



Clinical Benefit of Sorafenib Combined with Paclitaxel and Carboplatin to a Patient with Metastatic Chemotherapy-Refractory Testicular Tumors

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ABSTRACT

Testicular cancer is one of the few tumor types that have not yet benefited from targeted therapy. Still no new active agents for treating this cancer have been identified over the past 15 years. Once patients are refractory to cisplatin-based chemotherapy, they will be expected to die from testicular cancer. This report describes a 21-year-old man who was refractory to chemotherapy and immunotherapy. Whole exome sequencing and low-depth whole genome sequencing confirmed the *KRAS* gene amplification, which may lead to the

tumor cells' progression and proliferation. After discussion at the molecular tumor board, the patient was offered paclitaxel, carboplatin, and sorafenib (CPS) based on a phase III clinical trial of melanoma with *KRAS* gene copy gains. After treatment with CPS, the patient achieved excellent curative effects. Because of a nearly 50% frequency of *KRAS* amplification in chemotherapy-refractory testicular germ cells, CPS regimen may provide a new therapy, but it still warrants further validation in clinical studies. *The Oncologist* 2019;24:e1437–e1442

KEY POINTS

- Chemotherapy-refractory testicular cancer has a very poor prognosis resulting in a lack of effective targeted therapies.
- *KRAS* gene amplification occurs in nearly 20% of testicular cancer and 50% of chemotherapy-refractory testicular cancer.
- *KRAS* amplification may activate the MAPK signaling pathway, and inhibition of MAPK by sorafenib combined with paclitaxel and carboplatin could be a viable option based on a phase III clinical trial of melanoma.
- To the authors' knowledge, this is the first report of response to sorafenib-based combination targeted therapy in a patient with chemotherapy-refractory testicular cancer.
- Clinical genomic profiling can confirm copy number variation of testicular cancer and provide insights on therapeutic options.

PATIENT STORY

A previously healthy 21-year-old man presented with right-sided scrotal pain and swelling of the right testicle. His family history was not significant for cancer. A physical examination revealed a mildly enlarged and firm right testicle. The tumor markers for testicular cancer, such as alpha-fetal protein (AFP), beta-human chorionic gonadotropin (β -HCG), and LDH, were 433.1 ng/mL (normal value [NV], 0–8.78 ng/mL), 6890 IU/L (NV, 0–5.00 IU/L), and 978 IU/L (NV, 120–250 IU/L),

respectively. The patient's enhanced computed tomography (CT) scan showed multiple lesions in the thoracic region and a bulky enlarged lymph node measuring 2.7 cm \times 2.8 cm in the retroperitoneal region. To relieve the pain and determine the pathological diagnosis, a right-sided radical orchidectomy was performed upon patient consent. Histopathological examination revealed malignant germ cell tumors. Immunohistochemistry (IHC) revealed that the tumor was positive for SALL-4,

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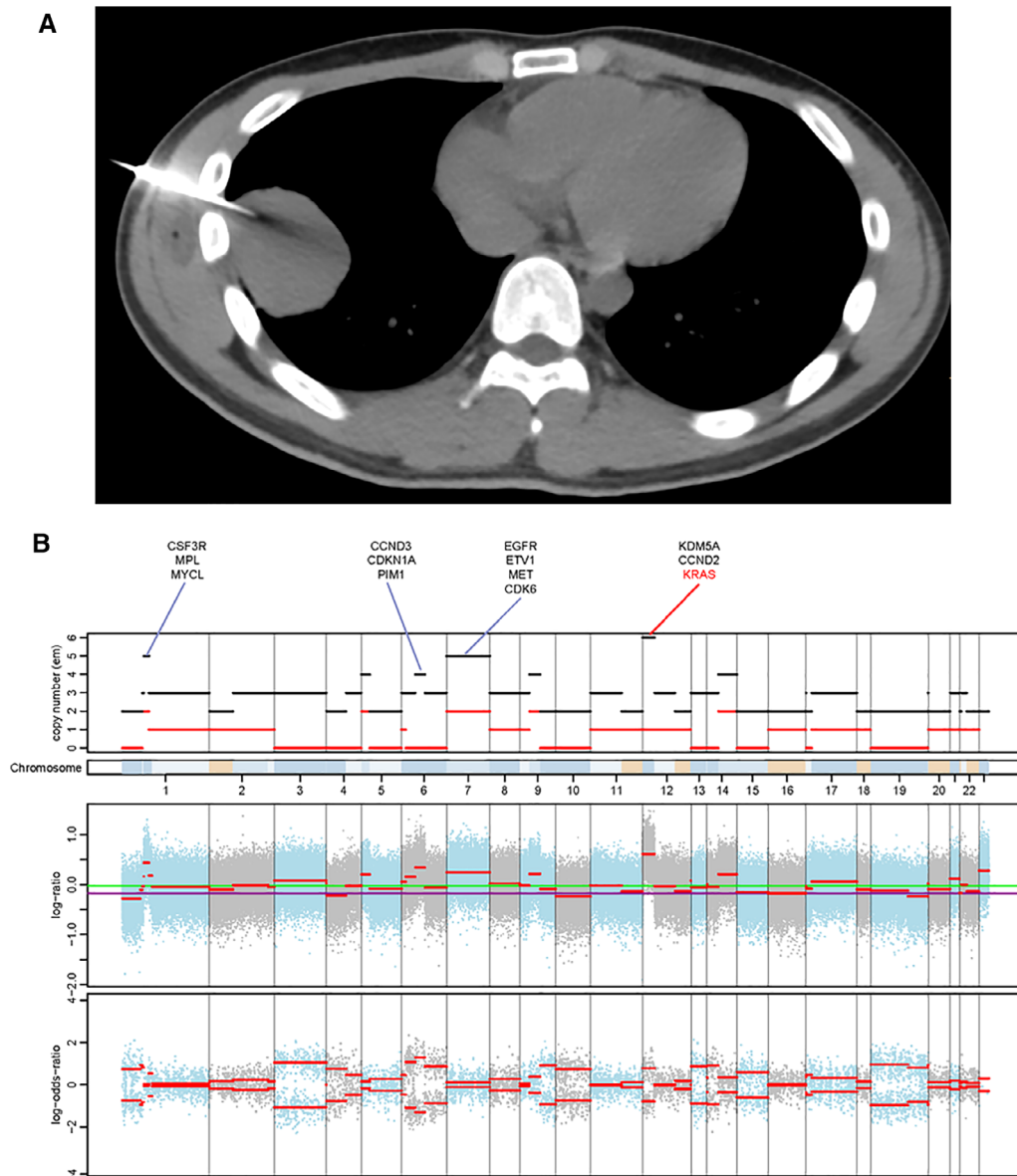


Figure 1. Puncture sampling and sequencing. **(A):** Right lung nodule puncture biopsy was performed under the guidance of computed tomography. **(B):** The Illumina NextSeq CN500 platform was used to sequence in paired-end mode, the Burrows-Wheeler aligner (BWA) was used to map reads to the GRCh37 Human reference genome, and the mean sequencing coverage of whole exome sequencing (WES) achieved a depth of 62.03 \times . GATK and VarScan were used to call mutations. CNVkit and FACETS were used to detect copy number variation (CNV). After sequencing, at CNV level 33, copy number gains have been detected involved 1,469 amplified genes, and within the gains, 22 oncogenes have been defined. Using OncoKB database, 13 oncogenes could be druggable variants

LIN28, Nanog, D240, CD30, CAM5.2, CK8/18, and Oct3/4, focally positive for PLAP, AFP, and EBC, and negative for CD117, EMA, CEA, HCG, and CD56. IHC diagnosis tends to be embryonal carcinoma. The American Joint Committee on Cancer prognostic TNM stage was IIIb (pT₃cN₂M_{1a}S2). Bleomycin, etoposide, and paraplatin (BEP) were administered as first-line chemotherapy. After two BEP cycles, the patient achieved a partial response and the tumor markers returned to normal levels. However, after the completion of four BEP cycles, the disease progressed, with the right lung metastasis becoming larger and a new metastasis appearing in the left lung. Because of rapid relapse after platinum-based therapy, the patient was considered to be chemotherapy refractory, and gemcitabine plus oxaliplatin (GEMOX) was adopted as the second-line chemotherapy.

However, after two GEMOX cycles, there was no antitumor response. Given the widespread and symptomatic progression despite several lines of conventional cytotoxic chemotherapy, the patient was recommended into a programmed cell death ligand 1 (PD-L1) clinical trial (Clinical trial: NCT03101488). After seven cycles of KN035, a PD-L1 antibody, he developed increasing chest pain and dyspnea and had radiographic disease progression, and his tumor markers continued to rise. Then, the patient presented with a headache, vomiting, and epileptic seizure. Brain magnetic resonance imaging suggested that a new metastasis had appeared in the left frontal lobe. Whole brain radiotherapy was used to relieve the symptoms from pressure in the intracranial space. Because there is still a lack of effective conventional therapies for cisplatin-refractory testicular germ cell

tumors (TGCTs), upon patient consent, a right lung nodule puncture biopsy was performed under the guidance of CT for more precise treatment (Fig. 1A). Whole exome sequencing and low-depth whole genome sequencing were used to detect both the copy number variation (CNV) and mutations. After sequencing, *KRAS* amplification was detected (Fig 1B).

MOLECULAR TUMOR BOARD

KRAS Amplification in TGCTs

TGCTs are the most common solid malignancy in young adult men, and in the past 20 years, their morbidity has increased by nearly 70% worldwide [1, 2]. The relationship with germ cell neoplasia in situ (GCNIS), the precursor lesion to malignant TGCTs, determines into which two major types TGCTs are divided: either GCNIS-related TGCTs or non-GCNIS-related TGCTs, based on the 2016 World Health Organization classification system [3]. GCNIS-related TGCTs, which occur most frequently, are mainly composed of seminomas and nonseminomatous germ cell tumors (NSGCTs). According to the postoperative pathology and immunohistochemistry, this patient was diagnosed with embryonal carcinoma belonging to NSGCTs, which tend to occur at younger ages. This type of testicular cancer is more likely to metastasize and has a higher mortality rate [4]. Although the mechanism of inducing testicular cancer is still unresolved, genetic effects might contribute to more than 40% based on previous research [5, 6]. TGCTs have a specific genetic hallmark of having an isochromosome for the short arm of chromosome 12 (i12p). This phenomenon was first described by Atkin and Baker in 1982; other studies revealed that the i12p was observed in more than 80% of all TGCTs [7–9]. Shen et al. used 137 samples to identify the molecular characterization of TGCTs and found that 87% of tumors present at least one i12p [10]. Although the exact mechanism of generating the i12p in TGCTs is still unknown, many proto-oncogenes are involved, such as *KRAS*, *CCND2*, and *GDF-3* [11]. The gain of i12p might cause overexpression of genes from 12p11.2–p12.1, especially for NSGCTs that have higher levels of expression than seminomas. Nearly 10% of TGCTs showed high levels of overexpression in this region.

KRAS is one gene located on chromosome 12p11.2–p12.1 that may activate many downstream pathways, such as the Raf/MEK/ERK and PI3 kinase pathways. Studies have revealed that *KRAS* gene overexpression is correlated with its amplification, which is involved in TGCT development [11, 12]. Based on existing data in the Cancer Genome Atlas database and other large-scale genomic studies found through “cBioPortal” (<http://www.cbioportal.org>), we systematically evaluated the frequency of *KRAS* alteration across tumors (Fig. 2) [13, 14]. The *KRAS* amplification frequency in TGCTs ranged from 8.72% to 20.56%. When TGCTs relapsed after chemotherapy, *KRAS* alterations, mainly amplification, were the most common genomic alterations (47.8% of seminomas and 51.2% of NSGCTs) [15]. *KRAS* amplification was also present in other tumors, but less frequently than in TGCTs. The *KRAS* amplification had higher frequency in TGCTs (19.70%), esophagogastric cancer (10.42%), and ovarian epithelial cancer (9.44%).

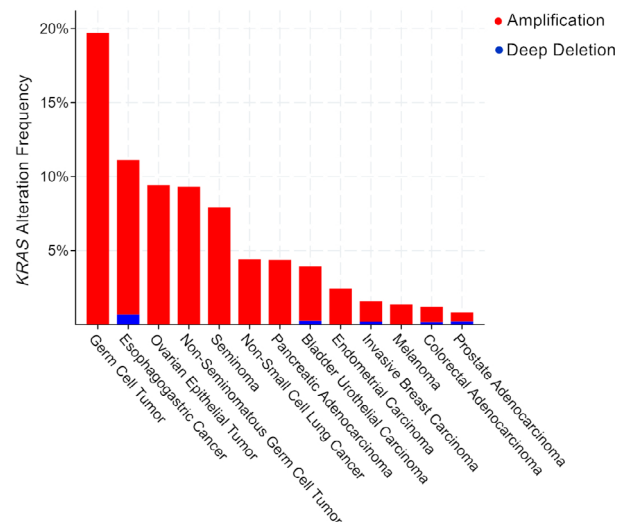


Figure 2. Frequencies of *KRAS* alteration across cancer types.

Molecular Findings and Implications

Owing to the positive response to platinum-based chemotherapy, more than 90% of TGCTs could be cured. However, when TGCTs are refractory to conventional chemotherapy, prognosis can be very poor, especially for patients with brain metastasis, because of the lack of effective treatment methods [4]. Genomic alterations might mediate the development of chemotherapy resistance; hence, genomic profiling might find potentially druggable targets and identify therapeutic opportunities for this patient. After sequencing, *KRAS* amplification was detected in this patient. As *KRAS* amplification may activate the RAS-RAF-MEK-ERK signaling pathway, leading to tumor cell proliferation and progression, we anticipated that therapy with agents targeting the *KRAS* pathway may work. Studies have shown that *KRAS* amplification is associated with chemotherapy resistance and tumor progression in solid tumors. A study that screened 1,039 colorectal cancer samples found that *KRAS* amplification frequency is only 0.67% in colorectal cancer but that it might be responsible for primary resistance to anti-EGFR therapy [16]. As for endometrial cancer, *KRAS* amplification is present in 3% and 18% of primary and metastatic tumors, respectively, and is significantly correlated with poor outcome [17]. Because disrupting the nucleotide-binding domain of *KRAS* is difficult, targeting *KRAS* directly has always failed; thus, targeting other effectors of the MAPK or PI3K pathways is another treatment option. Although clinical evidence for therapies to treat TGCTs with *KRAS* amplification is lacking, targeted drugs effective in treating other tumors with *KRAS* amplification might apply to TGCTs. Sorafenib, which is approved by the U.S. Food and Drug Administration for the treatment of hepatocellular carcinoma, renal cell carcinoma, and thyroid cancer, is a multikinase inhibitor, including RAF kinases, the tyrosine kinases, and VEGFR-2, which can inhibit the MAPK signaling pathway and target angiogenesis [18–21]. A phase III clinical trial revealed that the combination of carboplatin, paclitaxel, and sorafenib (CPS) can improve overall survival (OS) in patients with melanoma with *KRAS* gene copy gains, and the CPS regimen

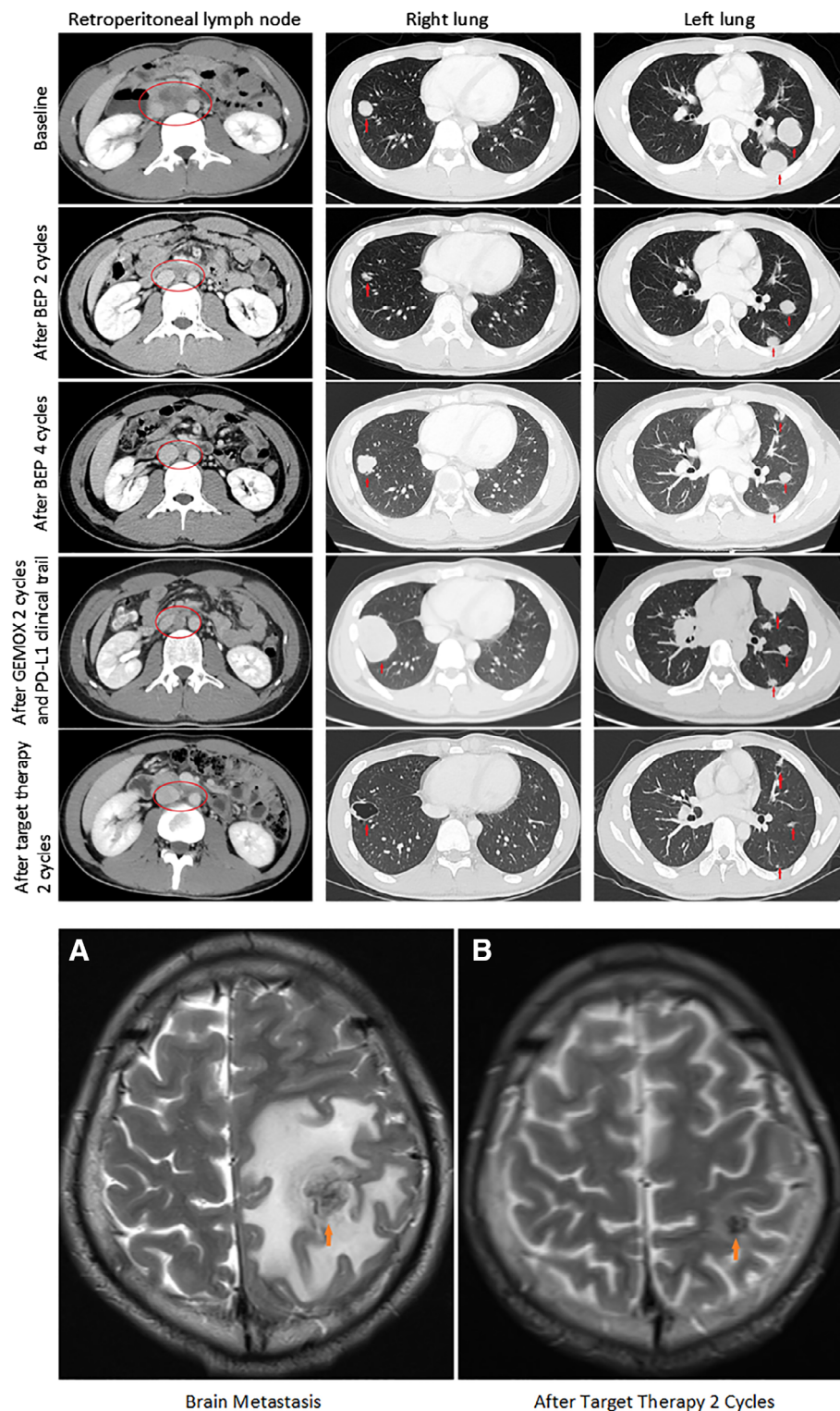


Figure 3. Imaging changes during treatment. **(A):** The thoracic region and lung imaging changes. The treatment time points are shown on the left. The cycles indicate the retroperitoneal lymph node metastasis, the arrows indicate metastatic lesions in the lung. **(B):** Brain magnetic resonance imaging before and after target treatment of metastatic lesions in the left frontal lobe. Abbreviations: BEP, bleomycin, etoposide, and paclitaxel; GEMOX, gemcitabine plus oxaliplatin; PD-L1, programmed cell death ligand 1.

had a better OS than just carboplatin and paclitaxel therapy (hazard ratio, 0.25; $p = .035$) [22, 23]. Another study showed that combined MEK and SHP2 inhibition can enhance the sensitivity of *KRAS* amplification models to MEK inhibition both in vivo and in vitro [24]. Some clinical

trials investigating RAF inhibitors, as single agents or in combination with MEK inhibitors or alternative pathway inhibitors, in treating solid cancers with *KRAS* amplification are under way (www.clinicaltrials.gov). Based on these findings, the board recommended the use of the CPS regimen.

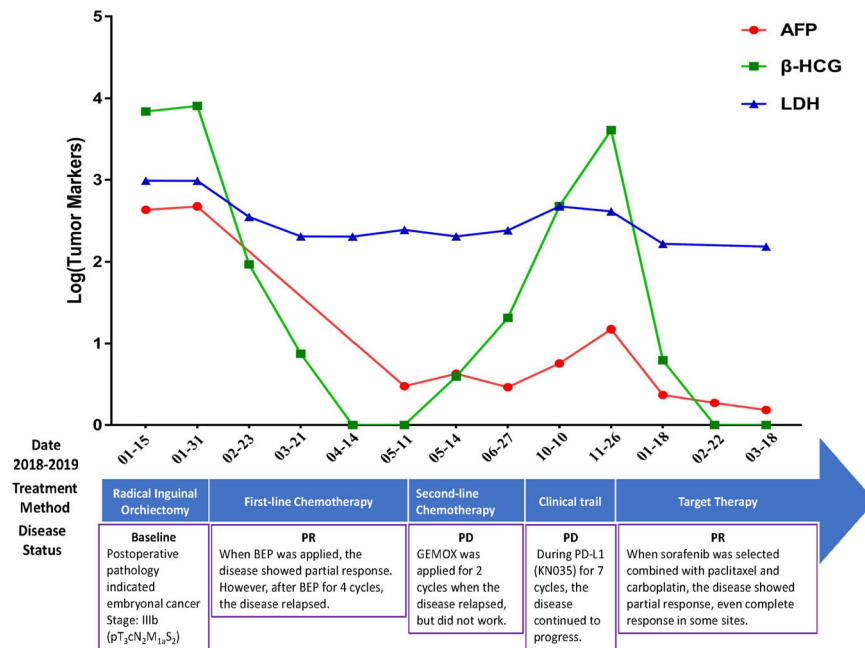


Figure 4. The patient's detailed treatment process, the condition change, and the tumor marker change. Abbreviations: BEP, bleomycin, etoposide, and paraplatin; GEMOX, gemcitabine plus oxaliplatin; PD, progressive disease; PD-L1, programmed cell death ligand 1; PR, partial response.

PATIENT UPDATE

After two cycles of CPS targeted therapy, the tumors in the thoracic region and right lung achieved a complete response and even formed a cavity in the right lung, and the tumors in the left lung achieved a partial response (Fig. 3A). The brain metastasis also achieved a complete response, although radiotherapy may help (Fig. 3B). The patient's tumor markers returned to normal again. The systematic treatment of the disease and tumor biomarker changes are shown in Figure 4.

However, there are still some residual tumor lesions in the left lung that have not been completely eliminated. This may be due to tumor heterogeneity. Because the gene sequencing samples were taken from the right lung, tumors in the left lung did not achieve the same therapeutic effect with CPS as those in the right lung. A puncture biopsy of the left lung tumor nodules may be performed in the future if necessary.

CONCLUSION

Although nearly 90% of TGCTs can be cured by cisplatin-based chemotherapy, there are still nearly 10% of patients who will be refractory to chemotherapy. However, there is still a lack of effective therapies in treating chemotherapy-refractory TGCTs. Meanwhile, TGCTs is one of the few tumors that has not yet benefited from targeted therapy. The *KRAS* amplification occurred in nearly 50% of chemotherapy-refractory TGCTs, which may provide a therapeutic target. As for this patient, a CPS regimen that can inhibit cell proliferation, target angiogenesis, and inhibit the MAPK signaling pathway activated by *KRAS* amplification has achieved excellent curative effects. Because of the high frequency of *KRAS* amplification in chemotherapy-refractory NSGCTs, CPS regimen may provide a new therapy. Regarding *KRAS* mutation and subsequent

activation of MAPK signaling pathway, *KRAS* point mutations affecting 12th, 13th, and 61st amino acids are well known. Given that *KRAS* and *NRAS* mutations are reported in 45% of seminomas [25], those patients may benefit from CPS regimen as well, although this warrants further validation in clinical studies.

GLOSSARY OF GENOMIC TERMS AND NOMENCLATURE

KRAS: Kirsten rat sarcoma viral oncogene
CCND2: Cyclin D2
GDF-3: Growth differentiation factor 3
EGFR: Epidermal growth factor receptor
VGFR2: Vascular endothelial growth factor receptor 2

AUTHOR CONTRIBUTIONS

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Data analysis and interpretation: Bijun Lian, Wenhui Zhang
Manuscript writing: Bijun Lian, Wenhui Zhang
Final approval of manuscript: Chengwei Shao, Zhiyong Liu, Jing Li, Xu Gao, Yinghao Sun

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DISCLOSURES

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