003) diluted at 1:5,000 for 1 hour at RT. The plates were washed 3 times with PBST. 50 µl of TMB liquid substrate (Sigma-Aldrich Inc., T0440-1L) were added to all wells. Plates were incubated for 3 min and stopped with 50 µl stop solution. Absorbance values were recorded at 405 nM (PHERAstar Plus).

**Mouse Delayed Type Hypersensitive (DTH) Model Dosing and Sensitization**
Eight week old C57Bl/6 female mice from Charles River were used for this animal model. In order to determine whether dual targeting of IL-17A and IL-6R would have any therapeutic benefit, we tested whether a combination of mAbs against mouse IL-17A and mouse IL-6R was more efficacious than the monotherapies alone. As MT-6194 does not cross react with mouse IL-17A or IL-6R we used surrogate rat monospecific monoclonal antibodies against either murine IL-17A or IL-6R. Mouse anti-murine IL-17A (BE0173) and rat anti-murine IL-6R (BE0047) as well as their respective isotype controls, mouse IgG1 (BE0083) and rat IgG2b (BE0090) were purchased from BioXcell. Dosing these test articles start on day 0 with an IP injection for each group (N=7). The IL-6R isotype control was given at 0.3mg/mouse while the IL-17A isotype control was given at 0.2mg/kg. Individual doses of the IL-6R and IL-17A antibodies were given at twice the concentration of the isotype controls: 0.6mg/mouse and 0.4mg/kg. A combination of both IL-6R and IL-17A antibodies was given to a group at the same concentration of the isotype control. A negative control group of mice were given an IP injection of PBS (N=4).

All mice were then sensitized with an immunization of 100µg ovalbumin (#vac-pova-100 from InvivoGen) emulsion in 100µl of a complete Freund’s adjuvant (CFA #7009 from Chondrex, Inc.) containing 100µg of killed mycobacterium tuberculosis. The emulsion was administered subcutaneously over two spots on the flank of the mouse.
**Challenge and Data Analysis**

14 days post immunization; DTH responses to the Ovalbumin were quantified using a 24 hour ear swelling assay. Pre-challenged ear thickness was determined using electronic micrometers. DTH responses were elicited by injecting 10µg of ovalbumin into the dorsal surface of the ear using a 100µl Hamilton syringe fitted with a 30 gauge needle. 24 hours after ear challenge, the increase in ear thickness over pre-challenged measurements was determined. P values were calculated by 2 way ANOVA Bonferroni’s Multiple Comparisons Test with GraphPad Prism®.

**Histochemistry**

Ear tissue obtained from the DTH model were preserved in 10% neutral buffered formalin. Subsequently, the tissues were paraffin-embedded and sectioned at 4µM thickness onto positively charged slides for traverse orientation to fully visualize ear thickness. The traverse sections were stained with hematoxylin and eosin (H&E). Quantitative data points were generated to measure the dermis thickness for each ear sample. Dermis thickness was calculated as the average of three independent thickness measurements throughout the tissue section. A representative section from each group of animals was selected to show the histopathological changes. Images were captured through ImageDx™ software (Reveal Biosciences, LLC).

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**Conflict of Interest**

The authors declare that they have no conflicts of interest with the contents of this article.

**Author contributions.** ML, RN and SG conceived the project, oversaw the work and verified all data analysis. RW & MS constructed and selected IL17 binding Fynomer molecules. AT and CS designed, constructed and expressed FynomAb bispecific molecules. DG oversaw selection of Fynomers and helped manage the project. VL performed all in vivo work and in vitro functional assays and assisted in writing the manuscript. RR designed and performed all biophysical characterization experiments. All authors approved the final version of the manuscript.
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Footnotes: SG current address; Salimetrics, LLC, 5962 La Place Court Suite 275, Carlsbad, CA 92008. RR; Neurocrine Biosciences, 12780 El Camino Real, San Diego, CA 92130. AT; NantKwest, Inc. 3530 John Hopkins Court, San Diego, CA 92121. ML; Salk Institute for Biological Studies, 10010 N Torrey Pines Rd La Jolla, CA 92037. DG; Grabulovski Consulting Services, Riedhofstrasse 57, CH - 8049 Zurich Switzerland
Table 1: Summary of kinetic binding data of MT-6194 to human and monkey IL-17A and IL-6R.

MT-6194 consistently had a similar affinity as tocilizumab for both human and cynomolgus monkey IL-6R and a higher affinity for human and monkey IL-17A than secukinumab. The data represent the average of four separate experiments.
Table 1

|          | huIL-17A |          | huIL-6R |          | cyIL-17A |          | cyIL-6R |
|----------|----------|----------|----------|----------|----------|----------|----------|
|          | kon (1/Ms) | koff (1/s) | KD (pM) | kon (1/Ms) | koff (1/s) | KD (pM) | kon (1/Ms) | koff (1/s) | KD (pM) |
| MT-6194  | 2.1x10^6  | 1.6x10^-4 | 76       | 4.7x10^5  | 2.3x10^-4 | 531      |
| Secukinumab | 6.5x10^5  | 9.2x10^-3 | 141      | -         | -         | -        |
| Tocilizumab | -        | -         | -        | 3.6x10^5  | 2.2x10^-4 | 612      |

|          | kon (1/Ms) | koff (1/s) | KD (pM) | kon (1/Ms) | koff (1/s) | KD (pM) |
|----------|----------|----------|----------|----------|----------|----------|
| MT-6194  | 3.0x10^6  | 2.3x10^-4 | 75       | 2.2x10^5  | 3.4x10^-4 | 1530     |
| Secukinumab | 1.1x10^6  | 2.7x10^-3 | 2280     | -         | -         | -        |
| Tocilizumab | -        | -         | -        | 2.3x10^5  | 3.6x10^-4 | 1510     |
Figure 1

A

B

C

D
Figure 1: Characterization of FynomAb MT-6194.

A. Cartoon showing the IL17A binding Fynomer (circle) fused to the C-terminus of the light chain of the anti-IL6R antibody. B. shows SDS PAGE analysis of bispecific MT6194 and monospecific tocilizumab. SDS-PAGE analysis was performed under both reducing and non-reducing conditions on 4-20% Tris-glycine gradient gel. Lane 1 and 6; MW ladder, Lane 2; MT6194 non-reduced, Lane 3; tocilizumab non-reduced, Lane 4; MT6194 reduced, Lane 5; tocilizumab reduced. 5µg were loaded for non-reduced lanes and 8µg for the reduced lanes. The increase size of the antibody light chain compared to tocilizumab can be seen under reducing conditions where the heavy chain molecular weight remains unchanged. C. Analytical HPLC-SEC of MT6194. Panel A: MT-6194. Panel B: tocilizumab. X-axis = time. Y-axis = absorbance at 280nM. 10 µg of each protein were injected onto a Zenix-C SEC-300 column (Sepax Technologies) using PBS as the mobile phase with a flow rate of 1mL/min.
Figure 2
Figure 2: Dual binding of sIL-6R and IL-17A measured by SPR.
SPR measurements were all performed using the BioRab ProteOn XPR36. An anti-human Ig was coupled to a GLM chip by standard amine coupling. Either MT-6194 or a control antibody was initially captured as the ligand and then either IL-17A or sIL-6R was flowed over as the analyte. The black line represents IL17A binding to MT-6194. The red line represents an additional signal seen after subsequent addition of sIL6R.
Figure 3

A

HT-29 Gro ELISA

- MT-6194
- Secukinumab

| Drug   | IC50  |
|--------|-------|
| MT-6194| 0.7565|
| Secukinumab | 1.252 |

B

HEK Blue™ IL-6R Blockade Assay

- MT-6194
- Tocilizumab

| Drug   | IC50  |
|--------|-------|
| MT-6194| 8.957 |
| Tocilizumab | 11.15 |
**Figure 3: In vitro inhibition of functional activity by MT-6194.**

Panel A. Inhibition of Groα release from HT-29 cells as a measure of IL-17A inhibition. HT29 cells were stimulated with IL-17A (1.9nM) in the presence of various concentrations of MT-6194. Secukinumab was used as a positive control. Groα levels in the supernatants were measured by ELISA using the DuoSet ELISA kit from R&D systems. Panel B: Inhibition of the effect of IL-6 on HEK-Blue cells. HEK Blue cells containing the STAT3-inducible SEAP reporter gene release SEAP into the culture supernatant when stimulated by IL-6. MT-6194 at concentrations from 500nM to 0.2nM were added to the cells 30 minutes prior to adding IL-6. SEAP was measured using the HEK-BlueTM detection reagent.
**PK of MT-6194 in Cynomolgus Monkey Compared to Tocilizumab**

**Figure 4**

| Monkey | Rsq   | HL_Lambda_z | Cmax  | Clast | AUClast | Vz_F_obs | Cl_F_obs |
|--------|-------|-------------|-------|-------|---------|----------|----------|
| 1      | 0.979809583 | 81.01105327 | 182.2676544 | 10.72923072 | 10356.49304 | 0.050331428 | 0.000430646 |
| 2      | 0.976956879 | 81.59537841 | 156.737256 | 6.91582944 | 12207.00919 | 0.045202422 | 0.000383991 |
| 3      | 0.983131384 | 78.51531263 | 193.90176 | 13.5731232 | 13102.10836 | 0.038687452 | 0.00034154 |
| 4      | 0.9988446 | 64.0509907 | 185.82252 | 14.8658016 | 7244.305485 | 0.053612262 | 0.000580181 |
Figure 4: PK data for MT6194 in four individual cynomolgus monkeys plus a tocilizumab control.

Monkeys were injected i.v. with a 5 mg/kg of MT-6194 based on individual weights. Blood was withdrawn at 5 min, 30 min, 2 hr, 6 hr, 24 hr, day 3, day 4, day 5, day 7, day 10, day 13, day 17, day 22, and day 27 after administration. Serum concentrations for each monkey were calculated using the standard curve and 4-parameter fit analysis (X value logarithmic, Y value linear). For each time-point and monkey, the average nM serum concentration of duplicates was multiplied by the corresponding dilution factor and converted to µg/ml.
Figure 5A
Figure 5A: Dual blockade of IL-17A and IL-6R in a murine DTH model.

Monospecific monoclonal antibodies against either murine IL-17A (BE0173) or IL-6R (BE0047), as well as their respective isotype controls, were used for dosing each group of mice (N=7) by i.p. injection. The IL-6R isotype control antibody was given at 0.3mg/mouse while the IL-17A isotype control was given at 0.2mg/kg. All mice were first sensitized with an immunization of 100µg ovalbumin emulsion in 100µl of a complete Freund’s adjuvant containing 100µg of killed mycobacterium tuberculosis. The emulsion was administered subcutaneously over two spots on the flank of the mouse.
Figure 5B
Figure 5B: Photomicrograph of transverse sections of H&E-stained OVA/CFA immunized mouse ear tissue.
Ears sensitized with 10µg OVA into the dorsal surface 24 hours prior to sacrifice. (Magnification: 40X, black bar = 100 µm).
(A) Positive Control Group: Inflamed ear that was treated with isotype control antibodies. Mouse IgG1 at 0.3mg and Rat IgG2b at 0.2mg/kg. (B) IL-6R Antibody Group: Inflamed ear that was treated with IL-6R antibody at 0.6mg. (C) IL-17A Antibody Group: Inflamed ear that was treated with IL-17A antibody at 0.4mg/kg. (D) Dual Treated Antibody Group: Less inflamed ear that was treated with IL-6R antibody at 0.3mg and IL-17A antibody at 0.2mg/kg. (E) Negative Control Group: Non-inflamed ear that was immunized with OVA/CFA but never treated or challenged with OVA.
A bispecific antibody that targets IL-6 receptor and IL-17A for the potential therapy of patients with autoimmune and inflammatory diseases

Michael Lyman, Vincent Lieuw, Robyn Richardson, Anjuli Timmer, Christine Stewart, Steve Granger, Richard Woods, Michela Silacci, Dragan Grabulovski and Roland Newman

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