Communication

DOI: 10.5582/irdr.2018.01018

Aurora B: A new promising therapeutic target in cancer

Paolo Chieffi*

Dipartimento di Psicologia, Università della Campania, Caserta, Italy.

Summary **A critical step for maintenance of genetic stability is chromosome segregation, which requires a high coordination of cellular processes. Loss of mitotic regulation is a possible cause of aneuploidy in human epithelial malignancy and it is thought to create an abnormal nuclear morphology in cancer cells. Serine/threonine protein kinase Aurora B gene plays a regulatory role from G2 to cytokinesis, encompassing key cell cycle events such as centrosome duplication, chromosome bi-orientation, and segregation. The overexpression of Aurora B has been observed in several tumour types, and has been linked with a poor prognosis for cancer patients. Therapeutic inhibition of Aurora kinase showed great promise as a probable anticancer regime because of its important role during cell division.**

Keywords: Aurora kinase, Aurora B, serine-threonine kinase, mitosis, cytokinesis, cancer, inhibitors

1. Aurora B and cancer

In the last decade, a large number of studies have linked the aberrant expression of Aurora kinases to cancer and this has lead to a great effort on the development of Aurora kinases inhibitors.

Aurora B is involved in chromosome segregation, spindle-checkpoint and cytokinesis, and alteration of each of these steps could induce aneuploidy, one of main features and driving force of cancer cells (*1-3*).

In vitro studies performed with several Aurora B inhibitors, dominant negative mutants or RNAi, show that Aurora B deficiency interferes with the cell cycle. Treated cells cannot divide after mitosis and become tetraploid, with two copies of centrosomes. Moreover, cells expressing catalytically inactive Aurora B do not arrest in mitosis in the presence of nocodazole or taxol. These observations concur with Aurora B's presumed roles: spindle checkpoint suppression allows cells to go through mitosis, despite a number of chromosomes being oriented in a syntelic manner (both kinetocores attached to the same pole), while the lack of phosphorylation of cleavage furrow components prevents cytokinesis. The effects of longer depletion of Aurora B seem to be cell line dependent. Some cells either enter additional cell cycles but, because of cell division failure, they become massively polyploid, whereas other cell lines undergo apoptosis or arrest in a pseudo G1 state. These differences are probably due to the p53-dependent post-mitotic checkpoint (*4-6*).

Aurora B is located on chromosome 17p13.1, a chromosomal region that has not been frequently associated with amplification in tumours, with the exception of glioblastoma (*7*). Aurora B gene is dramatically up-regulated in highly proliferating compared with non-proliferating cells. Although Aurora B overexpression has been shown in many tumours types, this is not the result of gene amplification, and it is still under debate whether the observed overexpression of Aurora B is a reflection of the high proliferative rate of neoplastic cells or whether it is causally related to tumorigenesis.

Aurora B is overexpressed in several human cancers, such as non small cell lung carcinoma (*8*), mesothelioma (*9*), glioblastoma (*7*) oral cancer (*10*), malignant endometrium, hepatocellular carcinoma (*11*), testicular germ cell tumours (*12-15*), ovarian (*16,17*), thyroid (*18,19*), colon (*20*) and prostate (*21,22*). Aurora B expression is positively correlated with poor prognosis and displays a tendency to group in higher grades of malignancy in different neoplastic lesions. Aurora B expression directly correlates with Gleason grade in prostate cancer (*21,22*), Duke's grade

Released online in J-STAGE as advance publication April 15, 2018.

**Address correspondence to:*
Dr. Paolo Chieffi, Dipartimento di Psicologia, Università della Campania, Viale Ellittico, 31 81100 Caserta, Italy.
E-mail: paolo.chieffi@unicampania.it

in colorectal cancer (*20*) and dedifferentiation in ovary and thyroid carcinoma (*16,18*). In thyroid tumours, an increase of Aurora B expression has been observed in papillary and anaplastic thyroid carcinomas. In the late stages of thyroid tumour progression a further increase of Aurora B expression was observed indicating that Aurora B overexpression might confer a growth advantage to neoplastic cells (*18,19*). In all lesions overexpressing Aurora B, phosphorylation of histone H3 was clearly detectable.

Several studies have suggested that commonly occurring gene polymorphisms of Aurora B are associated with cancer risk. An alternative splicing variant of Aurora B (Aurora B-Sv2) has been found frequently associated with advanced stages of hepatocellular carcinoma; and this variant appears to be more frequently associated with tumour recurrence and poor prognosis (*11*).

Aurora B kinase expression in epithelial ovarian cancer patients has been evaluated. Expression of Aurora B in poorly and moderately differentiated carcinomas of the ovary was significantly higher than in

Table 1. Clinical trials with Aurora kinase inihibitors

Drug	Tumor Type	Title of the Study	Phase	Sponsored by	ClinicalTrials.gov Identifier
VX-680 (MK0457)	Advanced Cancer	A Phase I, Open Label, Multi-centre Study to Assess the Safety, Tolerability, and Pharmacokinetics of AZD1152 in Japanese Patients With Acute Myeloid Leukaemia	Phase I	MERCK	NCT00104351
VX-680 (MK0457)	Leukemia	A Phase I/II Dose Escalation Study of MK0457 in Patients With Leukemia	Phase I	MERCK	NCT00111683
VX-680 (MK0457)	Carcinoma, Non-Small-Cell Lung	A Phase IIA Study Evaluating the Efficacy of MK0457 as a 5-Day Continuous Infusion in Patients With Advanced Non-Small Cell Lung Cancer (NSCLC)	Phase II	MERCK	NCT00290550
VX-680 (MK0457)	Leukemia	A Phase II Study of MK0457 in Patients With T315I Mutant Chronic Myelogenous Leukemia and Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia	Phase II	MERCK	NCT00405054
VX-680 (MK0457)	Chronic Myelogenous Leukemia, Leukemia, Lymphoblastic Acute Philadelphia Positive	A Phase I Dose Escalation of MK0457 in Combination With Dasatinib in Patients With Chronic Myelogenous Leukemia and Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia	Phase I	MERCK	NCT00500006
AZD1152	Acute Myeloid Leukemia	A Phase I, Open Label, Multi-centre Study to Assess the Safety, Tolerability, and Pharmacokinetics of AZD1152 in Japanese Patients With Acute Myeloid Leukaemia	Phase I	AstraZeneca	NCT00530699
AZD1152	Myeloid Leukemia	A Phase I/II, Open Label, Multi-Centre Study to Assess the Safety, Tolerability, Pharmacokinetics and Efficacy of AZD1152 in Patients With Acute Myeloid Leukaemia	Phase I Phase II	AstraZeneca	NCT00497991
AZD1152 + LDAC	Acute Myeloid Leukemia	A Randomised, Open-label, Multi-centre, 2- stage, Parallel Group Study to Assess the Efficacy, Safety and Tolerability of AZD1152 Alone and in Combination With Low Dose Cytosine Arabinoside (LDAC) in Comparison With LDAC Alone in Patients Aged 60 With Newly Diagnosed Acute Myeloid Leukaemia (AML)	Phase II	AstraZeneca	NCT00952588
AZD1152 + LDAC	Acute Myeloid Leukemia	A Phase I, Open-Label, Multi-Centre, Multiple Ascending Dose Study to Assess the Safety and Tolerability of AZD1152 in Combination With Low Dose Cytosine Arabinoside (LDAC) in Patients With Acute Myeloid Leukaemia (AML)	Phase I	AstraZeneca	NCT00926731
AZD1152	Solid Tumours	A Phase I, Open-Label, Multi-Centre Study to Assess the Safety, Tolerability and Pharmacokinetics of AZD1152 Given as a Continuous 48-Hour Intravenous Infusion in Patients With Advanced Solid Malignancies	Phase I	AstraZeneca	NCT00338182
AZD1152	Solid Tumours	A Phase I, Open-Label, Multi-Centre Study to Assess the Safety, Tolerability and Pharmacokinetics of AZD1152 Given as a Continuous 7- Day Intravenous Infusion in Patients With Advanced Solid Malignancies	Phase I	AstraZeneca	NCT00497679

well-differentiated carcinomas and overall, the Aurora B overexpression group demonstrated a significantly shorter progression-free survival and survival than a low expression group (*16*). In human colorectal cancer samples the correlation of Aurora B expression with overall survival was also evaluated, showing that patients with a high expression level of Aurora B lived significantly shorter lives compared with patients with low expression levels . Furthermore single-nucleotide polymorphism analysis showed that patients harboring G-allele in 885A>G showed a significantly decreased overall survival (*1*). These studies suggest that Aurora B expression could be used as a predictor of aggressive lesions and as a prognostic marker.

2. Aurora B as therapeutic target

The inhibition of Aurora B kinase has an anti-proliferative effect and causes regression in several animal models of human cancers, including breast, colon, lung, leukemia, prostate and thyroid (*15-22*). These observations strongly suggest a potential role for Aurora B inhibition in patients. To target the enzymatic activity of Aurora kinases, small molecules able to occupy the catalytic binding site have been identified, however due to the high homology of Aurora kinases catalytic subunit most of the inhibitors developed so far lack selectivity and inhibit both Aurora Kinases. Despite this lack of selectivity of Aurora Kinases inhibitors the number of Aurora inhibitors is rapidly increasing: approximately 15 Aurora inhibitors are under Phase I/II evaluation and others are in preclinical testing (Table 1) (*3,23*).

Inhibition of Aurora B by specific inhibitors interferes with normal chromosome alignment during mitosis and overrides the mitotic spindle checkpoint inducing endoreduplication. Aurora B seems to be more suitable as an anticancer drug target, since inhibition of Aurora B rapidly results in catastrophic mitosis, leading to cell death (*23-28*).

In order to block Aurora B functions, several anti-cancer drugs targeting its catalytic binding site have been developed. However, given the high homology of the catalytic domain of Aurora kinases, small molecules do not differentiate between them, and could be considered dual inhibitors (Aurora A and B inhibithors), however the phenotype observed in cells treated with these dual inhibitors closely resemble those exerted by specific inhibition of Aurora B. The first three small-molecule dual inhibitors of Aurora(s) described include Hesperadin, VX-680, and ZM447439. These three drugs have similar potency versus Aurora A, and Aurora B. Each induces a similar phenotype in cell-based assays, characterized by inhibition of phosphorylation of histone H3 on Ser^{10} and Ser^{28}, inhibition of cytokinesis, and development of polyploidy (*28-34*).

In addition, selective inhibitors of Aurora B have been developed: AZD1152 (highly potent and selective inhibitor of Aurora B), and GSK1070916 (a potent and selective inhibitor of Aurora B and Aurora C kinases) (*3,19,23*).

The role of Aurora B in human tumourigenesis remains to be fully clarified, however, overexpression of Aurora B occurs in many tumours and accumulating evidence indicates that its expression negatively correlates with patient's survival and prognosis.

Aurora B inhibitors have been obtained and are currently under clinical trials. However, antimitotic drugs frequently show the appearance of drug resistance, adverse side effects (neurotoxicity) and their clinical effects are not predictable. Aurora inhibitors lack neurotoxicity and could contribute to the treatment of human neoplasia (*31-34*).

Furthermore, preclinical data are predicting that the association of these inhibitors with conventional chemotherapy and radiotherapy display additive or even synergistic anticancer effect thus opening new avenues towards the cure of cancer.

References

1. Carvajal RD, Tse A, Schwartz GK. Aurora kinases: New targets for cancer therapy. Clin Cancer Res. 2006; 12:6869-6875.
2. Glover DM, Leibowitz MH, McLean DA. Parry H. Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. Cell. 1995; 81:95-105.
3. Portella G, Passaro C, Chieffi P. Aurora B: A new prognostic marker and therapeutic target in cancer. Curr Med Chem. 2011; 18:482-496.
4. Chieffi P, Franco R, Portella G. Molecular and cell biology of testicular germ cell tumors. Int Rev Cell Mol Biol. 2009; 278:277-308.
5. Chieffi P, Chieffi S. Molecular biomarkers as potential targets for therapeutic strategies in human testicular germ cell tumours: An overview. J Cell Physiol. 2013; 228:1641-1646.
6. Picascia A, Stanzione R, Chieffi P, Kisslinger A, Dikic I, Tramontano D. Proline-rich tyrosine kinase 2 regulates proliferation and differentiation of prostate cells. Mol Cell Endocrinol. 2002; 186:81-87.
7. Zeng WF, Navaratne K, Prayson RA, Weil RJ. Aurora B expression correlates with aggressive behaviour in glioblastoma multiforme. J Clin Pathol. 2007; 60:218-221.
8. Vischioni B, Oudejans JJ, Vos W, Rodriguez JA, Giaccone G. Frequent overexpression of aurora B kinase, a novel drug target, in non-small cell lung carcinoma patients. Mol Cancer Ther. 2006; 5:2905-2913.
9. López-Ríos F, Chuai S, Flores R, Shimizu S, Ohno T, Wakahara K, Illei PB, Hussain S, Krug L, Zakowski MF, Rusch V, Olshen AB, Ladanyi M. Global gene expression profiling of pleural mesotheliomas: Overexpression of aurora kinases and P16/CDKN2A deletion as prognostic factors and critical evaluation of microarray-based prognostic prediction. Cancer Res. 2006; 66:2970-2979.
10. Qi G, Ogawa I, Kudo Y, Miyauchi M, Siriwardena BS, Shimamoto F, Tatsuka M, Takata T. Aurora-B expression

and its correlation with cell proliferation and metastasis in oral cancer. Virchows Arch. 2007; 450:297-302.
11. Yasen M, Mizushima H, Mogushi K, Obulhasim G. Miyaguchi K, Inoue K, Nakahara I, Ohta T, Aihara A, Tanaka S, Arii S, Tanaka H. Expression of Aurora B and alternative variant forms in hepatocellular carcinoma and adjacent tissue. Cancer Sci. 2009; 100:472-480.
12. Chieffi P, Troncone G, Caleo A, Libertini S, Linardopoulos S, Tramontano D, Portella G. Aurora B expression in normal testis and seminomas. J Endocrinol. 2004; 181:263-270.
13. Esposito F, Libertini S, Franco R, Abagnale A, Marra L, Portella G, Chieffi P. Aurora B expression in post-puberal testicular germ cell tumours. J Cell Physiol. 2009; 221:435-439.
14. Santillo A, Falvo S, Chieffi P, Di Fiore MM, Senese R, Chieffi Baccari G. D-aspartate induces proliferative pathways in spermatogonial GC-1 cells. J Cell Physiol. 2016; 231:490-495.
15. Chieffi P, Franco R, Fulgione D, Staibano S. PCNA in the testis of the frog, *Rana esculenta*: A molecular marker of the mitotic testicular epithelium proliferation. Gen Comp Endocrinol. 2000; 119:11-16.
16. Staibano S, Franco R, Mezza E, Chieffi P, Sinisi A, Pasquali D, Errico ME, Nappi C, Tremolaterra F, Somma P, Mansueto G, De Rosa G. Loss of oestrogen receptor beta, high PCNA and p53 expression and aneuploidy as markers of worse prognosis in ovarian granulosa cell tumours. Histopathol. 2003; 43:254-262.
17. Chen YJ, Chen CM, Twu NF, Yen MS, Lai CR, Wu HH, Wang PH, Yuan CC. Overexpression of Aurora B is associated with poor prognosis in epithelial ovarian cancer patients. Virchows Arch. 2009; 455:431-440.
18. Sorrentino R, Libertini S, Pallante PL, Troncone G, Palombini L, Bavetsias V, Spalletti-Cernia D, Laccetti P, Linardopoulos S, Chieffi P, Fusco A, Portella G. Aurora B overexpression associates with the thyroid carcinoma undifferentiated phenotype and is required for thyroid carcinoma cell proliferation. J Clin Endocrinol Metab. 2005; 90:928-935.
19. Libertini S, Abagnale A, Passaro C, Botta G, Barbato S, Chieffi P, Portella G. AZD1152 negatively affects the growth of anaplastic thyroid carcinoma cells and enhances the effects of oncolytic virus dl922-947. Endocr Relat Cancer. 2011; 18:129-141.
20. Tatsuka M, Katayama H, Ota T, Tanaka T, Odashima S, Suzuki F, Terada Y. Multinuclearity and increased ploidy caused by overexpression of the aurora- and Ipl1-like midbody-associated protein mitotic kinase in human cancer cells. Cancer Res. 1998; 58:4811-4816.
21. Chieffi P, Cozzolino L, Kisslinger A, Libertini S, Staibano S, Mansueto G, De Rosa G, Villacci A, Vitale M, Linardopoulos S, Portella G, Tramontano D. Aurora B expression directly correlates with prostate cancer malignancy and influence prostate cell proliferation. Prostate. 2006; 66:326-333.
22. Sinisi AA, Chieffi P, Pasquali D, Kisslinger A, Staibano S, Bellastella A, Tramontano D. EPN: A novel epithelial cell line derived from human prostate tissue. In Vitro Cell Dev Biol Anim. 2002; 38:165-172.
23. Borisa AC, Bhatt HG. A comprehensive review on Aurora kinase: Small molecule inhibitors and clinical trial studies. Eur J Med Chem. 2017; 140:1-19.
24. Pero R, Lembo F, Chieffi P, Del Pozzo G, Fedele M, Fusco A, Bruni CB, Chiariotti L. Translational regulation of a novel testis-specific RNF4 transcript. Mol Reprod Dev. 2003; 66:1-7.
25. Fedele M, Franco R, Salvatore G, Paronetto MP, Barbagallo F, Pero R, Chiariotti L, Sette C, Tramontano D, Chieffi G, Fusco A, Chieffi P. PATZ1 gene has a critical role in the spermatogenesis and testicular tumours. J Pathol. 2008; 215:39-47.
26. Esposito F, Boscia F, Franco R, Tornincasa M, Fusco A, Kitazawa S, Looijenga LH, Chieffi P. Down-regulation of oestrogen receptor-β associates with transcriptional co-regulator PATZ1 delocalization in human testicular seminomas. J Pathol. 2011; 224:110-120.
27. Esposito F, Boscia F, Gigantino V, Tornincasa M, Fusco A, Franco R, Chieffi P. The high-mobility group A1-oestrogen receptor β nuclear interaction is impaired in human testicular seminomas. J Cell Physiol. 2012; 227:3749-3755.
28. Vicini E, Loiarro M, Di Agostino S, Corallini S, Capolunghi F, Carsetti R, Chieffi P, Geremia R, Stefanini M, Sette C. 17-β-estradiol elicits genomic and non-genomic responses in mouse male germ cells. J Cell Physiol. 2006; 206:238-245.
29. Boscia F, Passaro C, Gigantino V, Perdonà S, Franco R, Portella G, Chieffi S, Chieffi P. High levels of GPR30 protein in human testicular carcinoma *in situ* and seminomas correlate with low levels of estrogen receptor-beta and indicate a switch in estrogen responsiveness. J Cell Physiol. 2015; 230:1290-1297.
30. Chieffi P, Boscia F. New discovered molecular markers as promising therapeutic targets in germ cell tumors. Exp Opin Orphan Drugs. 2015; 3:1021-1030.
31. Chieffi P. Recent advances in molecular and cell biology of testicular germ-cell tumors. Int Rev Cell Mol Biol. 2014; 312:79-100.
32. Chieffi P. Molecular targets for the treatment of testicular germ cell tumors. Mini Rev Med Chem. 2007; 7:755-759.
33. Chieffi P. New perspective on molecular markers as promising therapeutic targets in germ cell tumors. Intractable Rare Dis Res. 2016; 5:137-139.
34. Chieffi P. An up-date on epigenetic and molecular markers in testicular germ cell tumors. Intractable Rare Dis Res. 2017; 6:319-321.

(Received February 20, 2018; Revised March 13, 2018; Accepted March 24, 2018)