IA-14069, A NOVEL SMALL-MOLECULE INHIBITOR DIRECT-TARGETING TUMOR NECROSIS FACTOR-\(\alpha\), ATTENUATES COLLAGEN INDUCED ARTHRITIS

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**Background:** Rheumatoid arthritis (RA) is systemic autoimmune disease that is characterized by autoreactive immune cells and various cytokines-mediated inflammation in multiple joints, leading to cartilage degradation, bone erosion and finally irreversible joint destruction\(^1\). The inflammatory cytokine tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) is known to play a central role in several chronic immune-mediated inflammatory disorders\(^2\).

**Objectives:** Despite the great success of anti-TNF-\(\alpha\) biological drugs in the treatment of RA, no chemical drug targeting TNF-\(\alpha\) is available. Here we report that IA-14069, a novel small molecule inhibitor, binds directly to TNF-\(\alpha\) and inhibits TNF-\(\alpha\) activities both in vitro and in vivo.

**Methods:** IA-14069 was screened and identified by competitive binding assay using TNF-\(\alpha\) and TNF receptor. In vitro neutralization activity of IA-14069 against TNF-\(\alpha\) was determined using MTT assay. The direct binding IA-14069 to TNF-\(\alpha\) was demonstrated by surface plasmon resonance and bead pull-down assays. The inhibition of TNF-\(\alpha\)-TNFR interactions by direct binding of IA-14069 to TNF-\(\alpha\) was analyzed by flow cytometry. Levels of phosphorylated I\(\kappa\)B (p-I\(\kappa\)B) and NF-\(\kappa\)B p65 were analyzed by western blot. IA-14069 was orally administrated to TNF-\(\alpha\) transgenic (TNF-\(\alpha\)-k\) mice at 3.3 mg/kg twice week or at 25, 50 or 100 mg/kg 3 times per week for preventive or therapeutic managements. IA-14069 was screened and identified by competitive binding assay using TNF-\(\alpha\) and TNF receptor. In vitro neutralization activity of IA-14069 against TNF-\(\alpha\) was determined using MTT assay. The direct binding IA-14069 to TNF-\(\alpha\) was demonstrated by surface plasmon resonance and bead pull-down assays. The inhibition of TNF-\(\alpha\)-TNFR interactions by direct binding of IA-14069 to TNF-\(\alpha\) was analyzed by flow cytometry. Levels of phosphorylated I\(\kappa\)B (p-I\(\kappa\)B) and NF-\(\kappa\)B p65 were analyzed by western blot.

**Results:** IA-14069 potently inhibits both TNF-\(\alpha\)-induced cytokotoxicity (IC\(_{50}\) < 0.7 \(\mu\)M) which directly binds to TNF-\(\alpha\) and TNF-\(\alpha\)-triggered signaling (p-I\(\kappa\)B and NF-\(\kappa\)B p65) activities. The therapeutic as well as preventive anti-RAs of IA-14069 were demonstrated in TNF-\(\alpha\)-k\) models. IA-14069 and MTX had synergistic effects in the CIA therapeutic model. According to pharmacokinetic analysis, IA-14069 showed significant bioavailability. In addition, no in vivo toxicity was observed even under treatment of excessive amount of IA-14069.

**Conclusion:** The data indicate that IA-14069 can be a novel and potential TNF-\(\alpha\) inhibitor for the treatment of RA and other inflammatory diseases.

**References:**