



The advantages, potentials and challenges of the single-molecule nanopore DNA sequencing

The recent availability of new, less expensive high-throughput DNA sequencing technologies has yielded a dramatic increase in the volume of sequence data that must be analyzed. The various technologies that constitute this new paradigm continue to evolve, and further improvements in technology robustness and process streamlining will pave the path for translation into clinical diagnostics (1). Nanopore DNA sequencing offers the possibility of a label-free, single-molecule approach that can be performed without the need for sample amplification. A single-molecule method for sequencing DNA that does not require fluorescent labelling could reduce costs and increase sequencing speeds. An exonuclease enzyme might be used to cleave individual nucleotide molecules from the DNA, and when coupled to an appropriate detection system, these nucleotides could be identified in the correct order (2). A number of key technical problems are solved through the new approach, published on-line on February 22nd 2009 (2). A protein nanopore with a covalently attached adapter molecule can continuously identify unlabelled nucleoside 5'-monophosphate molecules with accuracies averaging 99.8%. Methylated cytosine can also be distinguished from the four standard DNA bases: guanine, adenine, thymine and cytosine. The operating conditions are compatible with the exonuclease, and the kinetic data show that the nucleotides have a high probability of translocation through the nanopore and, therefore, of not being registered twice. This highly accurate tool is suitable for integration into a system for sequencing nucleic acids and for analysing epigenetic modifications (2).

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Management of hypertension in angiogenesis inhibitor-treated patients

Hypertension (HTN) is one of the most frequent comorbid conditions found in cancer patients and observed side-effects of systemic inhibition of vascular endothelial growth factor (VEGF) signaling. It is an established risk factor for coronary heart disease, stroke, heart failure, and end-stage renal disease. Incidence of intracerebral hemorrhage has been recently reported in patients with metastatic renal cell carcinoma treated with tyrosine kinase inhibitors targeting the vascular endothelial growth factor receptor (VEGFR) probably related to uncontrolled HTN at diagnosis.

The incidence and severity of HTN in cancer patients are dependent on the type of drugs, dose, and schedule used age of patients, as well as the presence of coexisting cardiac diseases.

The Joint National Committee on Prevention, Detection, Evaluation, and the Treatment of High Blood Pressure (JNC7) classification system consists of three main BP categories: normal, prehypertension, and HTN, which are shown in Table 1.

HTN *de novo* or worsening control of a preexisting one after the introduction of antiangiogenic treatment may indicate many possible underlying mechanisms: renal thrombotic microangiopathy, glomerular lesions, but more commonly it is isolated HTN secondary to treatment itself.

The mechanism of action by which angiogenic inhibitors causes 'isolated HTN' is uncertain. Measured BP is the product of the cardiac output by systemic vascular resistance (SVR). Drugs which increase either one as in case of $\beta 1$ + or α + inotropic drugs are known to increase BP. In the case of angiogenic inhibitors, heart failure with marked decrease of ejection fraction have been reported. Intracoronary VEGF infusions in VIVA trial induced vasodilatation thus decreasing BP level. So the HTN induced by antiangiogenic drugs is probably related to an increase in SVR.

Mechanisms inducing high SVR include neurohormonal factors (such as renin, and aldosterone, catecholamines, epinephrine, norepinephrine, endothelin I), vascular rarefaction (decrease in the density of microvessels), and endothelial dysfunction associated with a decrease in nitric oxide (NO) production and an increase in oxidative stress.

Angiotensin-converting enzyme (ACE) inhibitors (33.3%) and beta blockers (29%) were most commonly used to manage HTN in *de novo* hypertensive patients. Other antihypertensive classes included diuretics (26.6%), calcium channel blockers (CCBs) (22.7%), angiotensin 2 receptor antagonist (ARA) (15%), and others (9.7%).

Hurwitz et al. reported that all the HTN occurring in the phases I and II bevacizumab trials in mCRC patients was readily responsive to standard oral antihypertensive agents including diuretics, ACE inhibitors, and CCBs.

Globally, several messages could be proposed:

- (i) Patients suitable to angiogenic inhibitor therapy must be assessed at baseline for existing kidney disease with a screening BP, urine analysis for proteinuria, and a calculated estimate of renal function [creatinine clearance or glomerular filtration rate (GFR)]. Repeat screening should be carried out every week for the eight first weeks and before any infusion (for anti-VEGF humanized antibodies and VEGF-Trap) or cycle (for oral tyrosine kinase inhibitors). BP measurement maybe carried out either with home BP or office monitoring.
- (ii) HTN definition level should be adapted according to JNC7 recommendations for earlier management.
- (iii) Elevated BP under angiogenic inhibitors maybe secondary to renal thrombotic microangiopathy, glomerular damage, or more frequently related to the VEGF vascular effect. Indeed, patients who under angiogenic inhibitors developed proteinuria of grade 1+ by dipstick analysis, mechanic hemolytic anemia, or reduced renal function (GFR, <60 ml/min per 1.73 m²) should be referred to a nephrologist for additional evaluation.
- (iv) BP-lowering drugs should be individualized to the patient's clinical circumstances; ACE inhibitors or ARA should be preferred for those patients with proteinuria, chronic kidney disease risks, or metabolic syndrome; nondihydropyridine CCB should be avoided in treating patients receiving CYP450 inhibitors. Dihydropyridine CCB should be preferred in elderly or black patients. Angiogenic inhibitors should be withheld only from patients who experienced hypertensive crisis.
- (v) One test dose of 5–10 mg isosorbide dinitrate maybe administered in case of *de novo* HTN or added to previous antihypertensive treatment

Table 1. Hypertension grading and therapeutic recommendations comparison between NCI and JNC7 classifications

NCI ^a		JNC7b equivalence	
Grade	Hypertensive adverse event and recommendations	Class	Recommendations
0	None	Normal SBP	
1	Asymptomatic, transient (<24 h) increase by >20 mmHg (diastolic) or to >150/100 mmHg if previously within normal limits; not requiring treatment	Prehypertension	Requiring treatment in high cardiovascular risk patients
		Normal	
2	Recurrent, persistent, or symptomatic increase by >20 mmHg (diastolic) or to >150/100 mmHg if previously within normal limits; monotherapy may be indicated	Prehypertension	Requiring treatment in high cardiovascular risk patients
		Stage 1 hypertension	Treatment required
3	Requiring therapy or more intensive therapy than previously	Stage 1 hypertension	Treatment required
		Stage 2 hypertension	Treatment required
4	Hypertensive crisis		

a Common Terminology for Adverse Events (v 2.0) Grading of Hypertension in Cancer Trials [Cancer Therapy Evaluation Program. Common terminology criteria for adverse events (version 2.0; 30 April 1999). Rockville, MD: National Cancer Institute, National Institutes of Health. <http://ctep.cancer.gov/reporting/ctc.html>. 28 March 2006, date last accessed].

b JNC7 Blood Pressure Classification for Adults [24]: Normal systolic blood pressure (SBP) < 120 and diastolic blood pressure (DBP) < 80 mmHg; Prehypertension SBP 120–139 or DBP 80–89 mmHg; stage 1 hypertension SBP 140–159 or DBP 90–99 mmHg; stage 2 hypertension SBP ≥ 160 or DBP ≥ 100 mmHg.

in case of disequilibrium of previously controlled BP. Prompt return of pretreatment BP in spite of continuous anti-VEGF treatment may justify long-acting oral nitrates prescription. On the same idea, antihypertensive properties of phosphodiesterase inhibitors or Nebivolol should be evaluated in prospective clinical trials.

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New perspectives in the treatment of advanced or metastatic gastric cancer

Gastric cancer is the fourth most common cancer worldwide. Metastatic gastric cancer remains an incurable disease, with a relative 5-year survival rate of 7%-27%.

Chemotherapy is the main treatment option for patients with advanced disease. Chemotherapy, which improves overall survival (OS) and quality of life, is the main treatment option. Meta-analysis has demonstrated that the best survival results obtained in earlier randomized studies were achieved with three-drug regimens containing a fluoropyrimidine, an anthracycline, and cisplatin (ECF). Although there has been little progress in improving median OS times beyond the 9-month plateau achievable with the standard regimens, the availability of newer agents has provided some measure of optimism. A number of new combinations incorporating docetaxel, oxaliplatin, capecitabine, and S-1 have been explored in randomized trials. Some combinations, such as epirubicin-oxaliplatin-capecitabine, have been shown to be as effective as (or perhaps more effective than) ECF, and promising early data have been derived for S-1 in combination with cisplatin. One factor that might contribute to extending median OS is the advancement whenever possible to second-line cytotoxic treatments.

Intravenous 5-FU remains the most widely used agent and has been the cornerstone of old combination regimens such as FAM (5-FU, doxorubicin, and mitomycin C), FAMTX (5-FU, doxorubicin, and methotrexate), ELF (etoposide, leucovorin, and 5-FU), and ECF (epirubicin, cisplatin, and continuous infusion 5-FU).

Combination chemotherapy has been shown to be associated with a statistically significant ($p = 0.001$) survival benefit compared with monotherapy in a meta-analysis of several clinical trials. Finally, the meta-analysis also showed that three-drug combinations have a significant survival benefit compared with two-drug combinations.

The main hope for significant advances in the near future is the combination of new targeted biological agents with existing chemotherapy first-line regimens. A number of different classes of targeted agents have shown promising activity in clinical studies of advanced gastric cancer, including epidermal growth factor receptor (EGFR) and human epidermal growth factor (HER)-2-targeted monoclonal antibodies, antiangiogenic and antiangiogenic/antitumor compounds, and the proteasome inhibitor bortezomib.

High response and/or disease control rates have been reported for EGFR-targeted cetuximab combined with irinotecan and infusional 5-FU and leucovorin and VEGF-targeted bevacizumab combined with irinotecan and cisplatin. In particular, the FOLCETUX study has demonstrated that the addition of cetuximab to the FOLFIRI regimen increased survival in 38 untreated patients with confirmed advanced gastric/gastroesophageal adenocarcinoma. Trastuzumab exhibits activity in human gastric cancer cells that overexpress HER2/neu. A phase II trial has determined the efficacy and tolerability of trastuzumab plus cisplatin in patients with advanced gastric cancer with HER2/neu overexpression/amplification. Preliminary results showed that 6 (35%) out of 17 assessable patients achieved response, 3 (17%) stabilization. There was no grade 4 toxicity. In considering such studies, it is notable that the first-line cytotoxic regimens that have been selected for combination with biological agents tend to be those that are generally considered not to be optimal for the treatment of advanced gastric cancer. This begs the ques-

tion as to whether the impressive potential of these targeted agents might be more profitably explored in the future within combinations that include standard cytotoxic backbones such as ECF, DCF, EOX, or perhaps S-1 plus cisplatin. Indeed, a number of randomized phase III studies incorporating targeted agents in first-line regimens have recently been initiated: the ToGA (Trastuzumab with Chemotherapy in HER2-Positive Advanced Gastric Cancer) study is investigating the effect on progression-free survival of trastuzumab in combination with a fluoropyrimidine plus cisplatin *versus* chemotherapy alone in patients with HER-2-positive advanced gastric cancer, AVAGAST (Avastin® in Gastric Cancer) is investigating OS time in advanced gastric cancer patients receiving either capecitabine and cisplatin plus bevacizumab or chemotherapy alone plus placebo, and the REAL-3 study is investigating the benefit of adding panitumumab to an EOX regimen in patients with locally advanced or metastatic esophagogastric adenocarcinoma.

However, new biological agents could be useful in the management of advanced disease after the failure of first-line treatment. In this context, it is possible that targeted agents may have a future role as single-agent maintenance treatments. Two recent phase II studies have pursued this concept. The multicenter AIO phase II trial has evaluated tolerability and efficacy of sunitinib in highly pretreated Caucasian patients with unresectable metastatic cancer of stomach, esophagogastric junction or lower esophagus.

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Prepared by Vladimir Baltić

programs of gene expression and carcinogenesis since cobalt at higher concentrations is a known carcinogen.

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Prepared by Vladimir Baltić

Alterations of histone modifications by cobalt compounds

Post-translational modifications of nucleosomal histones play critical roles in all aspects of eukaryotic chromosome dynamics, including replication, recombination, repair, segregation and gene expression. Such modifications include acetylation and methylation of lysines (K) and arginines (R), citrullination of arginines, phosphorylation of serines (S) and threonines (T), sumoylation and ubiquitination of lysines and adenosine diphosphate-ribosylation. Each modification can affect chromatin structure that may regulate gene transcription. Studies have shown that different histone modifications yield distinct functional consequences. For example, in general, trimethylation of histone H3 at K9 (H3K9me3), K27 (H3K27me3) and K36 (H3K36me3); dimethylation of histone H3 at K9 (H3K9me2); ubiquitination of histone H2A (uH2A) and the lack of histone H3 (AcH3) and H4 (AcH4) acetylation correlated with transcriptional repression in higher eukaryotes, whereas trimethylation of histone H3 at K4 (H3K4me3) and ubiquitination of histone H2B (uH2B) were associated with transcriptional activation. However, these modifications may interact with each other and their total sum may be the ultimate determinant of chromatin state that governs gene transcription.

Microarray data showed that exposed to 200 μ M of CoCl₂ for 24 h, A549 cells not only increased but also decreased expression of hundreds of genes involved in different cellular functions, including tumorigenesis. This study is the first to demonstrate that cobalt ions altered epigenetic homeostasis in cells. It also sheds light on the possible mechanisms involved in cobalt-induced alteration of histone modifications, which may lead to altered