<u>Editor's comments:</u>

I read with interest the manuscript entitled: *'Expression of PDCD1 (PD-1) Gene among non-small cell lung cancer (NSCLC) Patients with Real-Time PCR application'*.

However there are some points to address:

1) The legends of figures are incomplete. Each table or figure should be understandable even without consulting the text.

Response: corrected
Figure 1: Linear and logarithmic graphs of Real-Time PCR amplification and temperature curve for *PDCD-1* gene. The x axis contains the number of reaction cycles and a y axis called Delta Rn.

Figure 2: The fold change expression of *PDCD-1* gene. The results showed that the expression of *PDCD-1* gene 2.46 Fold was more common in patients with lung cancer than NSCLC. A: expression of *PDCD-1* gene in control. B: expression of *PDCD-1* gene patients

2) The sample size is too small and will be difficult to do any statistical analyses with these results.

Response: corrected
This article is the result of a student project that we could collect at the time we were able to attend the university. It was not possible to collect more samples.

3) The frequency of expression of the gene in patients (Fig 1) has no control. This must be included. If not indicated.

Response: corrected
The level of gene expression and folding is shown in Fig. 2.

4) In Fig 2. What do the different colours of the temperature curve indicate. It needs a legend. Fig 2 has be be labelled as A and B each of which should have a legend.

Response: Corrected
Figure was corrected and legend was modify.
Figure 2: The fold change expression of *PDCD-1* gene. The results showed that the expression of *PDCD-1* gene 2.46 Fold was more common in patients with lung cancer than NSCLC. A: expression of *PDCD-1* gene in control. B: expression of *PDCD-1* gene patients

5) Nothing is said on the role of the 22 various grades of disease determined. Why go for these grades?. The literature review is insufficient.
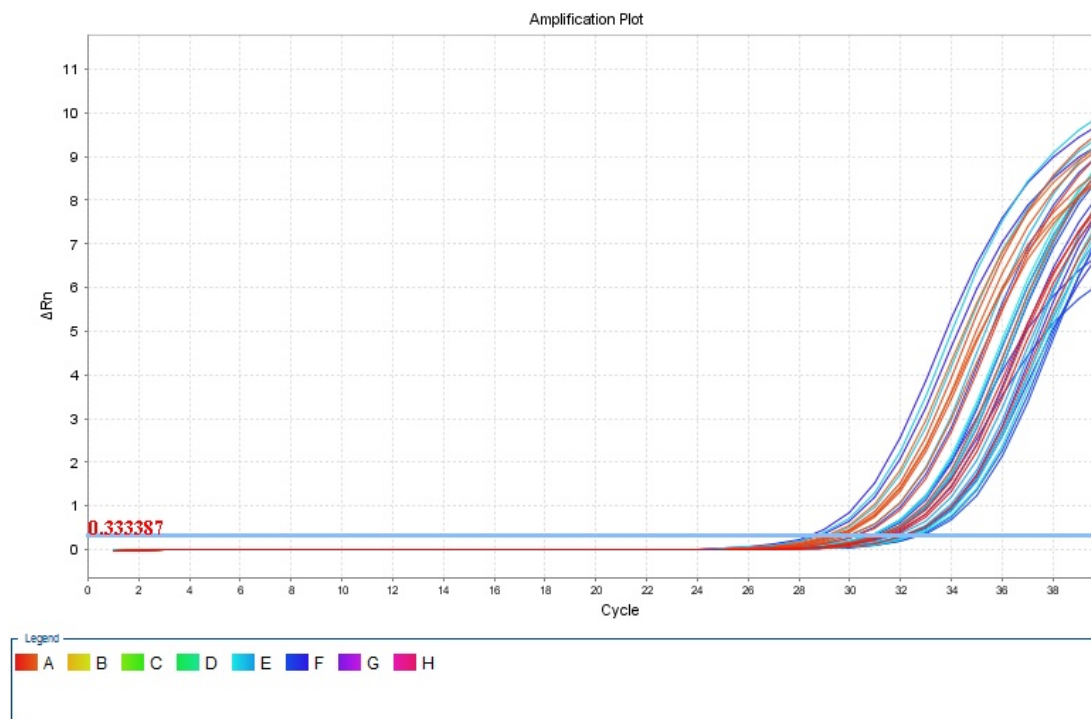
Response: Corrected
Because we only identified the grade in the patients studied and did not study the grads. So we focused on the expression of the PDCD-1 gene, which was the main work of the paper.

6) It must be shown that the expression of the house keeping gene is the same in all samples. This is not shown.

Amplification Plot PDCD shown in below:

Author's feedback: