Author's response to reviews

Title: Myositis specific autoantibodies in Korean patients with inflammatory myositis: Anti-140-kDa polypeptide antibody is primarily associated with rapidly progressive interstitial lung disease independent of clinically amyopathic dermatomyositis.

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Version:2
Date: July 25th, 2010

Author’s response to reviews: see over
Reply to reviewer 1.

1. The number of cases with each of the autoantibodies should be included in the abstract to assist readers in interpreting the data.

   → We added the number of cases for each of the autoantibodies in the abstract.

2. The term “myositis-specific autoantibodies” or MSA would imply finding the autoantibodies only in myositis patients and since the anti-p140 autoantibodies have mostly been reported in CADM this would seem to be a misnomer for these autoantibodies. The term MSA has also been reserved for those autoantibodies that have been extensively studied in non-myositis populations to assure their specificity, which does not yet seem to be the case for either anti-p140 or anti-p155/140. A more accurate description of the anti-p140 and anti-p155/140 might be autoantibodies frequently seen in myositis.

   → We agree that anti-p140 and anti-p155 antibodies have not been studied as extensively as the other classical MSAs. However, the specificity of anti-p140 antibodies has been examined in the study of Sato et al [reference #9 of the revised manuscript], in which this antibody was exclusively found in CADM patients without being found in 195 non-myositis patients. The specificity of anti-p155/140 antibodies to DM has been consistently reported since the identification of this antibody [references #12-14 of the manuscript]. Because these antibodies are frequently classified as MSA in recent review papers [Gunawardena H, et al. Rheumatology (Oxford). 2009 ;48:607-12; Mammen AL. Ann N Y Acad Sci.
2010;1184:134-53], we hope to use the term “MSA” for these antibodies in the manuscript.

3. Bohan and Peter criteria do not describe the specific rashes used to define DM nor how IBM is excluded – please clarify this.

→ We added the following paragraph in the Method section.

(Page 7, line 4) “DM was classified when heliotrope rash, Gottron’s sign, and/or Gottron’s papule were present. Patients with myositis overlap syndrome met both the above criteria and the criteria for another defined connective tissue disease. Patients with juvenile DM (age \( \leq 18 \)), ADM/CADM (classified according to the criteria by Sontheimer [5]), or inclusion body myositis (diagnosed by the presence of typical inclusions on a stained muscle biopsy under the light microscopy) were not included.”

4. Cancer-associated myositis is often defined as the diagnosis of cancer within 1 year of the diagnosis of myositis as in reference 17 – please clarify the definition used in this study.

→ The criteria of cancer-associated myositis were defined as the diagnosis of cancer within 3 years based on the previous literature [Chinoy H et al. Ann Rheum Dis 2007; 66:1345-9]. The related text was modified as such.

(Page 8, line 1) “Cancer-associated myositis was identified when cancer was detected
within 3 years of myositis diagnosis [14].”


5. There seems to be a usual over-representation of DM patients in this cohort compared to others. Also, myositis overlap with another connective tissue disease is not mentioned, and a remarkable 3 of 6 anti-synthetase patients had cancer. It would probably be useful to show the overlap and cancer patients as separate columns in Table 1 and comment on these unusual distributions and why they may have occurred.

→ The distribution of PM and DM of this study is similar to our previous report [Kang EH et al. Rheumatology 2005; 44:1282-6]. The number of myositis overlap cases (n = 3) has been noted in the table 1 and described in detail in the manuscript as below. The detailed clinical data of the patients with any malignancy history has been summarized separately in table 2.

(Page 10, line 5) “There were 3 myositis overlap cases (n = 1 for DM/systemic sclerosis, n = 1 for DM/systemic lupus erythematosus (SLE), n = 1 for PM/SLE).”
### Table 2. Clinical data of 12 patients who had malignancy

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>ILD</th>
<th>Time of detection† (months)</th>
<th>Primary site</th>
<th>cancer-associated myositis</th>
<th>Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>F</td>
<td>DM</td>
<td>-</td>
<td>-11</td>
<td>Esophagus</td>
<td>Anti-p155/140</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>F</td>
<td>DM</td>
<td>-</td>
<td>+18</td>
<td>Breast</td>
<td>Anti-Mi2</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>M</td>
<td>DM</td>
<td>-</td>
<td>-1</td>
<td>Stomach</td>
<td>Anti-p155/140</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>F</td>
<td>DM</td>
<td>-</td>
<td>0</td>
<td>Lung</td>
<td>Anti-p155/140</td>
</tr>
<tr>
<td>5</td>
<td>74</td>
<td>F</td>
<td>PM/SLE</td>
<td>+</td>
<td>-48</td>
<td>Stomach</td>
<td>Anti-PL-7</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>M</td>
<td>PM</td>
<td>-</td>
<td>0</td>
<td>Liver</td>
<td>none</td>
</tr>
<tr>
<td>7</td>
<td>36</td>
<td>F</td>
<td>DM/SLE</td>
<td>+</td>
<td>-1</td>
<td>Thyroid</td>
<td>Anti-PL-12</td>
</tr>
<tr>
<td>8</td>
<td>36</td>
<td>M</td>
<td>DM</td>
<td>-</td>
<td>0</td>
<td>Lymphoma</td>
<td>Anti-p155/140</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>F</td>
<td>DM</td>
<td>-</td>
<td>-2</td>
<td>Breast</td>
<td>Anti-p155/140</td>
</tr>
<tr>
<td>10</td>
<td>61</td>
<td>M</td>
<td>DM</td>
<td>+</td>
<td>+22</td>
<td>Lymphoma</td>
<td>Anti-Jo-1</td>
</tr>
<tr>
<td>11</td>
<td>68</td>
<td>M</td>
<td>DM</td>
<td>+</td>
<td>+2</td>
<td>Lung</td>
<td>none</td>
</tr>
<tr>
<td>12</td>
<td>65</td>
<td>M</td>
<td>PM</td>
<td>+</td>
<td>0</td>
<td>Stomach</td>
<td>Anti-Jo1</td>
</tr>
</tbody>
</table>

† Relative to time of myositis diagnosis.

DM = dermatomyositis, F = female, ILD = interstitial lung disease, M = male, MSA = myositis specific autoantibody, PM = polymyositis, SLE = systemic lupus erythematosus

6. As shown in Figure 1B, some of the patients assigned as anti-p155/140 or anti-p140 appear to have other IP bands that make their designation uncertain (also other patients, such as D, have IP bands in this region that apparently were not called as having these autoantibodies). For this reason, because of the large number of possible protein bands in this region, many investigators do not believe that protein immunoprecipitation alone can define with certainty the anti-p155/140 reactivity and
distinguish it from the anti-p140 or other reactivities, and that IP followed by immunoblotting or another validated method is needed. This is a key issue regarding the clinical associations claimed and these reactivities need to be validated by other methods.

→ It is true that the specificity of the antibodies is defined by their ability to recognize the autoantigen. However, anti-p140 and anti-p155/140 antibodies are usually distinguishable by comparing the sizes (or combination of sizes) of proteins precipitated by patient versus reference sera because they are mutually exclusive with other MSAs in general (references #10-13 of the revised manuscript); the p140 band precipitated together with p155 hardly indicates simultaneous presence of anti-p140 and anti-p155/140 antibodies, rather, co-presence of p155 and p140 indicates the presence of anti-p155/140. Based on this, high accuracy of immunoprecipitation to specifically detect anti-p140 or anti-p155/140 antibodies in inflammatory myositis patients has been shown by our authors [reference #28 of the manuscript]. In addition, distinctive clinical features have been demonstrated in association with each MSA defined by immunoprecipitation in our study, which are generally in parallel with the results of previous studies [references #9-15 of the manuscript]. However, we are aware that it is one of the limitations of our study and commented on this in the discussion section.

(Page 15, line 1) “Second, we did not further examine the specificity of each MSA beyond immunoprecipitation, which let us reserve the term “anti-CADM-140 antibody” for anti-p140 antibody. However, high accuracy of immunoprecipitation to specifically detect anti-p140 or anti-p155/140 antibodies in inflammatory myositis
patients has been previously reported [28]. In addition, distinctive clinical features
have been demonstrated in association with each MSA defined by
immunoprecipitation in our study, which are generally in parallel with the results of
previous studies [9-15].”

and anti-TIF1-{gamma} antibodies have clinical significance for patients with
dermatomyositis. Rheumatology (Oxford) 2010 May 25. [Epub ahead of print]

7. It is odd that the prior studies of Korean myositis autoantibodies (i.e. Rider et al.
1999 A&R), which showed different results, were not reference in this paper. Were
the patients in this study previously published in prior studies?

→ The 49 sera used in this study were collected from the patients diagnosed as
having inflammatory myositis at Seoul National University Hospital from 1993 to
2007. However, they do not represent the pool of consecutively collected sera during
this period. We carefully examined the patient cohort of our study. Thirty out of fifty
patients included in our previous study [Rider LG et al. Arthritis Rheum 1999;
42:1285-90 (reference #19 of the revised manuscript) met the enrollment criteria of
this study. However, only four of their sera were available for the present study.
Consequently, twenty-six patients (9 PM, 17 DM) were dropped during the
consecutive enrollment in this study; these 26 patients were followed up for 78 ±
59.9 months and there was one DM patient with rapidly progressive ILD. None of
them had malignancy.

Despite the reviewer’s comment, we do believe that the result of the present study
is not different from our previous report; both studies showed that anti-ARS and anti-Mi2 antibodies are common in Korean myositis patients. We modified the related text to describe in detail the study cohort in the Method section. In addition, we commented on the possible selection bias of the patient cohort in the discussion.

(Page 6, line 18) “Forty nine serum samples (n = 11 for PM, n = 38 for DM) were available from seventy-five patients (n = 20 for PM, n = 55 for DM) consecutively diagnosed as having definite inflammatory myositis according to the Bohan and Peter criteria [18] from March 1993 to November 2007 at the Rheumatology Clinic of Seoul National University Hospital. The remaining twenty-six sera had been examined for the presence of certain MSAs in our previous study [19], but were not available for the current study. DM was classified when heliotrope rash, Gottron’s sign, and/or Gottron’s papule were present. Patients with myositis overlap syndrome met both the above criteria and the criteria for another defined connective tissue disease. Patients with juvenile DM (age ≤ 18), ADM/CADM (classified according to the criteria by Sontheimer [5]), or inclusion body myositis (diagnosed by the presence of typical inclusions on a stained muscle biopsy under the light microscopy) were not included”


(Page 14, line 15) “The limitations of this study are as follows. First, 26 sera were dropped out during consecutive enrollment, which leaves the possibility of selection
bias. However, the clinical implications of anti-p140 and anti-p155/140 antibodies observed in this study are unlikely to be affected, because there was only one patient with rapidly progressive ILD and none with malignancy among these 26 patients during 78 ± 59.9 months of follow-up (data not shown).”

Reply to reviewer 2.

1. In the “patients and sera” section, how were these patients collected and over what time period. It states that “serum was collected during first admission”. Does this mean that only patients admitted to the hospital are included in this analysis? If this is the case, then why? If not please make it clear in this section just how these patients entered the study and whether they were consecutively enrolled from inpatient and outpatient sources.

→ All new onset myositis patients were admitted in Seoul Nation University Hospital to make a comprehensive diagnosis through clinical, biochemical, electrophysiological, and histological examinations and to assess internal organ involvements. Forty nine serum samples used in this study were collected among the patients diagnosed as having definite inflammatory myositis according to the Bohan and Peter criteria from March 1993 to November 2007 at the Rheumatology Clinic of Seoul National University Hospital. However, twenty-six patients who met the above criteria were not included in this study due to lack of their sera. We modified the related text as follows.

(Page 6, line 18) “Forty nine serum samples (n = 11 for PM, n = 38 for DM) were available from seventy-five patients (n = 20 for PM, n = 55 for DM) consecutively
diagnosed as having definite inflammatory myositis according to the Bohan and Peter criteria [18] from March 1993 to November 2007 at the Rheumatology Clinic of Seoul National University Hospital. The remaining twenty-six sera had been examined for the presence of certain MSAs in our previous study [19], but were not available for the current study. DM was classified when heliotrope rash, Gottron’s sign, and/or Gottron’s papule were present. Patients with myositis overlap syndrome met both the above criteria and the criteria for another defined connective tissue disease. Patients with juvenile DM (age ≤ 18), ADM/CADM (classified according to the criteria by Sontheimer [5]), or inclusion body myositis (diagnosed by the presence of typical inclusions on a stained muscle biopsy under the light microscopy) were not included.”

2. Clarify the definition of cancer-associated myositis in this same section (Patients and sera) according to what is specified in the literature. That is, establish parameters in terms of the timing of the myositis onset and the timing of the cancer onset. I see the mention of the cancer timing in the results but a definition should be provided earlier.

The criteria of cancer-associated myositis were defined as the diagnosis of cancer within 3 years based on the previous literature [Chinoy H et al. Ann Rheum Dis 2007; 66:1345-9]. The related text was modified as such.

(Page 8, line 1) “Cancer-associated myositis was identified when cancer was detected within 3 years of myositis diagnosis [14].”

[14] Chinoy H, Fertig N, Oddis CV, Ollier WE, Cooper RG. The diagnostic utility of

3. Was the cancer onset and type in the synthetase positive patients similar to the other pts with cancer that had the p155/140? The finding of malignancy in synthetase positive pts has been reported but is unusual. Do the authors there is anything unusual about these 3 pts? Were they Jo-1 positive or was it other synthetase?

→ Three cases out of 6 anti-ARS-positive patients had cancer-associated myositis (n = 2 for anti-Jo-1, n = 1 for anti-PL-12). The detailed clinical information on patients with any malignancy history was summarized in table 2 (please, refer to the answer to the reviewer 1’s comment #5). We added comments on anti-ARS positive patients with cancer-associated myositis in the discussion.

(Page 14, line 9) “Another interesting finding with regard to cancer-associated myositis was that 3 out of 6 anti-ARS positive patients had cancers, which, however, was not statistically significant. In fact, cancer-associated myositis in the presence of other MSAs than anti-p155/140 antibodies (such as anti-ARS or anti-Mi2) has been previously reported [14, 26, 27]. Although anti-p140 positive patients have been shown to have low prevalence of malignancy [9, 10], the presence of other MSAs rather than anti-p155/140 antibodies does not seem to rule out the presence of cancer in inflammatory myositis patients.”

4. A lot of the details are missing regarding the follow-up of this cohort such as data regarding imaging, PFTs and clinical details. How was this cohort followed after
presentation?

→ Detailed description how the patients were followed up was added in the Method section.

(Page 7, line 10) “Clinical information regarding disease manifestations, laboratory data, radiographic data, and the presence of internal malignancies was obtained by medical chart review. Chest radiography (CXR) and/or high resolution computed tomography (HRCT) were performed at baseline and repeated every 12 months or when they had new onset respiratory symptoms. Patients were diagnosed as having ILD based on the radiographic evidence in CXR and/or HRCT findings. Rapidly progressive ILD was defined to be present when ILD showed radiographic deterioration causing hypoxia within one month from respiratory symptom onset. All patients underwent cancer screening, including chest radiography, computed tomography for abdomen and pelvis, and endoscopy for stomach and colon. Breast and gynecologic examinations were done for female patients. Patients negative at initial cancer screening were re-examined whenever suspected for malignancy during follow-up.”

5. The authors note that anti-p140 was associated with rapidly progressive ILD and that this is different from the Japanese reports (that note this autoAb in CAMD)? However, if consecutive myositis pts including those seen in the outpatient area were not included in this Korean cohort then they would miss those patients with mild disease as they would not be admitted to the hospital.
As we explained in our answer to reviewer 2’s question #1, twenty-six patients (9 PM, 17 DM) were not included during consecutive enrollment. We are aware that there could have been a selection bias in the study cohort. However, there was only one patient with rapidly progressive ILD and none with malignancy among these 26 patients during 78 ± 59.9 months of follow-up. Therefore, we believe that the result of our study is unlikely to be affected. However, this is one of the limitations of our study and we described in detail the study cohort in the method section, and added a comment on this in the discussion section.

Forty nine serum samples (n = 11 for PM, n = 38 for DM) were available from seventy-five patients (n = 20 for PM, n = 55 for DM) consecutively diagnosed as having definite inflammatory myositis according to the Bohan and Peter criteria [18] from March 1993 to November 2007 at the Rheumatology Clinic of Seoul National University Hospital. The remaining twenty-six sera had been examined for the presence of certain MSAs in our previous study [19], but were not available for the current study. DM was classified when heliotrope rash, Gottron’s sign, and/or Gottron’s papule were present. Patients with myositis overlap syndrome met both the above criteria and the criteria for another defined connective tissue disease. Patients with juvenile DM (age ≤ 18), ADM/CADM (classified according to the criteria by Sontheimer [5]), or inclusion body myositis (diagnosed by the presence of typical inclusions on a stained muscle biopsy under the light microscopy) were not included.”

The limitations of this study are as follows. First, 26 sera were dropped out during consecutive enrollment, which leaves the possibility of selection
bias. However, the clinical implications of anti-p140 and anti-p155/140 antibodies observed in this study are unlikely to be affected, because there was only one patient with rapidly progressive ILD and none with malignancy among these 26 patients during 78 ± 59.9 months of follow-up (data not shown).”

6. I don’t follow the rationale of the authors noted in the next to the last page of the discussion when they talk about low CK levels and grey zones. This requires clarification.

Our intention to state that “the discrepancy between our result and the Japanese result might be due to the definitional grey zone between classic DM and CADM rather than a true ethnic difference” was to question if the high prevalence of anti-p140 antibodies in Korean patients with classic DM resulted from the current classification criteria for CADM/ADM; because the absence of muscle weakness for up to 6 months is a critical component for the diagnosis of ADM/CADM according to the criteria proposed by Sontheimer [reference #5 of the revised manuscript], those with mild muscle weakness but with minimal or no elevation in muscle enzyme levels are often diagnosed as having DM despite their clinical similarities to CADM patients [Kameda H, et al. J Rheumatol 2005;32:1719-26; Nawata Y et al. J Rheumatol 1999;26:1527-33]. To clarify this, we summarized the clinical characteristics of the nine anti-p140 positive patients in table 4, and found that the majority of those who were positive for anti-p140 antibody in our study show high muscle enzymes levels. Therefore, we omitted the related paragraph.

7. The statements regarding the poor cancer-related outcome of the anti-p155/140 subset
may be interesting but not enough details are provided and the numbers are very small. The authors do recognize the small number issue in their discussion but there is little support or data provided on the cancer-associated outcomes.

We added table 2 (please, refer to the answer to the reviewer 1’s comment #5) and figure 2 (survival graph by Kaplan-Meier analysis) to describe in detail the clinical characteristics of cancer-associated myositis patients and modified the related text.

(Page 14, line 3) “However, our study is adopting a small number of patients to conclude with a certainty. We are cautious to claim the prognostic value of this antibody in cancer-associated myositis until it is proven via multivariate analyses that appropriately adjust other prognostic factors. Further studies employing a larger number of cancer-associated myositis patients are warranted to confirm this result and to determine if anti-p155/140 antibody predates or follows the onset of cancer, if its titer correlates with cancer progression, or if certain types of cancers are more prone to develop anti-p155/140 antibody. Another interesting finding with regard to cancer-associated myositis was that 3 out of 6 anti-ARS positive patients had cancers, which, however, was not statistically significant. In fact, cancer-associated myositis in the presence of other MSAs than anti-p155/140 antibodies (such as anti-ARS or anti-Mi2) has been previously reported [14, 26, 27]. Although anti-p140 positive patients have been shown to have low prevalence of malignancy [9, 10], the presence of other MSAs rather than anti-p155/140 antibodies does not seem to rule out the presence of cancer in inflammatory myositis patients.”

Reply to reviewer 3.
1. As there seems to be other p140 autoantigens recognized by MSA (e.g. NXP1) it would save confusion by using additional nomenclature (e.g. anti-CADM 140, anti-p140 (MDAG-5) etc) to delineate what p140 autoantigen is being recognized by the patients described here.

→ Because we did not confirm the specificity of antibody beyond immunoprecipitation, we reserved the term “anti-CADM 140” or “anti-p140 (MDAG-5)”. However, we understand that there could be a confusion regarding the nomenclature of anti-p140 antibody, and added a comment on this in the discussion section.

(Page 15, line 1) “Second, we did not further examine the specificity of each MSA beyond immunoprecipitation, which let us reserve the term “anti-CADM-140 antibody” for anti-p140 antibody. However, high accuracy of immunoprecipitation to specifically detect anti-p140 or anti-p155/140 antibodies in inflammatory myositis patients has been previously reported [28]. In addition, distinctive clinical features have been demonstrated in association with each MSA defined by immunoprecipitation in our study, which are generally in parallel with the results of previous studies [9-15].”

2. Mention is made of only Japanese and NA studies – but there is no referencing of UK studies.

Because all of our study subjects were adult patients, we did not include the references on anti-p155/140 antibodies found in juvenile DM patients. We added a
UK reference [14] together with a recent Spanish reference [15] and modified the manuscript.

(Page 6, line 8) “However, clinical implications regarding these novel antibodies in adult PM and DM patients have been limited to a few ethnic cohorts [9-15].”


3. Cancer-associated myositis needs defining (e.g. occurring within 3 years of onset of myositis)

The criteria of cancer-associated myositis were defined as the diagnosis of cancer within 3 years based on the previous literature [14]. The related text was modified as such.

(Page 8, line 1) “Cancer-associated myositis was identified when cancer was detected within 3 years of myositis diagnosis [14].”

Comments to the editor
In addition to the modifications according to the reviewers’ comments, there was correction on the clinical data of cancer–associated myositis patients.

1) We described only 11 cases classified as cancer-associated myositis in the previous manuscript and did not include the malignancy history of one PM patient who had early gastric cancer 48 months before the diagnosis of myositis. Because this patient was positive for anti-ARS, and there were 3 other cancer-associated myositis patients positive for anti-ARS, we included this information in the table 2 of the revised manuscript to present the whole data on the patients with any history of cancer.

2) We found that there was a mistake in calculating the interval time between the diagnosis of myositis and cancer onset for 2 patients. This was corrected in the table 2 of the revised manuscript. Please, note the time of detection for patient 1 (-11 months) and 2 (+18 months) in table 2 of the revised manuscript.