

Novel class of DNA-based compounds act as antagonists for TLR7 and 9: In vitro and in vivo studies in MRL-lpr and NZBW/F1 mouse models

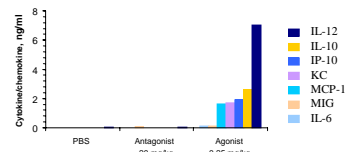
Fu-Gang Zhu, Ekambar R. Kandimalla, Dong Yu, Daqing Wang, and Sudhir Agrawal
Idera Pharmaceuticals, Inc, 345 Vassar Street, Cambridge, MA 02139, USA.

Introduction

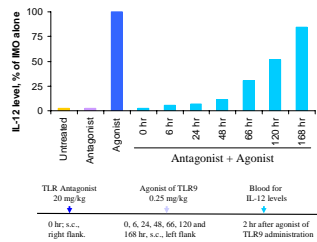
- Toll-like receptors (TLR) recognize pathogen-associated molecular patterns (PAMP) and induce pathogen specific innate and adaptive immune responses. Of the eleven TLRs (TLR1-11) known in mammals, TLR3 (double-stranded viral and synthetic RNA), TLR7/8 (single-stranded viral and synthetic RNA, and ribonucleosides), TLR9 (unmethylated CpG motif containing bacterial and synthetic DNA) recognize nucleic acid molecular patterns.
- Recent studies suggest involvement of TLRs in autoimmune diseases in animals and human subjects with susceptible background.
- Our structure-activity relationship studies of DNA have led us to identify a novel class of DNA-based compounds, which act as antagonists of TLR9 and 7¹⁻³.
- Our earlier studies showed that antagonist candidates inhibit activity of TLR9 and 7 agonists in vitro and in vivo in mice and human cell-based assays.
- In the present study we have evaluated the effects of novel antagonist candidates in lupus prone MRL-lpr and NZBW/F1 mouse models.

1. Bhargava D et al. presented at 10th American Association of Immunologists (AAI) annual meeting, Boston, MA, Mar. 12-16, 2006.
2. Wang D et al. presented at 2nd Annual Meeting of the Organon/Orion Therapeutics, New York, NY, October 19-21, 2006.
3. Wang D et al. presented at the American College of Rheumatology (ACR) annual meeting, Washington, DC, November 10-15, 2006.

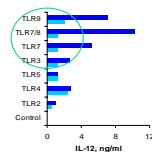
In vivo immune stimulation of an antagonist and an agonist in mice



TLR antagonist: Sustained inhibitory effect on agonist of TLR9 induced IL-12 in vivo

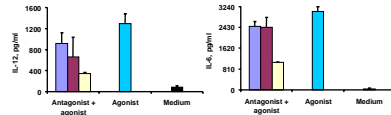


TLR antagonist: Specificity of inhibitory activity



TLR Antagonist 10 mg/kg, Agonist of TLR 0.25 mg/kg, Blood for IL-12 levels 1 hr after, s.c., left flank; 2 hr after agonist of TLR9 administration, right flank.
 TLR2 agonist - MALP2 (0.5 mg/kg)
 TLR3 agonist - poly(I:polyC) (20 mg/kg)
 TLR4 agonist - LPS (0.25 mg/kg)
 TLR5 agonist - Flagellin (0.25 mg/kg)
 TLR7 agonist - Loxosorbite (100 mg/kg)
 TLR7/8 agonist - 2826k (0.1 mg/kg)
 TLR9 agonist - IMO (0.25 mg/kg)

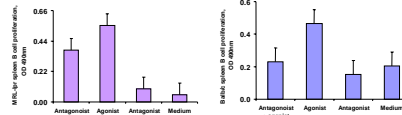
Effect of an antagonist on NZBW/F1 mouse spleen B cell cytokine secretion



Purified spleen B cells from NZBW/F1 were cultured with 1, 3, 10 µg/ml antagonist in the presence and absence of 1 µg/ml agonist for 24 h.

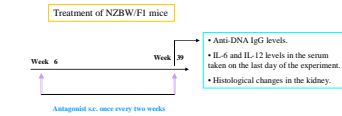
Antagonist (1 µg/ml) + Agonist (1 µg/ml)
 Antagonist (3 µg/ml) + Agonist (1 µg/ml)
 Antagonist (10 µg/ml) + Agonist (1 µg/ml)
 Agonist (1 µg/ml)
 Medium

Effect of an antagonist on mouse B lymphocyte proliferation

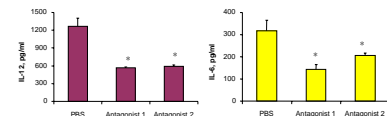


Purified spleen B cells from MRL-lpr and Balb/c mice were cultured with 1 µg/ml antagonist in the presence and absence of 0.3 µg/ml agonist for 72 h.

NZBW/F1 mouse studies - Experimental Protocol

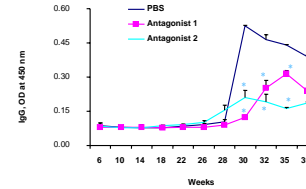


The long term effect of antagonist candidates on serum cytokine levels in NZBW/F1 mice



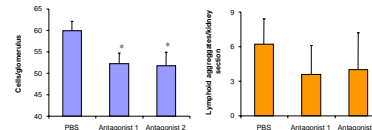
* = P < 0.05
 Serum IL-6 and IL-12 levels on the last day of the experiment (week 39).

Serum anti-DNA IgG levels in NZBW/F1 mice



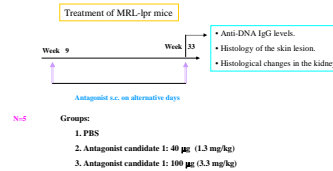
Serum anti-DNA total IgG levels were significantly lower in mice treated with antagonist 1 or 2 than in PBS treated mice (* = p values < 0.05).

Effect of antagonist candidates on renal disease development in NZBW/F1 mice

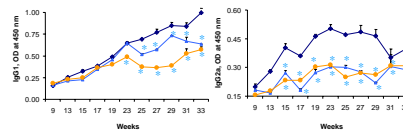


* = P < 0.05
 Kidneys were examined for histological changes at the end of experiment (week 39).

MRL-lpr mouse studies - Experimental Protocol

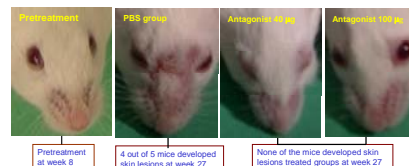


Serum anti-DNA IgG levels in MRL-lpr mice

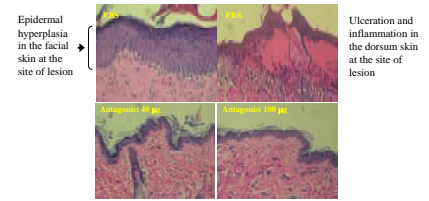


Serum anti-DNA IgG1 and IgG2a levels were significantly lower in mice treated with antagonist at both 40 and 100 µg/dose (* = p values < 0.05).

Effect of antagonist treatment on the development of skin lesions in MRL-lpr mice

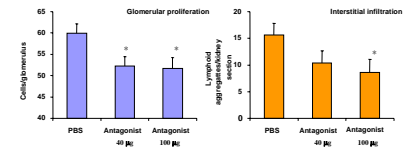


Effect of an antagonist candidate on the development of skin lesions in MRL-lpr mice (histological changes)



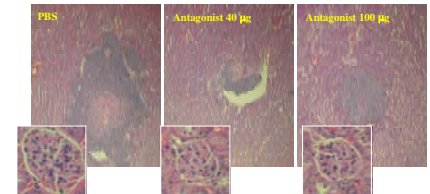
Extensive epidermal hyperplasia, ulceration and inflammation were observed in the skin sections of all five mice in PBS treated group. None of the skin sections of the mice treated with antagonist at 40 or 100 µg dose showed pathological changes observed in untreated mice.

Effect of an antagonist candidate on renal disease development in MRL-lpr mice



* = P < 0.05
 Kidneys were examined for histological changes at the end of experiment (week 33).

Effect of an antagonist candidate on inflammation and glomerular proliferation in the kidney of MRL-lpr mice



Conclusions

- A novel class of synthetic DNA-based antagonist candidates has been identified.
- Antagonist candidates have shown to block immune stimulation by agonists of TLR9 and 7 in preclinical studies.
- Evaluation of antagonist candidates in MRL-lpr and NZBW/F1 mouse models shows that:
 - Antagonist treatment is effective in preventing development of butterfly skin rash in MRL-lpr mice.
 - Antagonists are effective in reducing serum anti-DNA antibody levels in both MRL-lpr and NZBW/F1 mice.
 - MRL-lpr and NZBW/F1 mice treated with antagonists showed improved renal histopathology.
- TLR antagonists show potential as novel candidates for the treatment of lupus and other autoimmune diseases.