EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Aurora A Functional Single Nucleotide Polymorphism (SNP) Correlates With Clinical Outcome in Patients With Advanced Solid Tumors Treated With Alisertib, an Investigational Aurora A Kinase Inhibitor

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ARTICLE INFO

Article history:
Received 13 September 2017
Received in revised form 12 October 2017
Accepted 13 October 2017
Available online xxxx

Keywords:
Aurora A kinase inhibitor
Alisertib
SNP
Prognosis
Predictive biomarker
Correlative analysis

ABSTRACT

Background: Alisertib (MLN8237) is an investigational, oral, selective Aurora A kinase inhibitor. *Aurora A* contains two functional single nucleotide polymorphisms (SNPs; codon 31 [F/I] and codon 57 [V/I]) that lead to functional changes. This study investigated the prognostic and predictive significance of these SNPs.
Methods: This study evaluated associations between *Aurora A* SNPs and overall survival (OS) in The Cancer Genome Atlas (TCGA) database. The *Aurora A* SNPs were also evaluated as predictive biomarkers for clinical outcomes to alisertib in two phase 2 studies (NCT01045421 and NCT01091428). *Aurora A* SNP genotyping was obtained from 85 patients with advanced solid tumors receiving single-agent alisertib and 122 patients with advanced recurrent ovarian cancer treated with alisertib plus weekly paclitaxel (n = 62) or paclitaxel alone (n = 60). Whole blood was collected prior to treatment and genotypes were analyzed by PCR.
Findings: TCGA data suggested prognostic significance for codon 57 SNP; solid tumor patients with VV and VI alleles had significantly reduced OS versus those with II alleles (HR 1.9 [VI] and 1.8 [VV]; $p < 0.0001$). In NCT01045421, patients carrying the VV alleles at codon 57 (n = 53, 62%) had significantly longer progression-free survival (PFS) than patients carrying IV or II alleles (n = 32, 38%; HR 0.5; $p = 0.0195$). In NCT01091428, patients with the VV alleles at codon 57 who received alisertib plus paclitaxel (n = 47, 39%) had a trend towards improved PFS (7.5 months) vs paclitaxel alone (n = 32, 26%; 3.8 months; HR 0.618; $p = 0.0593$). In the paclitaxel alone arm, patients with the VV alleles had reduced PFS vs modified intent-to-treat (mITT) patients (3.8 vs 5.1 months), consistent with the TCGA study identifying the VV alleles as a poor prognostic biomarker. No significant associations were identified for codon 31 SNP from the same data set.
Interpretation: These findings suggest that *Aurora A* SNP at codon 57 may predict disease outcome and response to alisertib in patients with solid tumors. Further investigation is warranted.

1. Introduction

Aurora A kinase (AAK), a member of the conserved serine/threonine protein kinase family, is a key mitotic regulator with a critical role in

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https://doi.org/10.1016/j.ebiom.2017.10.015

centrosome maturation and separation, spindle assembly, chromosome alignment, and cytokinesis (Barr and Gergely, 2007; Marumoto et al., 2003). Overexpression and/or amplification of AAK has been observed in a variety of cancers (Dar et al., 2008; Hoque et al., 2003; Mazumdar et al., 2009) and tends to be associated with a poor patient outcome (Nikonova et al., 2013). Furthermore, AAK inhibition results in mitotic progression abnormalities leading to cell death (Marumoto et al., 2003; Gorgun et al., 2010; Zhou et al., 2013). As such, AAK represents an attractive target for anti-cancer therapy.

Aurora A is an oncogene located on chromosome 20q13.2, a locus frequently amplified in solid tumors (Bischoff et al., 1998). Two functional single nucleotide polymorphisms (SNPs) (rs2273535 and rs1047972) in *Aurora A* gene, located at codon 31 and codon 57 in the NH_2-terminal region of the Aurora A protein, have been reported to be associated with functional consequences and increased cancer risk (Chen et al., 2007, 2015; Ewart-Toland et al., 2005; Kimura et al., 2005; Miao et al., 2004). The T91A SNP at codon 31 results in a phenylalanine to isoleucine (F31I) change; homozygous T/T at this locus codes for FF, T/A leads to FI, and A/A codes for II. This SNP is associated with increased frequency of aneuploidy in human colon tumors (Ewart-Toland et al., 2005), increased breast cancer risk, especially in Asian patients (Dai et al., 2004), and low penetrance cancer susceptibility for other cancer types including lung and oesophageal cancer (Ewart-Toland et al., 2005). The G169A SNP at codon 57 results in a valine to isoleucine amino acid substitution (V57I); homozygous A/A genotype at this locus codes for II, A/G leads to IV, and G/G codes for VV. Studies indicate the II variant reduces AAK activity (Kimura et al., 2005), and may be a protective factor for risk of developing cancer, especially in Caucasian patients and those with breast cancer (Dai et al., 2004). Studies investigating the association between *Aurora A* SNPs and clinical outcomes are limited. The heterozygous A/T at codon 31 (FI) has been shown to be associated with a significantly higher risk of tumor relapse, shorter disease-free survival, and shorter median survival time in oesophageal cancer patients treated with preoperative chemoradiation (Pan et al., 2012). In particular, in patients treated with cisplatin-based chemoradiation, this genotype at codon 31 was associated with poor response and shorter survival (Pan et al., 2012). The variant I31/V57 haplotype also carried a significant risk for a low rate of complete response and higher recurrence rate (Pan et al., 2012).

The investigational oral agent alisertib (MLN8237) is a selective, small-molecule AAK inhibitor that has demonstrated preclinical activity against a broad range of tumor types (Manfredi et al., 2011; Sehdev et al., 2013). In pilot studies, alisertib demonstrated antitumor activity with manageable toxicity in patients with solid tumors (Cervantes et al., 2012; Melichar et al., 2015) and haematological malignancies (Goldberg et al., 2014; Kelly et al., 2014). Two phase 2 clinical trials investigating single-agent alisertib (NCT01045421) in patients with advanced solid tumors, or alisertib in combination with weekly paclitaxel (NCT01091428) in patients with recurrent ovarian cancer, have recently been completed (Coleman et al. 2014 ESMO Congress) (Melichar et al., 2015). In NCT01045421, an overall response rate (ORR) of 13% was reported in the response-evaluable population, with patients in the breast cancer and small-cell lung cancer (SCLC) cohorts showing response rates of 18% and 21%, respectively (Melichar et al., 2015). In NCT01091428, alisertib plus weekly paclitaxel significantly increased median progression free survival (PFS) compared with paclitaxel alone (hazard ratio [HR] 0.740 [80% CI 0.563, 0.971]) (Falchook et al. manuscript submitted in parallel).

Although alisertib has demonstrated promising clinical activity, its efficacy has been variable (Friedberg et al., 2014; Goldberg et al., 2014; Matulonis et al., 2012; Melichar et al., 2015). Therefore, it is important to identify potential predictive marker(s) that could be used to select patients likely to benefit from alisertib treatment (Hadley and Hendricks, 2014; Kap et al., 2016). This study aimed to assess the potential association between two *Aurora A* SNP genotypes (rs2273535 and rs1047972) at codons 31 and 57, and clinical endpoints in patients from the NCT01045421 and NCT01091428 trials.

2. Methods

2.1. Study Design and Participants

The Cancer Genome Atlas (TCGA) database was searched for 10,403 patients with solid tumors who had recorded information on the SNPs of interest. RNA sequencing (RNA-seq) and overall survival (OS) data from 10,034 patient samples were available to assess the association between *Aurora A* SNP genotypes and OS. Since the SNPs are not somatic mutations and there are no reported cases with somatic mutations at those sites, variants found from RNA-Seq can be thought of as germline SNPs. The genotypes of a particular SNP were called based on the allele fraction of its SNP allele.

Details of both phase 2 alisertib studies have been published previously (Melichar et al., 2015); additional study information (patient consent, treatment regimen, and study endpoints) can be found in the appendix. In brief, NCT01045421 was an open-label, multicentre study of single-agent alisertib in 249 adult patients with advanced, relapsed solid tumors including breast cancer, small cell lung cancer (SCLC), non-SCLC (NSCLC), head and neck squamous-cell carcinoma (HNSCC), and gastro-oesophageal adenocarcinoma (GE). NCT01091428 was a randomised, open-label study of alisertib plus weekly paclitaxel compared with paclitaxel alone in 142 adult patients with previously treated recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer. Correlative analyses of Aurora A SNPs and clinical efficacy outcomes were conducted in the subgroup of patients (85 of 249) from the study NCT01045421 and the subgroup of patients (122 of 142) from the study NCT01091428.

2.2. Procedures

To analyze *Aurora A* SNP data in TCGA, SNPs were genotyped based on RNA-seq results from patients (see appendix for details). Once genotypes at the SNP sites were assigned to each sample, the potential association between genotype and OS was investigated. At each SNP site (i.e. codon 31 and codon 57), survival curves for the three strata (homozygous reference, heterozygous, and homozygous SNP) were plotted and HR were calculated using the Cox proportional hazard model with respect to homozygous reference (FF at codon 31 and II at codon 57), with no clinical covariates considered; the p-value was computed using $\chi 2$ (Marumoto et al., 2003) test.

Whole blood samples were collected prior to administration of alisertib or paclitaxel, and genomic DNA was isolated from peripheral blood mononuclear cells. *Aurora A* SNP genotypes were analyzed by real-time polymerase chain reaction (PCR; see appendix for details). Whole exome next-generation sequencing (NGS) was also carried out on a subset of tumor samples (from 47 patients) from NCT01045421.

Details of the statistical methodology utilized in both phase 2 studies have been previously described (Melichar et al., 2015). In this analysis, a correlative study was performed to assess the relationship of *Aurora A* SNP genotypes with alisertib treatment outcomes (PFS, best tumor size change, and best response). For NCT01045421, a Cox regression model was used to analyze possible associations between *Aurora A* SNPs and PFS, stratified by tumor indication. The p-value was adjusted for tumor indication. Analysis of variance (ANOVA) was used to analyze best tumor size change, adjusting for tumor indication and baseline tumor size. A simple X2 (Marumoto et al., 2003) test on the contingency table was used to test the independence between genotype and patient response status. For NCT01091428, PFS was analyzed using log-rank test to compare treatment arms in different populations.

3. Results

3.1. Prognostic Implication of Aurora A SNP at Codon 57

We analyzed *Aurora A* SNP data derived from 10,403 cancer patients with 33 different cancer types in the TCGA database; VV

Please cite this article as: Niu, H., et al., *Aurora A* Functional Single Nucleotide Polymorphism (SNP) Correlates With Clinical Outcome in Patients With Advanced Solid Tumors Treated With Alisertib, an ..., EBioMedicine (2017), https://doi.org/10.1016/j.ebiom.2017.10.015