ABSTRACT

Objectives To evaluate the prognostic relevance of four functional single nucleotide polymorphisms (SNPs) in CD133 (rs2240688A>C, rs10022537T>A, rs7686732C>G, and rs3130C>T) on overall survival (OS) of non-small cell lung cancer (NSCLC) patients.

Design Retrospective cohort study.

Setting Department of General Surgery, in a general hospital, Henan Province, China.

Participants NSCLC patients aged ≥18 years, who were not receiving preoperative neoadjuvant therapies and had a blood sample available for genotyping, were eligible for inclusion. Those participants who were pregnant or breastfeeding, had a previous history of cancer, had other primary tumours, or who had had primary tumours of the skin and nasopharynx, were excluded from the study.

Outcome measures The primary endpoint was OS, which was calculated from the date of enrolment until the date of death or date of last follow-up.

Results There was a total of 1383 participants, with a median age of 63 years; 726 (52.5%) were male. Compared with the rs2240688 AA genotype, the variant AC/CC genotypes were independently associated with OS (HR 1.27, 95% CI 1.12 to 1.45 for AC genotype; HR 2.32, 95% CI 1.91 to 2.80 for CC genotype). Higher hazard ratios for associations between CD133 rs2240688 polymorphism and OS were observed in patients with adjuvant chemotherapy and radiotherapy for curative intent (HR 1.90, 95% CI 1.52 to 2.26).

Conclusions The study confirmed the significant association between the SNP rs2240688 A>C of CD133 and OS of NSCLC patients. Larger population-based studies in different ethnic groups are necessary to further validate the role and mechanisms of CD133 in NSCLC.

INTRODUCTION

Lung cancer remains the most common cause of cancer-related deaths in China and worldwide.1 The number of patients newly diagnosed with lung cancer was estimated at 1.8 million worldwide in 2012 and 500 000 in China. The number is expected to reach one million in China by 2025.2 Approximately 83% of lung cancer patients have non-small cell lung cancer (NSCLC).3 In addition, despite improvements in technologies and development of multiple treatments including surgery, radiotherapy, chemotherapy and utilisation of other biological agents, the prognosis of NSCLC is very poor due to recurrence and metastasis, with an overall 5-year survival rate <16%.4–6 Hence, it is necessary to identify biomarkers for the prevention, early diagnosis, monitoring of progression and therapeutic effects of NSCLC.

It is widely conceived that cancer stem cells (CSCs) are able to be self-renew and to produce heterogeneous lineages of cancer cells.7–10 CSCs have been hypothesised as the origin of cancer due to their potent tumour-driving capability on tumour initiation, growth, metastasis and relapse.9–10 The cell surface marker CD133, which is located in cellular protrusions, is related to tumorigenesis and cancer progression.11 The CD133 antigen, also known as prominin-1, has been used extensively as a biomarker of CSCs among different types of tumours, including colon cancer,12 liver cancer,13 gastric cancer,14
The expression of CD133 is significantly correlated with the development and prognosis of NSCLC. As single nucleotide polymorphisms (SNPs) are considered to harbour information about genetic variation in functionality of the genome and susceptibility to tumour development, we hypothesised that potential functional SNPs in CD133 may influence the function of CD133 and, consequentially, prognosis. A recent case–control study conducted by our team in a Chinese population showed that the rs2240688 variant AC/CC genotypes were associated with a statistically increased risk of lung cancer. However, another study found that rs2240688C variant genotypes were negatively associated with lung cancer and have a protective effect on overall survival (OS). Considering the limited number of studies involving CD133 genetic variants with NSCLC and their inconsistent results, we investigated the associations between four functional SNPs in CD133 and the prognosis of NSCLC in a Chinese population.

**METHODS**

**Study populations**

The sample in this retrospective cohort study included 1383 patients with histologically confirmed NSCLC, who were treated at the Department of General Surgery, Henan Provincial People’s Hospital between January 2006 and December 2014. All participants with NSCLC in a case–control study which identified the relationship between polymorphisms of CD133 and the risk of lung cancer were included in the previous retrospective cohort study.

Eligibility for inclusion were the following criteria: (1) willing to participate in the study and sign an informed consent form; (2) aged ≥18 years with pathologically confirmed NSCLC; (3) had not received preoperative neoadjuvant therapies (including chemotherapy and radiotherapy); and (4) had a blood sample available for genotyping four selected SNPs in CD133. Those participants who were pregnant or breastfeeding, had previous cancer history, had other primary tumours, or had primary tumours of the skin and nasopharynx, were excluded from this study. A personal identification number was assigned to every study subject at enrolment and specified on each case report form in order to maintain confidentiality.

The present study was performed in accordance with the Declaration of Helsinki, and the protocol and informed consent form have been reviewed and approved by the Institutional Review Boards of Henan Provincial People’s Hospital. Written informed consent was provided by all participants.

**Initial screening, assessment and follow-up**

In this retrospective cohort study, blood samples and medical data were obtained from the biobank for lung cancer patients at the Department of General Surgery, Henan Provincial People’s Hospital. This biobank consists of an electronic database of demographic and clinicopathological data (including age, sex, smoking status, histological tumour type, tumour-node-metastasis stage, chemotherapy or radiotherapy treatment), as well as blood samples. At the time of inclusion, written informed consent was obtained, and each participant was interviewed to obtain a detailed medical history. Blood samples were separated by centrifugation within 2 hours of collection. Serum samples were stored in aliquots below −70°C until analysis.

Details on surgical treatment were recorded including dates, types of surgeries, and complications. From the electronic database, we identified patients treated with adjuvant chemotherapy and we classified patients as being treated with adjuvant chemotherapy if the patient received platinum-based chemotherapy within 3 months of surgery. We classified patients as having received radiotherapy if they received external beam radiation, radioactive implants, radioisotopes, brachytherapy or other types of radiotherapy within 6 months of cancer diagnosis. We classified patients as having received curative intent radiotherapy if the patients with early disease (stages I and II) were treated with surgical resection in addition to radiotherapy. We classified patients as having received palliative radiotherapy if the patients with advanced or distant disease (stages III and IV) were treated with radiotherapy or in combination with other treatments for supportive care. Family history of cancer was defined as any types of cancer present in first degree relatives of the participants (parents, siblings and children).

OS was evaluated for all the patients with regular follow-up at 3-month intervals for the first 2 years after surgery, at 6-month intervals for years 3–5, and yearly thereafter according to the hospital guidelines. The patients who failed to attend follow-up visits were telephoned or their family members were contacted. Follow-up of patients for the present study was performed until July 2016.

**SNP selection and genotyping**

Potential SNPs should be common (≥5% minor allele frequency) in Chinese populations. Candidate CD133 gene SNPs with potentially functional significance (that is, located in the promoter, the transcription factor-binding site, exon and 3′-untranslated region (UTR), or the coding regions with amino acid changes) were selected based on NCBI dbSNPs (http://www.ncbi.nlm.nih.gov) and SNPInfo Web Server (http://www.snpinfo.niehs.nih.gov/snpfunc.htm). As a result, four CD133 candidate SNPs (rs2240688 A>C, rs10022537 T>A, rs7686732 C>G, rs3130 C>T) were identified and chosen in our model analysis. Three SNPs (rs2240688, rs7686732, rs3130) were located in the 3′-UTR of CD133, and rs10022537 was located within the intron of the CD133 gene.
Table 1 Demographic and clinicopathological characteristics of non-small cell lung cancer patients recruited from Henan Provincial People's Hospital between January 2006 and December 2014

| Characteristics | Lung adenocarcinoma (n=793) | Lung squamous cell cancer (n=331) | Others (n=259) | Total (n=1383) |
|-----------------|-----------------------------|-----------------------------------|----------------|----------------|
| **Age (years)** |                             |                                   |                |                |
| <65             | 476 (60.0)                  | 151 (45.6)                        | 154 (59.5)     | 781 (56.5)     |
| ≥65             | 317 (40.0)                  | 180 (54.4)                        | 105 (40.5)     | 602 (43.5)     |
| **Sex**         |                             |                                   |                |                |
| Male            | 372 (46.9)                  | 206 (62.2)                        | 148 (57.1)     | 726 (52.5)     |
| Female          | 421 (53.1)                  | 125 (37.8)                        | 111 (42.9)     | 657 (47.5)     |
| **Smoking status** |                           |                                   |                |                |
| Non-smoker      | 182 (23.0)                  | 24 (7.3)                          | 33 (12.7)      | 239 (17.3)     |
| Former smoker   | 322 (40.6)                  | 165 (49.8)                        | 100 (38.6)     | 587 (42.4)     |
| Current smoker  | 289 (36.4)                  | 142 (42.9)                        | 126 (48.6)     | 557 (40.3)     |
| **Pack-years**  |                             |                                   |                |                |
| ≤25             | 158 (25.9)                  | 23 (7.5)                          | 28 (12.4)      | 209 (15.1)     |
| 26–50           | 233 (38.1)                  | 121 (39.4)                        | 96 (42.5)      | 450 (32.5)     |
| >50             | 220 (36.0)                  | 163 (53.1)                        | 102 (45.1)     | 485 (35.1)     |
| **Stage**       |                             |                                   |                |                |
| I-II            | 234 (29.5)                  | 110 (33.2)                        | 50 (19.3)      | 394 (28.5)     |
| III             | 271 (34.2)                  | 155 (46.8)                        | 102 (39.4)     | 528 (38.2)     |
| IV              | 288 (36.3)                  | 66 (19.9)                         | 107 (41.3)     | 461 (33.3)     |
| **Family history** |                           |                                   |                |                |
| Yes             | 121 (15.3)                  | 45 (13.6)                         | 39 (15.1)      | 205 (14.8)     |
| No              | 672 (84.7)                  | 286 (86.4)                        | 220 (84.9)     | 1178 (85.2)    |
| **Surgery**     |                             |                                   |                |                |
| No              | 274 (34.5)                  | 101 (30.5)                        | 166 (64.1)     | 541 (39.1)     |
| Lobectomy       | 271 (34.2)                  | 67 (20.2)                         | 79 (30.5)      | 417 (30.2)     |
| Segmentectomy   | 130 (16.4)                  | 62 (18.7)                         | 3 (1.2)        | 195 (14.1)     |
| Wedge resection | 118 (14.9)                  | 101 (30.5)                        | 11 (4.3)       | 230 (16.6)     |
| **History of radiotherapy** |             |                                   |                |                |
| No              | 263 (33.2)                  | 100 (30.2)                        | 143 (55.2)     | 506 (36.6)     |
| Palliative therapy | 116 (14.6)                  | 112 (33.8)                        | 27 (10.4)      | 255 (18.4)     |
| Curative intent | 414 (52.2)                  | 119 (36.0)                        | 89 (34.4)      | 622 (45)       |
| Adjuvant chemotherapy |                   |                                   |                |                |
| No              | 444 (56.0)                  | 177 (53.5)                        | 155 (59.9)     | 776 (56.1)     |
| Yes             | 349 (44.0)                  | 154 (46.5)                        | 104 (40.1)     | 607 (43.9)     |

Genomic DNA was extracted from the buffy coat fraction of each blood sample with a DNA blood Mini Kit (Qiagen Inc, Valencia, California, USA) according to the manufacturer’s instructions. The genotyping methods of the four CD133 SNPs are described in detail elsewhere.19

**Statistical analysis**

We expect 3-year survival rates of 35% in patients with variant genotypes of rs2240688 (AC/CC) and 27% in patients with rs2240688 AA genotype. Based on a difference of 15% between groups on the primary outcome, assuming a 10% drop-out rate, a total of 1234 participants (at 1:1 ratio, 617 subjects in each group) are required to provide 80% power, with the use of a two-sided significance level of 0.05.

All statistical tests were performed using SAS 9.3 software (Cary, North Carolina, USA). Descriptive analysis results were presented as median and interquartile range (IQR) for continuous variables and frequencies (percentage) for categorical variables. Distributions of
## Table 2  Associations between CD133 genotypes and overall survival among non-small cell lung cancer patients recruited from Henan Provincial People’s Hospital between January 2006 and December 2014

| Genotypes | No. of patients N (%) | No. of deaths N (%) | MST (months) | HR (95% CI) | Adjusted HR (95% CI)* |
|-----------|-----------------------|---------------------|--------------|-------------|------------------------|
| **rs2240688** | | | | | |
| AA        | 652                  | 463 (71.0)          | 20.3         | 1.0         | 1.0                    |
| AC        | 555                  | 434 (78.2)          | 15.6         | 1.29 (1.13 to 1.47) | 1.27 (1.12 to 1.45) |
| CC        | 172                  | 143 (83.1)          | 8.2          | 2.22 (1.84 to 2.68) | 2.32 (1.91 to 2.80) |
| **Recessive** | | | | | |
| AA/AC     | 1207                 | 897 (74.3)          | 18.1         | 1.0         | 1.0                    |
| CC        | 172                  | 143 (83.1)          | 8.2          | 1.98 (1.66 to 2.36) | 2.07 (1.73 to 2.48) |
| **Dominant** | | | | | |
| AA        | 652                  | 463 (71.0)          | 20.3         | 1.0         | 1.0                    |
| AC/CC     | 727                  | 577 (79.4)          | 13.0         | 1.43 (1.27 to 1.62) | 1.43 (1.26 to 1.61) |
| **rs10022537** | | | | | |
| TT        | 913                  | 689 (75.5)          | 17.2         | 1.0         | 1.0                    |
| TA        | 413                  | 311 (75.3)          | 14.5         | 1.06 (0.93 to 1.22) | 1.10 (0.96 to 1.27) |
| AA        | 39                   | 30 (76.9)           | 15.2         | 1.14 (0.79 to 1.64) | 1.00 (0.69 to 1.44) |
| **Recessive** | | | | | |
| TT/TA     | 1326                 | 1000 (75.4)         | 16.8         | 1.0         | 1.0                    |
| AA        | 39                   | 30 (76.9)           | 15.2         | 1.12 (0.78 to 1.61) | 0.96 (0.67 to 1.39) |
| **Dominant** | | | | | |
| TT        | 913                  | 689 (75.5)          | 17.2         | 1.0         | 1.0                    |
| TA/AA     | 452                  | 341 (75.4)          | 14.5         | 1.07 (0.94 to 1.22) | 1.13 (0.99 to 1.29) |
| **rs7686732†** | | | | | |
| CC        | 398                  | 286 (71.9)          | 17.1         | 1.0         | 1.0                    |
| CG        | 88                   | 63 (71.6)           | 15.9         | 1.06 (0.80 to 1.39) | 1.12 (0.85 to 1.49) |
| GG        | 5                    | 4 (80.0)            | 20.1         | 1.33 (0.49 to 3.56) | 1.26 (0.47 to 3.41) |
| **Dominant** | | | | | |
| CC/CG     | 486                  | 349 (71.8)          | 17.0         | 1.0         | 1.0                    |
| GG        | 5                    | 4 (80.0)            | 20.1         | 1.31 (0.49 to 3.52) | 1.24 (0.46 to 3.34) |
| **Recessive** | | | | | |
| CC        | 398                  | 286 (71.9)          | 17.1         | 1.0         | 1.0                    |
| CG/GG     | 93                   | 67 (72.0)           | 15.9         | 1.07 (0.82 to 1.39) | 1.13 (0.86 to 1.49) |
| **rs3130†** | | | | | |
| CC        | 134                  | 92 (68.7)           | 18.0         | 1.0         | 1.0                    |
| CT        | 269                  | 201 (74.7)          | 17.0         | 1.13 (0.88 to 1.44) | 1.14 (0.89 to 1.47) |
| TT        | 92                   | 64 (69.6)           | 14.4         | 0.98 (0.72 to 1.36) | 0.97 (0.70 to 1.34) |
| **Dominant** | | | | | |
| CC/CT     | 403                  | 293 (72.7)          | 17.3         | 1.0         | 1.0                    |
| TT        | 92                   | 64 (69.6)           | 14.4         | 0.91 (0.69 to 1.19) | 0.89 (0.68 to 1.18) |
| **Recessive** | | | | | |
| CC        | 134                  | 92 (68.7)           | 18.0         | 1.0         | 1.0                    |
| CT/TT     | 361                  | 265 (73.4)          | 16.8         | 1.09 (0.86 to 1.38) | 1.09 (0.86 to 1.39) |

*Adjusted for age, sex, smoking status, histopathology type, stage, chemotherapy and radiotherapy.

†Genotyping of the two SNPs rs7686732 and rs3130 was only carried out for a portion of the participants.

MST, median survival time; SNPs, single nucleotide polymorphisms.
categorical variables, including demographic variables, prognostic factors and clinicopathological characteristics, were compared using the \( \chi^2 \) test/Fisher’s exact test as appropriate. The primary endpoint was OS, which was calculated from the date of enrolment until the date of death or date of last follow-up. Survival curves of OS were estimated by the Kaplan-Meier method and compared by the log-rank test. The associations of \( CD133 \) SNPs with OS were estimated by calculating hazard ratios (HR) and corresponding 95% confidence intervals (CI) from both univariate and multivariate Cox proportional hazards regression models, followed by stratification analysis by age, sex, smoking status, histopathology type, stage, family history, and application of chemotherapy and radiotherapy. In addition, the associations of \( CD133 \) SNPs with OS were analysed under specific genetic models: genotypic, recessive and dominant models, but only the dominant model was used in the stratification analysis of \( CD133 \) rs2240688 polymorphism as rs2240688 (AC/CC) are variant genotypes. All these analyses were performed with or without adjustment for demographic variables and selected clinicopathological characteristics. All tests were two-sided and a value of \( p < 0.05 \) was considered to be statistically significant for all analyses.

RESULTS

Baseline characteristics of the study population

There were a total of 1383 participants with histologically confirmed NSCLC included in this retrospective cohort, including 793 (57.3%) lung adenocarcinomas, 331 (23.9%) lung squamous cell cancers, and 259 (18.7%) other types of NSCLC. Table 1 summarises the baseline characteristics of the study population by histopathology type. There were 726 (52.5%) males and 657 (47.5%) females, with ages ranging from 28 to 92 years (median 63 years; IQR 54–70 years). There were 394 (28.5%), 528 (38.2%) and 461 (33.3%) participants with stage I–II, III and IV NSCLC, respectively. There were a total of 842 (60.9%) participants who received surgical treatment, including 417 (30.2%) with lobectomy, 195 (14.1%) with segmentectomy and 230 (16.6%) with wedge resection. There were 607 (43.9%) participants who underwent adjuvant chemotherapy and 877 (63.4%) who underwent radiotherapy, including 622 (45.0%) for curative intent and 255 (18.4%) for palliative therapy.

Association of \( CD133 \) genotypes with OS

The enrolled NSCLC patients who returned for at least one follow-up visit had been followed for a median of 14.4 months (IQR 24.4 months). At the end of the study, 339 (24.5%) patients were alive and 1044 (75.5%) patients had died of any cause during follow-up.

The genotype distributions of the selected four SNPs in \( CD133 \) and their associations with OS of NSCLC patients are shown in Table 2. In all patients, variant genotypes of rs2240688 (AC/CC) were statistically significantly associated with OS (log-rank \( p < 0.001 \) under a recessive model). Compared with rs2240688 AA genotype, the variant AC/CC genotypes were associated with a statistically poorer OS of NSCLC (HR 1.29, 95% CI 1.13 to 1.47 for AC genotype; HR 2.22, 95% CI 1.84 to 2.68 for CC genotype). As shown in multivariate survival analysis using Cox proportional hazards regression, rs2240688 variant genotypes remained significantly associated with OS (HR 1.91, 95% CI 1.12 to 1.56 and HR 2.22, 95% CI 1.84 to 2.68 for CC genotype) after adjustment for age, sex, smoking status, histopathology type, stage, chemotherapy and radiotherapy. However, the association between genotype distribution of the other three SNPs (rs10022537, rs7686732, rs3130) and OS of NSCLC patients was not observed.

Figure 1 shows the Kaplan-Meier curves for the OS among all NSCLC patients stratified by rs2240688 genotypes. The median OS was 20.3 months (95% CI 18.7 to 22.7) for participants with rs2240688 AA genotype, 15.6 months (95% CI 13.0 to 17.1) with rs2240688 AC genotype, and 8.2 months (95% CI 7.1 to 9.9) with rs2240688 CC genotype.

\( CD133 \) genotypes and OS of NSCLC by clinicopathological characteristics

Stratified analysis was further performed for rs2240688 A>C by age, sex, smoking status, histopathology type, stage, family history of cancer, and application of chemotherapy and radiotherapy (Table 3). Compared with the rs2240688 AA genotype, the association between OS and rs2240688 AC/CC variant genotypes also remained statistically significant in the subgroup of all ages, all sexes, former smoker, current smoker, lung adenocarcinoma,
### Table 3  Stratified analysis for associations between CD133 rs2240688 polymorphism (dominant for the C allele) and overall survival among non-small cell lung cancer patients recruited from Henan Provincial People’s Hospital between January 2006 and December 2014

| Variables                  | rs2240688 (death/patients) | MST (Months) | HR (95% CI) | Adjusted HR (95% CI)* |
|----------------------------|-----------------------------|--------------|-------------|-----------------------|
|                            | AA                          | AC/CC        |             |                       |
| Age (years)                |                             |              |             |                       |
| <65                        | 254/367                     | 324/411      | 20.1 vs 14.1| 1.36 (1.16 to 1.61)   | 1.44 (1.22 to 1.70) |
| ≥65                        | 209/285                     | 253/316      | 21.2 vs 11.9| 1.54 (1.28 to 1.85)   | 1.46 (1.21 to 1.76) |
| Sex                        |                             |              |             |                       |
| Male                       | 249/334                     | 326/390      | 18.1 vs 11.8| 1.53 (1.30 to 1.80)   | 1.45 (1.23 to 1.71) |
| Female                     | 214/318                     | 251/337      | 23.0 vs 16.8| 1.32 (1.10 to 1.59)   | 1.45 (1.21 to 1.74) |
| Smoking status             |                             |              |             |                       |
| Non-smoker                 | 82/116                      | 89/120       | 18.3 vs 16.8| 1.19 (0.88 to 1.60)   | 1.28 (0.94 to 1.74) |
| Former smoker              | 188/278                     | 241/309      | 26.0 vs 13.4| 1.54 (1.27 to 1.86)   | 1.52 (1.25 to 1.84) |
| Current smoker             | 193/258                     | 247/298      | 19.0 vs 11.6| 1.43 (1.18 to 1.72)   | 1.44 (1.19 to 1.74) |
| Histopathology type        |                             |              |             |                       |
| Lung adenocarcinoma        | 253/377                     | 313/414      | 23.0 vs 16.0| 1.40 (1.19 to 1.66)   | 1.44 (1.22 to 1.70) |
| Lung squamous cell cancer  | 118/161                     | 132/170      | 21.0 vs 12.0| 1.40 (1.09 to 1.79)   | 1.32 (1.03 to 1.71) |
| Others                     | 92/114                      | 132/143      | 14.6 vs 9.0 | 1.54 (1.18 to 2.01)   | 1.51 (1.14 to 2.00) |
| Stage                      |                             |              |             |                       |
| I-II                       | 78/169                      | 140/224      | 53.9 vs 18.0| 2.09 (1.58 to 2.77)   | 2.28 (1.72 to 3.03) |
| III                        | 217/272                     | 219/256      | 19.0 vs 15.5| 1.15 (0.95 to 1.39)   | 1.17 (0.97 to 1.42) |
| IV                         | 168/211                     | 218/247      | 13.4 vs 11.2| 1.42 (1.16 to 1.73)   | 1.43 (1.16 to 1.75) |
| Family history             |                             |              |             |                       |
| Yes                        | 71/102                      | 80/103       | 22.2 vs 11.6| 1.52 (1.10 to 2.09)   | 1.56 (1.12 to 2.17) |
| No                         | 392/550                     | 497/624      | 20.0 vs 13.2| 1.42 (1.24 to 1.62)   | 1.41 (1.23 to 1.61) |
| Surgery                    |                             |              |             |                       |
| No                         | 191/232                     | 274/306      | 12.3 vs 9.6 | 1.36 (1.13 to 1.64)   | 1.38 (1.15 to 1.66) |
| Lobectomy                  | 157/210                     | 179/206      | 26.0 vs 17.0| 1.38 (1.11 to 1.71)   | 1.40 (1.12 to 1.74) |
| Segmentectomy              | 61/95                       | 61/100       | 25.0 vs 21.6| 1.14 (0.80 to 1.62)   | 1.27 (0.87 to 1.84) |
| Wedge resection            | 54/115                      | 63/115       | 48.9 vs 23.4| 1.72 (1.19 to 2.48)   | 1.75 (1.20 to 2.54) |
| History of radiotherapy    |                             |              |             |                       |
| No                         | 196/237                     | 230/266      | 15.0 vs 12.0| 1.17 (0.97 to 1.42)   | 1.24 (1.02 to 1.51) |
| Palliative therapy         | 103/125                     | 113/130      | 19.0 vs 16.8| 1.19 (0.91 to 1.56)   | 1.16 (0.88 to 1.53) |
| Curative intent            | 164/290                     | 234/331      | 31.2 vs 12.8| 1.90 (1.55 to 2.32)   | 1.90 (1.55 to 2.33) |
| Adjuvant chemotherapy      |                             |              |             |                       |
| No                         | 294/384                     | 326/391      | 19.3 vs 16.2| 1.23 (1.05 to 1.44)   | 1.22 (1.04 to 1.43) |
| Yes                        | 169/268                     | 251/336      | 22.7 vs 9.9 | 1.78 (1.46 to 2.16)   | 1.86 (1.52 to 2.26) |

*Adjusted for age, sex, smoking status, histopathology type, stage, chemotherapy and radiotherapy. MST, median survival time.

Higher HRs for associations between CD133 rs2240688 polymorphism and OS were also observed in patients with adjuvant chemotherapy (HR 1.86, 95% CI 1.52 to 2.26) and radiotherapy for curative intent (HR 1.90, 95% CI 1.55 to 2.33), compared with patients without adjuvant chemotherapy (HR 1.22, 95% CI 1.04 to 1.43) and those without radiotherapy (HR 1.24, 95% CI 1.02 to 1.51). Higher HRs for associations between CD133 rs2240688 polymorphism and OS were also observed in patients with stage I-II and wedge resection surgery (table 3).

**DISCUSSION**

It is generally accepted that SNPs represent genetic variation in functionality of the genome and they are potential functional biomarkers for cancer aetiology. CSCs are responsible for tumour initiation, growth, migration, aggressiveness, metastasis, drug resistance and pluripotency. In this study, information was collected to determine...
the role of CSCs in the clinical outcomes of NSCLC. We focused on the CD133 gene that has been used to isolate CSCs. Four potential functional SNPs in the CD133 gene locus were selected from SNP websites and peer-reviewed literature by using the candidate gene approach.

The prognostic and clinicopathological values of CD133 protein and mRNA expression have been indicated in other studies. In this hospital-based cohort study, we found that the variant genotypes (AC/CC) of rs2240688 A>C in the miRNA binding site of the stem cell marker gene CD133 was associated with a significantly poorer prognosis for NSCLC patients. The association remained statistically significant (HR 1.27, 95% CI 1.12 to 1.45 for AC genotype; HR 2.32, 95% CI 1.91 to 2.80 for CC genotype) after adjustment for age, sex, smoking status, histopathology type, stage, chemotherapy and radiotherapy. Additionally in the stratified analysis, the poorer prognosis associated with rs2240688 A>C variant genotypes did remain statistically significant in most subgroups. It was validated that rs2240688 A-to-C transition gained a new binding site of the microRNA has-miR-135a/b, which may play a pivotal role in modulating the effect of the SNP on CD133 expression. The rs2240688 A>C variant genotypes are located in the 3'-UTR of CD133. SNPs in the 3'-UTR have been shown to have functional effects on the control of mRNA stability and efficiency through the regulation of miRNA, including miR-34a, −101,−128, −137 and −138. It is inferred that SNPs in a target-binding site could alter the miRNA–mRNA interaction and thus affect the expression of miRNA targets. Considering the tumour-driving capability of CSCs on tumour growth and metastasis, the present study suggests that CD133 might modify their metastasis competence of NSCLC by the miRNA binding site polymorphisms, which could be a putative target for improved therapies for treatment. Our subgroup analysis results showed that rs2240688 A>C variant genotypes had more effects on the prognosis of NSCLC among patients receiving adjuvant chemotherapy or radiotherapy. This may be due to its association with resistance to chemotherapy and radiotherapy. Higher HRs for associations between CD133 rs2240688 polymorphism and OS were also observed in patients with stage I-II and wedge resection surgery. It may due to the effect of the rs2240688 A>C variant genotypes on tumour growth and metastasis which would result in greater impact among early stage patients.

However, the prognostic value of CSCs marker CD133 in NSCLC remains controversial. Another study in China found that the rs2240688 variant genotypes were associated with a favourable survival. Several studies found no significant association between the expression level of CD133 and OS of NSCLC patients. The inconsistent results may be explained in part by the different ethnic population, in addition to the different sampling methods used to select the populations under study. A meta-analysis showed that NSCLC patients with higher CD133 expression had poor OS only in Asian patients, but not in Caucasian patients. Therefore, high quality and interethnic studies with large samples should be undertaken to confirm the prognostic and clinical value of CD133.

This study had several limitations that should be taken into account. First, selection bias cannot be excluded even though inclusion/exclusion criteria were determined to minimise the bias. Potential confounding factors, such as clinicopathological characteristics, may be associated with SNPs in the CD133 gene and also exert an effect on the overall mortality in our cohort of NSCLC patients. However, the independent association between SNPs in the CD133 gene and OS of NSCLC patients was determined by using multivariate Cox proportional hazards regression models. Moreover, the HRs were largely very similar in all subgroups and similar to the overall HR, which implied no confounding by these factors. Second, in the hospital-based cohort study, all participants were recruited from a single hospital in Henan Province. Therefore, our study setting may limit the generalisability of our results. Finally, our study made many statistical comparisons, which might increase type I error.

CONCLUSIONS
This study confirmed a significant association between the SNP rs2240688 A>C of CD133 and OS for NSCLC patients. Larger population-based studies in different ethnic groups are necessary to further validate the role and mechanisms of CD133 in NSCLC.

Contributors Q-FL, Z-FZ and YH conceived and designed the experiments. G-JH and G-YY performed the experiments. Q-FL and G-YY analysed the data. Q-FL, Z-FZ and YH contributed to the writing of the manuscript. All authors contributed to and have approved the final manuscript.

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Patient consent Obtained.

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