

Introduction

ARRY-438162 is a potent, selective inhibitor of MEK1/2 (mitogen activated protein kinase kinase) which has entered clinical trials in humans for the treatment of rheumatoid arthritis. ERK1/2 (extracellular signal-regulated kinase), which is directly phosphorylated by MEK1/2, is elevated in the synovium of rheumatoid arthritis patients (1). ERK1/2 activation has been seen in synovial macrophages and synovial fibroblasts upon challenge by TNF-alpha and IL-1beta, two of the major contributors to joint destruction in RA (2,3). Due to the cytokine signaling associated with ERK1/2 activation, and the inhibition of cytokine production observed with MEK 1/2 inhibition, we believe a MEK1/2 inhibitor may be a useful drug in the treatment of RA. Previously we have presented data on the activity of a MEK1/2 inhibitor in animal models of inflammation and arthritis, and this activity could be directly linked to the inhibition of the ERK1/2 in the synovial tissue of diseased joints. Here we show that ARRY-162 also effects differentiation and function of osteoclast without dramatically altering osteoblast differentiation. Furthermore, ARRY-162 has the ability to inhibit inflammation and bone resorption in a model of rat adjuvant-induced arthritis using a therapeutic dosing regimen.

References

- Schett G, Tohidast-Akrad M, Smolen JS, Schmid BJ, Steiner CW, Bitzan P, Zenz P, Redlich K, Xu Q, Steiner G. Arthritis and Rheumatism. 2000;43(11): 2501-12.
- Gortz B, Hayer S, Tuerck B, Zwerina J, Smolen J, Schett G. Arthritis Research and Therapy. 2005;7(5): 1140-1147.
- Lu H, Sun T, Yao L, Zhang Y. Chinese Medical Journal. 2000;113(10): 872-6.

Methods

All *in vivo* studies were performed in accordance with Array BioPharma Inc. IACUC guidelines and in harmony with the Guide for Laboratory Animal Care and Use.

In vitro Osteoclast differentiation and resorption

- Human osteoclast precursors (Lonza Walkersville Inc) were seeded at 10,000/well in the presence of differentiation media. To determine differentiation, ARRY-162 was added and cells were incubated at 37°C, in a humidified atmosphere of 5% CO₂ for 7 days. Osteoclasts were identified by staining for tartrate-resistant acid phosphatase. To determine bone resorption, osteoclast precursors were seeded on a OsteoAssay plate (Lonza) and allowed to differentiate. On day 7, ARRY-162 was added and the serial supernatant samples were assayed for the calcium (Calcifluor Assay, Lonza).

In vitro osteoblast differentiation

- Human mesenchymal stem cells (Lonza) were seeded at 10,000/well in the presence of differentiation media with or without ARRY-162. After 14 days of culture, cells were rinsed and stained for the presence of calcium using the Von Kossa stain.

Adjuvant-Induced Arthritis in the Rat

- Male Lewis rats (~175 g); n=8 per treatment group
- Lipoidal amine (LA), Freund's complete adjuvant (100 µl injection of 7mg LA, intradermal at the base of the tail, Day 0)
- Endpoints:
 - Changes in body weight (QD)
 - Microscopic histology (day 21)(inflammation and bone resorption)
 - Changes in ankle diameter (QD)
 - Paw weight (day 21)
 - mCT density and area measurements (day 21)
- Treatment : Days 14-21
 - Vehicle 4 ml/kg, PO, QD
 - ARRY-162 3, 10 or 30 mg/kg, PO, QD
 - Dexamethasone 0.1 mg/kg, PO, QD
 - Enbrel 10 mg/kg SC, Q3D
- On day 21, animals were euthanized, blood was collected for cytokine analysis, and both hind paws were removed and processed for histology.

Results

In Vitro Osteoclast and Osteoblast Differentiation and Function

Osteoclast Differentiation

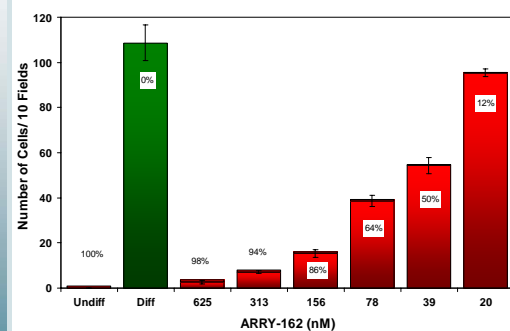


Figure 1. ARRY-162 inhibits *in vitro* osteoclast differentiation (IC₅₀ = 39 nM). Osteoclasts were identified by staining for tartrate-resistant acid phosphatase.

Osteoclast Resorption

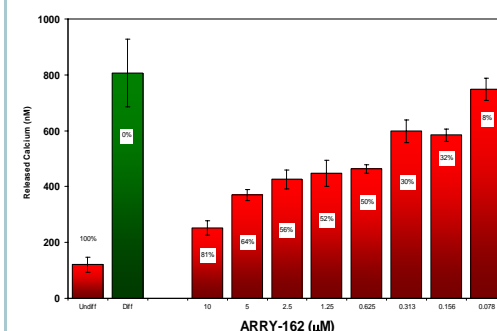


Figure 2. ARRY-162 inhibits *in vitro* osteoclast resorption (IC₅₀ = 625 nM). Resorption was determined on day 10 following treatment with ARRY-162.

Osteoblast Differentiation

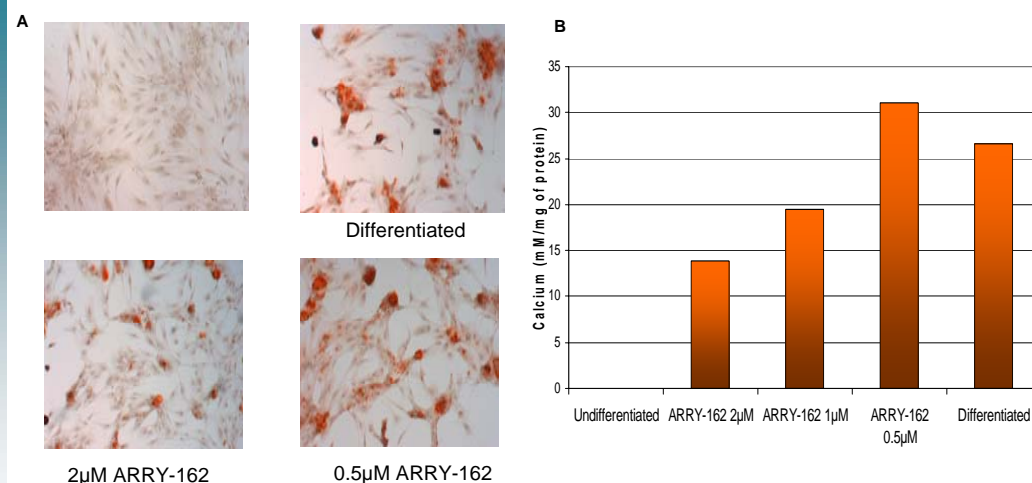


Figure 3. Inhibition of MEK 1/2 by ARRY-162 weakly affects osteoblast differentiation. Human mesenchymal stem cells (Lonza) were seeded at 10,000/well in the presence of differentiation media with or without ARRY-162. A) After 14 days of culture, cells were rinsed and stained for the presence of mineralization by Alizarin red. B) Alizarin red was extracted from stained cells and quantitation was measured by determine optical density at 405nm.

Adjuvant-Induced Arthritis in the Rat

Paw Diameter

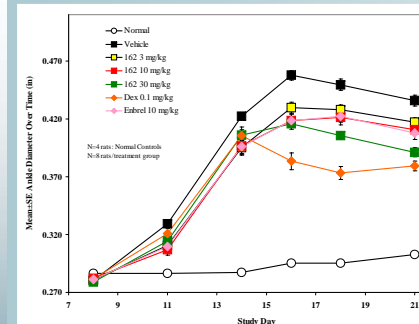


Figure 4. ARRY-162 demonstrated dose-related inhibition of ankle swelling, significant at 10 and 30 mg/kg when compared to vehicle control. The activity of ARRY-162 at 10mg/kg was equivalent to Enbrel in this model. Dosing was initiated on day 14.

Serum IL-6 Concentration

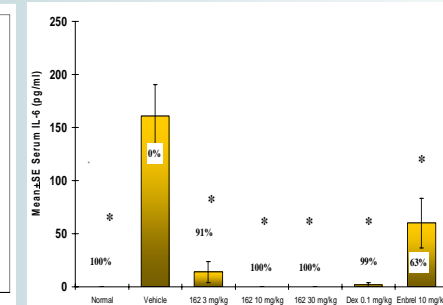


Figure 5. ARRY-162 demonstrated dose-related inhibition of serum IL-6 concentration, with complete inhibition at 10 mg/kg when compared to vehicle control. (Student's T-test; *p<0.05 to vehicle)

Relative Spleen Weight

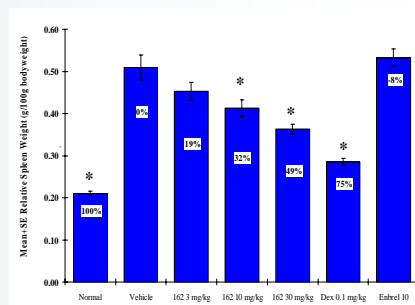


Figure 6. ARRY-162 demonstrated dose-related inhibition of relative spleen weights. All doses of ARRY-162 demonstrated better inhibition vs. Enbrel (Student's T-test; *p<0.05 to vehicle)

Joint Histopathology

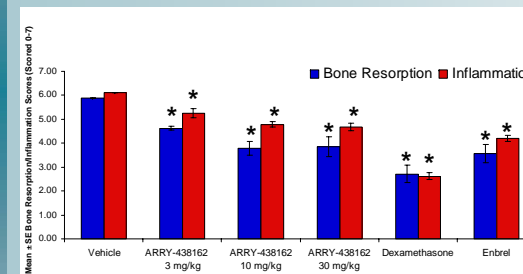


Figure 7. ARRY-162 significantly inhibits bone resorption and inflammation with delayed dosing when compared to vehicle.

Micro CT Scans of Ankle Joints

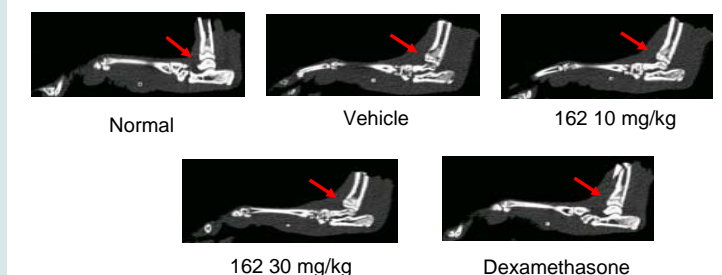


Figure 8. Representative ankles were imaged by micro computed tomography (mCT). Arrow points to growth plate.

Summary

These results demonstrate that:

- ARRY-162 is an excellent inhibitor of osteoclast differentiation (IC₅₀ = 39 nM) and bone resorption (IC₅₀ = 625 nM) *in vitro* with minimal effects on osteoblast differentiation.
- ARRY-162 is a potent disease modifying agent in rat AIA using therapeutic dosing in animals with established disease. Effects were significant and dose-related for bone resorption, cytokine production, and relative spleen weights.

In Conclusion:

- ARRY-162 not only alters the inflammatory response in pre-clinical models of arthritis but also has a direct effect on the bone marrow microenvironment. We show that inhibition of MEK with ARRY-162 directly inhibits *in vitro* osteoclast differentiation and resorption and this correlates with reduce bone resorption *in vivo*. Taken together, these results provide the framework for clinical trials with the specific MEK1/2 pathway inhibitor, ARRY-162, in various inflammatory and cancer bone diseases.

ARRY-162 is currently in a Phase II trial in rheumatoid arthritis patients.