78538-Answering Reviewers

1. The manuscript must be carefully proofread for grammar, spelling, and punctuation issues.

Ans: Thank you for your valuable comments. We have re-examined the manuscript and corrected the deficiencies in our grammar, spelling, and punctuation issues. Then we sent it to the professional English language editing company (Springer Nature Author Services) to assist in the revision. At present, the relevant revisions have been completed.

2. It is recommended that authors improve the design of diagrams.

Ans: We have modified it according to the requirements for Figures by WJCC-case report, please refer to the manuscript and **78538-Figures.pptx** for details.

1. The quality of Figure 4a, 4b, 4c, 4d are poor, especially the Ki67 picture, the hematoxylin counterstain is not enough, and the nuclei of the negative cells are not clearly displayed. Please improve the picture quality.

Ans: Thank you for your valuable comments. We have re-examined the pathological staining and took figures again (Figure 4A,4B,4C,4D), hoping to show better results. But due to this case contains a large amount of melanin (as shown in the HE (Figure 4E)), immunohistochemical staining cannot be performed directly according to the standard procedure. It is necessary to remove the melanin first, and then perform the immunohistochemical staining of the corresponding antibody. We use hydrogen peroxide to remove the melanin which is a strong oxidant. This step will have some impact on subsequent immunohistochemical staining. Therefore, there will be problems of weak nuclear hematoxylin staining and poor morphology. Our team has tried our best to present better and reliable pathological figures, and we hope that respected experts can understand our efforts.

2. Please supplement the relevant picture data of the molecular test results such as FISH of this case.

Ans: We have supplemented the relevant picture data of the molecular detection results such as FISH in this case, please refer to the attached **78538-Figures.pptx** and Chinese report pictures (FISH report 1, FISH report 2) for details.

3. Please add the picture of the PET-CT imaging data of this case.

Ans: Due to the characteristics of China's medical environment and the Chinese people's medical habits, this patient has undergone different or repeated imaging examinations (including PET/CT etc.) in many hospitals in different cities in China for past 22 years. He also underwent 3 operations. The second and third operations were performed in our hospital. We tried to contact the patient to inquire and retrieve the relevant imaging data for reference. Because of the patient said that it took a long

time, and the patient also moved house many times during this period. Many precious imaging examination films/ The CD-ROM data was lost or damaged during the moving process. So, we can only check from the medical records that the patient had performed relevant examinations to rule out the possibility of extracranial disease metastasis. We could not provide the PET/CT imaging data for experts' reference. Which is our deficiency, but the relevant medical records are true. The patient is happy and honored to share his longest surviving case and treatment experience of PIMM. I hope the experts can understand the efforts and sincerity of our team.

4. There are still some minor grammatical and spelling errors throughout this manuscript.

Ans: Thank you for your valuable comments. We have re-examined the manuscript and corrected the deficiencies in our grammar and spelling. Then we sent it to the professional English language editing company (Springer Nature Author Services) to assist in the revision. At present, the relevant revisions have been completed.

FISH REPORT:

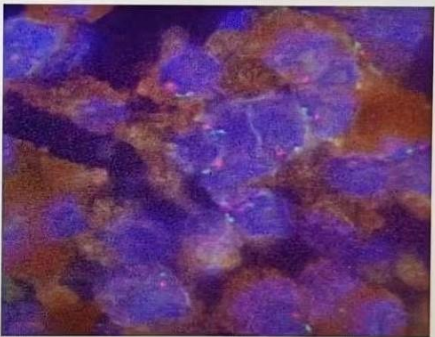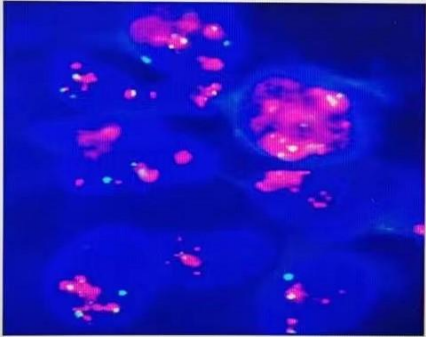| 临床诊断：黑色素瘤 | 送检日期：2021-09-06 | 医生：邓美玲 |
| 检测方法：荧光原位杂交法 | 标本类型：石蜡包埋组织 | 备注：无 |

检测项目： MDM2基因扩增检测。

病理质控结果：无HE

FISH 检测结果：阴性。(-)

检测结果附图：

| 阳性对照 | 检测样本 |

滤光片组:DAPI,TRITC,FITC

临床诊断：黑色素瘤   送检日期：2021-08-16   医生：胡婉明

检测方法：荧光原位杂交法   标本类型：石蜡包埋组织   备注：无

检测项目：恶性黑色素瘤FISH检测

检测结果：阳性 (+)

基因	染色体位置	结果
CCND1 信号数／计数细胞核	6q23	阴性 (-)
MYB 信号数／计数细胞核	6p25	阴性 (-)
RREB1 异常细胞数／计数细胞	6p25/6p11.1-q11.1	阳性 (+)
MYB 缺失细胞／计数细胞	6q23/6p11.1-q11.1	阴性 (-)

滤光片组：DAPI, TRITC, FITC, GOLD

异常信号判读标准：
CCND1信号数/计数细胞核 ≥ 2.5；MYB信号数/计数细胞核 ≥ 2.5；
MYB缺失细胞/计数细胞 ≥ 31%；RREB异常细胞数/计数细胞 ≥ 63%。