Title: ARG098, a novel anti-human Fas antibody, suppresses synovial hyperplasia and prevents cartilage destruction in a severe combined immunodeficient HuRAg mouse model.

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Author's response to reviews: see over
August 17, 2010

Professor John Isaacs,
Ms Judith Gorton,
The BioMed Central Editorial Team,

Dear Professor Isaacs,
Dear Ms Gorton,

RE: MS: 4752772533600702 entitled "ARG098, a novel anti-human Fas antibody, suppresses synovial hyperplasia and prevents cartilage destruction in a severe combined immunodeficient-HuRAg mouse model" (previous title,"ARG098, a novel anti-human Fas antibody, shows strong cytotoxic effects, suppresses synovial hyperplasia, and prevents cartilage destruction in rheumatoid arthritis")

Thank you for your mail on 2nd August 2010 and for the referees’ and Associate Editor’s comments concerning the above manuscript. I am pleased to learn that Musculoskeletal Disorders is interested, in principal, in publishing our manuscript with the extensively edit. We are attaching a revised manuscript and a letter to the referees. I hope that the revised manuscript is now acceptable for publication in Musculoskeletal Disorders.

We would like to receive the reply by the end of September, if possible.
Thank you for your consideration.

Sincerely yours,

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We found the reviewer’s comments very helpful and have revised the manuscript accordingly.

Major Compulsory Revisions:
1) The authors report the superior effect of ARG098 in inducing apoptosis in Jurkat cells and RA FLS compared to historical attempts. To make this claim, the authors need to show ARG098 side by side with another anti-Fas antibody such as CH-11. In particular, they should examine dose responses of another Fas antibody in Jurkat cells, and find concentrations of this antibody and ARG098 which induce comparable levels of apoptosis in Jurkat. Then, the two antibodies should be compared at these concentrations in RA FLS. Only then can the authors claim any superiority of the ARG098 compound.

Evaluation: the authors mention only unpublished data regarding relative efficacies of ARG098, 7C11, and CH-11 in the induction of RA FLS apoptosis. Given that a critical element of their manuscript is that ARG098 is an improvement upon other anti-Fas antibodies, it is unclear to me why this data isn’t shown in the manuscript. It is also unclear from their remark if the antibodies were studied in parallel in the same RA FLS cell lines. Finally, the 7C11-induced apoptosis they report as data not shown is 70% of that achieved by ARG098, suggesting little eventual improvement in efficacy in vivo. Again, it would have been helpful if the authors had attempted to compare these antibodies in vivo as well. The authors continue to make strong unsubstantiated claims in the abstract and conclusion (“These results suggest that ARG098 is a novel and efficacious for RA treatment”).

As we have shown in the additional figure in this letter, ARG098 induced cytotoxicity concentration dependently toward RA synoviocytes preincubated with 5 ng/mL of tumor necrosis factor-α for 5 days. ARG098 at a concentration 1000 ng/mL induced cytotoxicity in more than 80% (80.7 ± 0.70%). Whereas clone 7C11, a IgM subtype of anti-Fas antibody, at 1000 ng/mL induced cytotoxicity in 68.7 ± 3.43% and the efficacy was nearly same in more concentration, and clone CH-11, another IgM subtype of anti-Fas antibody, at 10000 ng/mL induced cytotoxicity 10.7 ± 1.50% in this condition. The epitope might make a difference among efficacies of these antibodies, but this explanation is still unclear.

We agree with the referee’s comment that these data are insufficient to strongly claim the superiority of ARG098 against 7C11 anti-Fas antibody.

So, we have revised the manuscript and the comparative statements against other anti-Fas antibodies in page 11-12 (previously, page 11 top paragraph) were deleted following the suggestion of Associate Editor comments.
Some of the strong claims in the abstract and conclusions were toned down as follows; “These results suggest that ARG098 is a novel and efficacious agent for RA treatment.” to “These results suggest that ARG098 might become a new therapy for RA.” in Abstract section. “ARG098 will be an efficacious RA therapy” to “ARG098 might be a potential RA therapy” in Conclusions section.

Additional figure - Cytotoxic effects of ARG098, CH-11, and 7C11 on RA synoviocytes preincubated with inflammatory cytokines.

RA synoviocytes were preincubated with 5 ng/mL of tumor necrosis factor-α for 5 days and then treated with human IgM, ARG098, CH-11, and 7C11 for 24 h. Each point represents mean ± SEM (n = 3). Cytotoxicity was measured with the WST assay.

2) While the authors attribute the historical lack of efficacy of anti-Fas antibodies on RA FLS to, among other things, their need for multimerization, a very good recent study by Pundt and colleagues (Arthritis Res Ther, 2009, 11/1/R16) demonstrated that RA FLS susceptibility to FasL-induced death was exquisitely dependent upon the proliferation rate of cells in vitro, as well as cell cycling status and culture confluency. The authors need to take into consideration that their positive results with ARG098 are secondary to tissue culture conditions –eg., there is no evidence in the methods section that they serum-starve their FLS or achieve cell cycle arrest.

Evaluation: The authors respond that they performed all of their experiments in cells cultured in 10% FBS. Pundt and colleagues have reported that proliferating FLS are least susceptible to FasL-induced apoptosis. Again, testing other anti-Fas antibodies or soluble FasL would have been helpful to the authors to allow us to draw some intrinsic data relative to published literature. Inclusion of 10% FBS in the medium will also mask intrinsic, imprinted properties of RA FLS, as demonstrated by Buckley and colleagues at the level of gene expression.
Following the suggestion of the Referee 1, we showed the results of other anti-Fas antibodies in this letter. Please see the answer for Major Compulsory Revisions 1). Cell death in some part of RA synoviocytes in this culture condition was also induced with another anti-Fas antibody clone 7C11 treatment, and this may suggest that the some part of RA synoviocytes were not Fas resistant. We can also understand that our positive in vitro results with ARG098 may be a secondary event depending on the tissue culture conditions such as proliferation rate, cell cycling status and culture confluency. We suppose that there are both proliferating and arresting synovial fibroblasts in our culture in 10% FBS. So, we have mentioned in Discussion section that “from our in vitro results with the experimental cell culture in 10% FBS we could not evaluate the intrinsic quantitative efficacy of ARG098”.

3) The authors ignore a number of important manuscripts published on this topic such as effects of PI3 kinase signaling in RA FLS resistance to Fas-dependent apoptosis from the group of Pope, and others. Additionally, they fail to examine the effect of their antibody on synovial macrophages, an important synovial population reported to be resistant.

Evaluation: The authors still have macrophages and dendritic cells in their culture after 5 passages, or are using mixed cell populations in early cultures??!! In this case, we don’t know what cell population we are studying, and it is even more difficult to extrapolate in vitro results to their in vivo results.

In our culture condition, we have not checked what cell population are there. However, as reviewer indicated, we speculate the majority of our tested cell population may be synovial fibroblast cells which are consisted with mixed cell status of proliferation rate and cell cycling, etc. We suppose that there are both proliferating and arresting synovial fibroblasts in our culture with 10% FBS. Although from these in vitro results we can not extrapolate intrinsic in vivo results, we believe that a part of in vivo effects are expected by our in vitro results. Therefore, following the suggestion of the reviewer, we have revised the ‘Cell isolation and cell culture’ in Method section and added “however, the majority of our tested cell population may be synovial fibroblast cells”.

4) Both introduction and discussion sections are unnecessarily long, a result of inadequate organization and internal redundancies – both sections could be shortened by 33-50%.

Evaluation: The authors have shortened both sections sufficiently in length, but organization within each section is still poor.
We have revised the Discussion section to be more appropriate and shorter.

5) Extreme care should be given to grammatical errors appearing throughout the manuscript.

Evaluation: The authors have greatly improved grammar throughout the manuscript but the manuscript will still require the firm hand of the editorial office.

Following the suggestion of the reviewer, the revised manuscript has been edited by a native English speaker.
We found the reviewer's suggestions very helpful and have revised the manuscript accordingly.

Minor changes suggested:
Abstract: "is a novel anti-rheumatoid arthritis (RA)" might be deleted.
"...ARG098 as an anti-RA agent" might be changed by "as a therapy for RA".
"ARG098 is a novel and efficacious agent for RA treatment" might be changed by
"might become a novel therapy for RA"
Conclusions: "ARG098 will be an efficacious RA therapy" might be changed by
"ARG098 might be a potential RA therapy".

As indicated by the reviewer, we have revised as follows (the corrections are highlighted);
The background in abstract to “The anti-human Fas/APO-1/CD95 (Fas) mouse/human chimeric
monoclonal IgM antibody ARG098 (ARG098) targets the human Fas molecule. The cytotoxic
effects of ARG098 on cells isolated from RA patients, on normal cells in vitro, and on RA synovial
tissue and cartilage in vivo using implanted rheumatoid tissues in an SCID mouse model
(SCID-HuRAg) were investigated to examine the potential of ARG098 as a therapy for RA.”
The conclusion in abstract to “These results suggest that ARG098 might become a new therapy for
RA.”
The conclusions to "Taken together, these results suggest that ARG098 might be a potential RA
therapy that directly suppresses synovial activity and decreases cartilage destruction when delivered
by intra-articular injection.”

Referee 2 further comments:
"I was a little bit worry about this manuscript due to its strong statements without convincing
arguments. Accordingly I had recommend reject the manuscript at first revision. However, in
my opinion authors modified the manuscript in the correct way (reducing strong statements,
improving English and shortening the extension) and, after to highlight that some strong
statement still remained and must be changed, I though the manuscript was suitable for
publication.
In my opinion Prof Tak arguments are correct and I have to agree with them. However, if the
authors can change the strong claims on efficacy, explain well if they used or not mixed cell
population and modify other parts of the text accordingly to the comment of Prof Tak, the work could finally be considered for acceptation."

Following the suggestions of the reviewers and the Associate Editor, we have revised to tone down about the efficacy of ARG098 and deleted the antibody comparison data in the manuscript. In Method and Discussion, we explained that the majority of the cells we used may be speculated to be RA synovial fibroblasts.
Associate Editor comments:

We found the Associate Editor’s suggestions very helpful and have revised the manuscript accordingly.

The manuscript is not acceptable in its current form but could be acceptable if you permit further revisions:

1. Some of the statements need to be toned down as suggested by Dr Canete (see below)

As indicated by the Associate Editor and Dr Canete’s suggestion, we have revised the manuscript. Please see the answers in Referee 2 in this cover letter.

2. The comparative statements against other anti-Fas antibodies on page 11 (top paragraph) need to be deleted or backed up by showing the data requested by Prof Tak.

We have shown the comparative data in this cover letter as the answer to Professor Tak, and the comparative statements against other anti-Fas antibodies in page 11-12 were deleted (previously page 11 top paragraph) following the suggestion of Associate Editor comments.

3. It should be clarified that the synovial lymphocytes used in apoptosis expts (page 11) are those extracted from synovial biopsies after 1 day of incubation, as stated on page 5 (I presume this is where they derive from).

To clarify the synovial lymphocytes, we revised as follows as the suggestion of Associate Editor comments (the corrections are highlighted);

In page 7 (Previously page 5) to “All experiments were performed using RA synoviocytes within the fifth passage, and using RA synovium-infiltrating lymphocytes without further passage.”

In page 12 (Previously page 11) to “ARG098 induced apoptosis in RA synovium-infiltrating lymphocytes, which did not adhere and were separated from the RA synoviums after 1 day of incubation as written in methods.”.

In page 12, the word integrated into “RA synovium-infiltrating lymphocytes”.
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Editorial comments:

We found the Editorial comments very helpful and have revised the manuscript accordingly.

Please also highlight (with 'tracked changes'/coloured/underlines/highlighted text) all changes made when revising the manuscript to make it easier for the Editors to give you a prompt decision on your manuscript.

We have highlighted the changes in the manuscript revision. Please see them.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

We have revised the Title page, Competing interests, Authors’ contributions and References as following the Instructions for authors.