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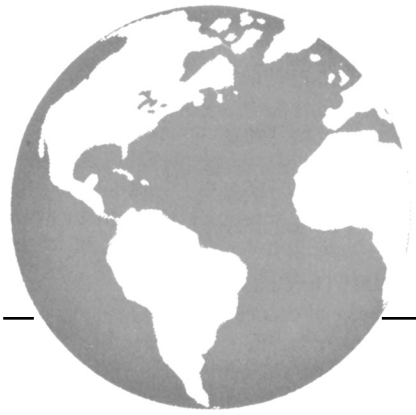

Drug resistance and its significance for treatment decisions in non-small-cell lung cancer

E. Tsvetkova MD and G.D. Goss MD**

ABSTRACT

Non-small-cell lung cancer (NSCLC) constitutes about 85% of all lung cancers. Approximately 50% of patients diagnosed with NSCLC present with advanced disease (stage III or IV) that is not amenable to curative treatment. The number of patients with stage IIIB or IV disease who are alive at 1 year after diagnosis has increased from 10% in the untreated population in the early 1980s to 50% in patients with a good performance status receiving treatment today. However, those statistics remain dismal, and the two dominant reasons are the large number of patients diagnosed with advanced-stage disease and the observed primary or secondary resistance to current therapies. The present article addresses the question of drug resistance in lung cancer, focusing on subjects that are currently topical and under intense scrutiny.

KEY WORDS

NSCLC, primary resistance, secondary resistance

1. INTRODUCTION

Lung cancer is the most common malignancy in the world and the leading cause of cancer-related death. In 2011 in the United States, 221,130 new cases of lung cancer were diagnosed, and 156,940 deaths were attributable to lung cancer [1]. In Canada, the incidences of lung cancer and of lung cancer–related death in 2011 were 25,400 and 20,600 respectively [2]. The lifetime probability of developing lung cancer is 8% for men and 6% for women [1].

Non-small-cell lung cancer (NSCLC) constitutes about 85% of all lung cancers. At the molecular level, NSCLC encompasses a heterogeneous group of neoplasms, and this heterogeneity affects therapeutic decision-making. Histologically, NSCLC includes squamous cell carcinomas, adenocarcinomas, large-cell carcinomas, adenosquamous carcinomas, and carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements [3].

Approximately 50% of patients diagnosed with NSCLC present with advanced disease (stage III or IV) that is not amenable to curative treatment. The overall median survival in stage IV NSCLC is about 10–12 months. Since the early 1980s, the number of patients with stage IIIB or IV disease who are alive at 1 year after diagnosis with NSCLC has increased from 10% in the untreated population to 50% in patients with a good performance status receiving treatment [4]. However, those statistics remain dismal, and concerted ongoing effort to improve outcomes is required.

The two dominant reasons for such dismal outcomes are the large number of patients diagnosed with advanced-stage disease and the observed primary or secondary resistance to current therapies.

Here, we address the question of drug resistance in lung cancer. A comprehensive review is beyond the scope of the present article, and so we instead focus on subjects that are currently topical and under intense scrutiny.

2. PLATINUM RESISTANCE

The treatment backbone of advanced NSCLC is platinum-based doublet chemotherapy. The goals of treatment in this setting are symptom palliation and prolongation of life.

Cisplatin enters cells predominantly by passive diffusion, although its uptake and efflux have been linked to a number of transporters. Once in the cell, cisplatin forms adducts with DNA, causing intrastrand and interstrand crosslinks. The DNA damage produced by cisplatin is detected and repaired by the nucleotide excision pathway. If the damage produced is not totally repaired, cells initiate cellular death through apoptosis or necrosis. Several signal transduction pathways, including the three main MAPK (mitogen-activated protein kinase) kinase subfamilies (Erk, Jnk, p38MAPK), Akt, and nuclear factor κB are activated [5,6].

Unfortunately, the major limitation of platinum-doublet combination treatment is primary or secondary drug resistance. The mechanisms of

drug resistance are multiple and often combinatorial. They include reduced intracellular accumulation of platinum secondary to either or both of impaired drug intake and increased outward transport. Recent research demonstrates that the transporters involved in maintenance of copper homeostasis are involved in the transport of platinum-containing drugs [7]. Studies with the yeast *Saccharomyces cerevisiae* showed that the yCtr1 protein, encoding a multiple transmembrane spanning protein, is required for copper transport into yeast cells. Identification of the Ctr1 family as a cisplatin transporter was also shown by recapitulating the cisplatin-resistance phenotype through the deletion of yCtr1 in yeast mutants [8] and subsequently demonstrating that yCtr1-mutant cells were defective in cisplatin accumulation. Furthermore, evidence suggests that a copper export system functions as an efflux transporter for platinum-based medications. Thus, factors affecting intracellular copper homeostasis (Ctr1 transporters and Atp7A and B eliminators) also influence the transport of platinum-based chemotherapeutics [6–8].

In the development of drug resistance, DNA repair capacity plays a crucial role. Two proteins important to that capacity are the excision repair cross-complementation group 1 (ERCC1) and BRCA1. ERCC1 is the lead enzyme in the nucleotide excision repair pathway [6,9,10]. A recent study has shown that increased levels of ERCC1 messenger RNA (mRNA) are related to clinical resistance to platinum-based chemotherapy in NSCLC [11]. In IALT (the International Adjuvant Lung Cancer Trial), patients with low levels of ERCC1 were shown to benefit from adjuvant platinum-based chemotherapy; those with high ERCC1 levels did not benefit. Conversely, patients with high ERCC1 levels who were on the control arm and who did not receive chemotherapy did prognostically better [12]. Those data suggest and support the hypothesis that ERCC1 plays a role in chemoresistance. However, certain polymorphisms in the *ERCC1* gene (T19007C and C8092A) are reported to be associated with response to platinum-based therapy. To date, more than 100 polymorphisms in the *ERCC1* gene have been reported, and their role in drug resistance is still unclear [13].

A growing body of evidence indicates that the breast cancer susceptibility gene 1 (*BRCA1*) confers sensitivity to apoptosis induced by antimicrotubule drugs (paclitaxel and vincristine), but induces resistance to agents that produce DNA damage (cisplatin and etoposide) and to radiotherapy [14–16]. Those preclinical findings are supported by a variety of experimental models in breast and ovarian cancer cells: inducible expression of *BRCA1* enhanced paclitaxel sensitivity [17]; inactivation of endogenous BRCA1 mediated by small interfering RNA led to paclitaxel and docetaxel resistance [18,19], and reconstitution of BRCA1–deficient cells with wild-type *BRCA1* enhanced sensitivity to paclitaxel and vinorelbine. This differential modulating effect of *BRCA1* mRNA expression was also observed in tumour cells isolated from malignant effusions of NSCLC and gastric cancer patients, where *BRCA1* mRNA levels correlated negatively with cisplatin sensitivity and positively with docetaxel sensitivity [20]. Two retrospective studies—in NSCLC [21] and ovarian cancer [22] patients—found that low or intermediate *BRCA1* mRNA levels correlated with significantly longer survival after platinum-based chemotherapy, and that survival in patients with higher *BRCA1* expression increased after taxane-based chemotherapy [22].

BRCA1 is recruited to the sites of DNA breaks, playing a central role in DNA repair and in cell-cycle checkpoint control. Binding of the mediator of DNA damage checkpoint 1 (Mdc1) protein to the phosphorylated tail of histone H2AX facilitates formation of BRCA1 nuclear foci at double-strand breaks [23]. The receptor-associated protein 80 (Rap80) acts upstream of BRCA1 and is required for the accumulation of BRCA1 to sites of DNA breaks [24,25]. Abraxas recruits Rap80 to form a complex with BRCA1. Both Abraxas and Rap80 are required for DNA damage repair, and cells depleted of Abraxas or Rap80 exhibit hypersensitivity to irradiation [24]. Many proteins involved in other cellular pathways have been implicated in drug resistance development; they include changes in promoter methylation of hMlh1 as a cause of acquired platinum-based chemotherapy resistance [26]; reduced expression of membrane-associated beta tubulin and the intermediate filament cytokeratin 18; altered signalling of protein kinase C and cAMP pathways; and expression of c-Fos [21].

3. RESISTANCE TO EPIDERMAL GROWTH FACTOR INHIBITOR

The introduction in 2005 of treatments using the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) changed survival and quality of life for a subgroup of patients with advanced NSCLC. Activating mutations in *EGFR* correlate with sensitivity to EGFR-TKIs and are used as predictive biomarkers of response and progression-free survival [27,28]. These mutations are present in approximately 40% of East Asian and in 10% of Caucasian NSCLC patients [29]. However, it has been very well documented in a number of large randomized trials that the benefit from this class of drugs is not confined to patients with sensitizing mutations but is also seen in the *EGFR* wild-type setting in a subgroup of patients [30,31].

Resistance to EGFR-TKIs may be primary or secondary. In adenocarcinoma, certain clinical features, such as Asian ethnicity, female sex, and non-smoker status correlate with the efficacy of the EGFR-TKIs, but no clinical profile predicts resistance to these drugs. Lung tumours can show *de novo* (primary) resistance to TKI therapy, even in the presence of an activating

mutation in *EGFR*. Among patients with *EGFR*-mutant tumours, a 75% relative risk is observed, indicating that approximately 25% of cases do not respond to EGFR-TKIs [32]. In some cases, the reason for the lack of response is the occurrence of second-site mutations in the *EGFR* kinase domain [29] that confer resistance even in the presence of activating mutations. Examples include the T790M mutation (which rarely occurs *de novo,* but is more common in the acquired-resistance setting) and small insertions or duplications in exon 20 (such as D770_N771, ins NPG, ins SvQ, ins G, and N771T) [32,33]. In the *EGFR* wild-type setting, activating mutations occurring at codons 12 and 13 in the GTPase domain of *KRAS* are observed in 15%–25% of NSCLC tumours and occur almost exclusively in those that are *EGFR* wild-type. For reasons that are poorly understood, *KRAS* mutations are found more frequently in tumours from former or current smokers than in tumours from never-smokers, in adenocarcinomas, and in tumours from Caucasians than in tumours from East Asians.

The initial observation that *KRAS*-mutant lung tumours are resistant to EGFR-TKI [34] has been validated [35]. Unfortunately, as happens with platinum-based therapy, all patients initially responding to EGFR-TKIs will experience relapse because of secondary or acquired drug resistance. To more precisely define acquired resistance to EGFR-TKIs, the following definition has been adopted: previous treatment with a single-agent EGFR-TKI (for example, gefitinib or erlotinib); a tumour that harbours an *EGFR* mutation known to be associated with drug sensitivity, or objective clinical benefit from treatment with an EGFR-TKI; systemic progression of disease (by the Response Evaluation Criteria in Solid Tumors or radiology criteria put forth by the World Health Organization) while on continuous treatment with gefitinib or erlotinib within the preceding 30 days; and no intervening systemic therapy between the cessation of gefitinib or erlotinib and the initiation of new therapy. This relatively simple definition should lead to a more uniform approach to the problem of acquired resistance [32].

The second-site *EGFR* mutations alluded to earlier in the primary resistance setting may also be responsible for acquired resistance. The T790M mutation in *EGFR* develops in 50% of *EGFR*-mutant tumours with acquired resistance to erlotinib or gefitinib [33]. At least two molecular mechanisms explain how T790M confers drug resistance. First, substitution of a bulky methionine for threonine at position 790 leads to altered drug binding in the adenosine triphosphate (ATP) pocket of EGFR. Second, introduction of the T790M mutation increases the ATP affinity of the *EGFR–L858R* mutant by more than an order of magnitude, in effect restoring ATP affinity back to the level of wild-type *EGFR*. That restoration closes the therapeutic window that was opened by the diminished ATP affinity of the oncogenic mutants [36,37].

Other pathway abnormalities, such as mutation or dysregulation of the cascade of kinases (Ras, Raf, PI3K/Akt/mTOR), are likely involved in the development of resistance. Furthermore, primary and acquired resistance to EGFR-TKIs both may also be mediated by non-mutation-based mechanisms. One example involves increased expression of hepatocyte growth factor (HGF), the ligand for the Met receptor tyrosine kinase [38]. Binding of HGF increases Met-mediated activation of the PI3K/Akt pathway and thus decreases the ability of an EGFR-TKI to effectively inhibit this signalling cascade. In contrast to the role of Met in acquired resistance (discussed shortly), primary resistance because of increased HGF activation of Met is channelled through Gab1, not ErbB3. Amplification of the *MET* oncogene is observed in up to 20% of *EGFR*-mutant NSCLCs after TKI failure, independently of the T790M mutation [39]. Cells with *MET* amplification seem to undergo a kinase switch and rely on Met signalling through the ErbB3 pathway to maintain activation of Akt by increased phosphorylation in the presence of EGFR-TKIs.

In addition to its role in *de novo* resistance (discussed earlier), the Met ligand HGF can play a part in acquired resistance to TKIs [38]. In one study, tumour cells with *MET* amplification were detected at a low frequency using high-throughput fluorescence *in-situ* hybridization in 4 patients with untreated *EGFR*-mutant tumours who all developed acquired resistance to gefitinib or erlotinib through *MET* amplification. By contrast, pre-existing amplification was found only rarely in tumours from patients (1 of 8) who did not develop resistance by *MET* amplification. Collectively, these data suggest that TKI therapy may select for pre-existing cells with *MET* amplification. Other mechanisms of acquired resistance include pathway crosstalk—for example, between the epidermal growth factor, insulin-like growth factor, vascular endothelial growth factor (VEGF), and HGF pathways. Those mechanisms are not discussed in detail here.

To better understand the EGFR-driven signalling role in gefitinib-induced cytotoxicity and resistance development, Sordella *et al.* generated isogenic cell lines with either wild-type or mutated *EGFR*. Mutant *EGFR* selectively activated Akt and Stat5 signalling, but not Erk1 or 2, to promote survival of lung cancer cells. Immunohistochemical analysis of advanced NSCLC showed that patients with phosphorylated Akt-positive tumours responded better to treatment (improved relative risk and time to progression) than did patients with Akt-negative tumours [40,41].

K-ras is a downstream mediator of EGFR-induced cell signalling. *KRAS* gene mutations, which occur in 15%–30% of lung adenocarcinomas, are generally exclusive of mutations in *EGFR* or the human epidermal growth factor receptor 2 (*HER2*) and are generally associated with resistance to EGFR-TKI [34].

First-generation EGFR inhibitors competitively bind to the ATP binding site on the internal domain

of the receptor and are reversible. Second-generation inhibitors recently introduced into clinical study covalently bind to the target and are considered irreversible. In the acquired resistance setting, this irreversible binding is considered to be superior in efficacy to reversible binding. Recently a number of second-generation EGFR-TKIS (for example, afatinib and dacomitinib) have entered into phase III clinical development, where it is hoped they will help to overcome both primary and secondary resistance in a percentage of patients.

4. RESISTANCE TO ANGIOGENESIS INHIBITORS

Bevacizumab, an antibody to the ligand VEGF, inhibits activation of the VEGF receptor pathway. In a randomized phase III study, bevacizumab in combination with carboplatin and paclitaxel has been shown to improve overall survival by 2 months in patients with advanced NSCLC [4]. However, biomarkers that predict the efficacy of angiogenic inhibitors in general, and of the TKIS specifically, have been elusive, with no marker identified to date.

Fibroblast growth factors (FGFS) constitute a complex family of angiogenic signalling molecules that play a crucial role in angiogenesis and inflammation as well as in tumour proliferation. The FGF pathway may function as a potential mechanism for resistance to VEGF and EGFR inhibitors. The FGF family consists of 18 members in 6 subfamilies. The FGF receptor (FGFR) tyrosine kinases are coded by four genes: *FGFR1, FGFR2, FGFR3,* and *FGFR4.* Signalling via FGF is implicated in cell proliferation, motility, and angiogenesis in NSCLC. Inhibition of FGFR signalling may be achieved in a number of ways, including antisense RNA, RNA interference, FGFR-TKI, and FGF antibodies, leading to cellular proliferation and tumour growth *in vitro*. *FGFR1* amplification has been observed in about 20% of squamous cell NSCLCS [42]. Tumours with *FGFR1* amplification are very sensitive to FGFR inhibitors *in vitro,* suggesting that *FGFR1* may be a target in this group of NSCLCS. Moreover, FGFR signalling has been implicated in epithelial-to-mesenchymal transition, and pathway activation has been associated with resistance to EGFR inhibitors. Notably, the predominant evidence of proliferation dependency on EGFR signalling in lung cancer came from squamous and large-cell lung cancer groups that are less responsive to EGFR-TKIS [39]. Small-molecule FGF signalling inhibitors include cediranib, vatalanib, sorafenib, semaxanib, pazopanib, brivanib, and so on, but newer, more specific inhibitors include FGFR1 trap antibodies.

5. RESISTANCE TO ANAPLASTIC LYMPHOMA KINASE INHIBITOR

One of the key driver mutations of survival and proliferation in lung cancer cells is translocation and functional dysregulation of the anaplastic lymphoma kinase (*ALK*) gene. These mutations were reported in 2007 in a subset of patients with advanced NSCLC and showed a positive correlation with female sex, non-smoker status, and adenocarcinoma [43]. In nearly all instances, *ALK* mutation positivity excludes *EGFR* and *KRAS* mutation positivity in tumours interrogated for oncogenic drivers. Fewer than 3 years after the mutation had been documented as a lung cancer oncogenic driver, an early-phase clinical trial reported impressive results for crizotinib treatment in pretreated patients with NSCLC containing *ALK* rearrangements (relative risk: 57%; mean duration of response: 6.4 months). Crizotinib is a small molecule that functions as a protein kinase inhibitor by competitive binding within the ATP-binding pocket of target kinases. About 4% of patients with NSCLC have a chromosomal rearrangement that generates a fusion gene between *EML4* (echinoderm microtubule-associated protein-like 4) and *ALK,* which results in constitutive kinase activity that contributes to carcinogenesis and seems to drive the malignant phenotype [44]. In addition to *EML4, ALK* can fuse with a number of other genes, resulting in similar constitutive kinase activity. The kinase activity of the fusion protein is inhibited by crizotinib.

At the same time that drug efficacy was reported, drug resistance to *ALK*-targeted therapy—both primary and secondary—was reported. Because crizotinib inhibits the Met receptor tyrosine kinase, tumours were tested for *MET* amplification. None was *MET*-amplified, arguing against inhibition of Met as a primary determinant of response. In a study investigating mechanisms of resistance to crizotinib [45] in patients with *ALK* mutation, 11 patients were evaluated; 4 (36%) developed secondary mutations in the tyrosine kinase domain of *ALK.* A novel mutation in the *ALK* domain, encoding a G1269A amino acid substitution that produces resistance to crizotinib *in vitro* was identified in 2 of the 4 patients. Two patients harboured new-onset *ALK* copy number gain. One patient had outgrowth of *EGFR*-mutant NSCLC without persistent *ALK* gene rearrangement. Two patients had *KRAS* mutation [45]. The conclusion was that resistance to Alk inhibitors develops via somatic kinase domain mutations, *ALK* gene fusion, and emergence of separate oncogenic drivers.

6. THE ROLE OF CANCER STEM CELLS

The origins of the various types of NSCLC are poorly understood, but many studies have indicated that several human cancers, including lung cancer, might arise from the malignant transformation of stem cells and their progenitors into cancer progenitor cells. Somatic genomic alterations (mutations, deletions, amplifications, chromosomal rearrangements, and so on) and change in DNA methylation might result in aberrant activation of distinct developmental cascades

in adult stem cells. These cancer progenitors may subsequently give rise to a heterogenous population of cancer cells. Such a scenario implicates the activation of numerous tumorigenic cascades that are mediated through distinct growth factor signalling pathways that assume a critical role for the growth and survival of the cancer cells.

Understanding cancer stem cells and their aberrant or activated signalling pathways is essential for the development of new anticancer drugs for the treatment of NSCLC [46]. Several signalling pathways have been identified as key regulators of stem cells—Hedgehog (Hh), Notch, Wnt, and transforming growth factor α/EGFR, among others. Some act in shaping and maintaining the stem cells; others act as direct regulators of the stem cells. The Hh signalling pathway regulates proliferation and differentiation in a time-and-position-dependent fashion by binding the Ptch1 receptor. Overexpression of Hh signalling elements results in the sustained growth and enhanced invasive properties of malignant cells in multiple myeloma, melanoma, small-cell lung cancer, and many gastrointestinal malignancies. Upregulated Hh pathway is correlated with molecular markers of proliferation, pathologic status, and advanced clinical stage [47]. Notably, there is evidence that, during cancer progression, the Hh–Gli cascade cooperates with other oncogenic drivers such as mutated *KRAS* and has crosstalk with various growth factors, including tyrosine kinase receptors (EGFR), Wnt/beta-catenin, and transforming growth factor β (TGF-β) and its receptors [48]. The persistent activation of receptor tyrosine kinases (EGFR, platelet-derived growth factor receptor, TGF-β/TGF-βR, and Wnt/beta-catenin) cooperating with Hh pathway may promote the acquisition of more aggressive features and treatment resistance by malignant cells. Wnt are factors that regulate cell growth, motility, and differentiation during embryogenesis. Wnt signalling has been reported to be involved in lung carcinogenesis through active canonical signalling and through Dishevelled overexpression in NSCLC. In lung cancer, several Wnt pathways play a role, with tumour cell proliferation and poor prognosis. *WNT7* (described as a tumour-suppressor gene) is downregulated in most cancer cells [49]. Notch signalling pathway functions in stem-cell maintenance, binary cell-fate decision, and induction of differentiation. Notch function in lung cancer exhibits properties suggesting both tumour promotion and inhibition depending on tumour cell type. Notch is thought to have growth-promoting properties in NSCLC and growth inhibitory properties in small-cell lung cancer. This group of stem-cell regulators presents ideal targets for molecularly targeted strategies in both small-cell lung cancer and NSCLC.

A number of inhibitors of Hh, Wnt, and Notch are in phase I or II development, and unfortunately, it is already known that they are unlikely to be "golden bullets" against lung cancer. On the other hand, it is known that some stem cells can be killed with adjuvant chemotherapy in the early disease setting. Combinations of adjuvant chemotherapy with this group of novel agents may therefore be a reasonable strategy to further overcome lung cancer drug resistance.

7. TREATMENT SELECTION

Although significant advances have been made both in understanding the biology of advanced NSCLC and in improving treatment outcomes for patients with the disease, the dream of targeted personalized medicine remains elusive for most patients. Since 2000, because of the advent of EGFR- and Alk-targeted inhibitors, median overall survival has been significantly improved for patients whose tumours have activating mutations of *EGFR* and constitutively active *ALK* fusion genes. Furthermore, a number of other oncogenic drivers have been identified and are currently the subjects of a plethora of clinical trials targeting them with novel agents. Examples include patients with tumours harbouring *BRAF, ROS,* and *HER2* mutations, among others. Unfortunately in Western white populations, less than 20% of the patient population with advanced NSCLC consists of individuals with *EGFR* and *ALK* mutations.

So, when a patient with good performance status and advanced NSCLC presents in the clinic for the first time, what should the treatment strategy be?

Currently, the strategy is based on physician and patient access to treatment and biomarker evaluation. In Canada, as of this writing, the only targeted agents registered for the treatment of advanced NSCLC are bevacizumab (which has not been covered here), gefitinib, and erlotinib. Access to those agents and to biomarker evaluation is not uniform across the country, and therefore the strategy outlined next is just one of several.

In the first instance, the tumour in a patient presenting for the first time should be analyzed for *EGFR* and *ALK* mutations, and if the result is positive, the patient should be treated with an EGFR or Alk inhibitor. Such treatment will maximize the likelihood of response and minimize the likelihood of toxicity. If biomarker (mutational) analysis is not available, then the patient should be treated with platinum-doublet chemotherapy, with or without bevacizumab depending on the histology of the tumour [4]. It is imperative that patients in whom mutational status is not known receive chemotherapy and not a targeted agent, because available data indicate a worse outcome for mutation-negative patients receiving a targeted agent, particularly EGFR-TKI inhibitors [50]. If, on the other hand, a patient is *EGFR*- and *ALK*-mutation–positive, then their likelihood of response to targeted therapy does not decrease with the line of therapy. It is therefore reasonable to start with chemotherapy

regardless of mutational status, provided that, at some time during their therapy, mutation-positive patients receive the targeted agent.

However, this field is moving rapidly, and the treatment strategies that apply today may be different in as little as a few months.

As knowledge of lung cancer biology and the mechanisms of resistance to treatment rapidly increase, patients, physicians, and their families can, over the next decade, look forward to more effective treatment with fewer toxicities and better long-term outcomes.

8. CONFLICT OF INTEREST DISCLOSURES

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Correspondence to: Glenwood D. Goss, The Ottawa Hospital Cancer Centre, 501 Smyth Road, Ottawa, Ontario K1H 8L6.
E-mail: ggoss@ottawahospital.on.ca

* The Ottawa Hospital Cancer Centre, Division of Medical Oncology, University of Ottawa, Ottawa, ON.