ELSEVIER

Critical Reviews in Oncology/Hematology 82 (2012) 171–186

CRITICAL REVIEWS IN
Oncology
Hematology
Incorporating Geriatric Oncology

www.elsevier.com/locate/critrevonc

# Optimizing targeted cancer therapy: Towards clinical application of systems biology approaches

Arend H. Sikkema [a], Wilfred F.A. den Dunnen [b], Sander H. Diks [a], Maikel P. Peppelenbosch [c], Eveline S.J.M. de Bont [a,*]

[a] *Beatrix Children's Hospital, Department of Pediatric Oncology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands*

[b] *Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands*

[c] *Department of Gastroenterology and Hepatology, Erasmus MC's, Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands*

Accepted 4 May 2011

## Contents

1. Introduction ..... 172
2. Molecular biology of cancer cells: targeting signaling kinases ..... 172
3. Application of kinome-targeted therapies: opportunities and challenges ..... 174
4. Dissecting the human phosphoproteome ..... 176
   4.1. Quantitative phosphoproteomics ..... 176
   4.2. Direct selective measurement of kinase activity ..... 178
      4.2.1. Reporter constructs ..... 178
      4.2.2. Peptide substrate-based kinase activity arrays ..... 178
      4.2.3. Quantitative measurement of kinase activity ..... 179
   4.3. Peptide array-based kinase activity profiling to assess aberrant signaling in disease and neoplasia ..... 180
5. Conclusions and future perspectives: bridging the gap ..... 180
   Conflict of interest statement ..... 181
   Role of funding source ..... 181
   **Reviewers** ..... 181
   Acknowledgements ..... 181
   References ..... 181
   Biographies ..... 186

## Abstract

In cancer, genetic and epigenetic alterations ultimately culminate in discordant activation of signal transduction pathways driving the malignant process. Pharmacological or biological inhibition of such pathways holds significant promise with respect to devising rational therapy for cancer. Thus, technical concepts pursuing robust characterization of kinase activity in tissue samples from cancer patients have been subject of investigation. In the present review we provide a comprehensive overview of these techniques and discuss their advantages and disadvantages for systems biology approaches to identify kinase targets in oncological disease.

*Abbreviations:* ATP, adenosine-5′-triphosphate; mRNA, messenger ribonucleic acid; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; RPPA, reverse phase protein array; FRET, fluorescence resonance energy transfer; KAYAK, kinase activity assay for kinome profiling; LMD, laser microdissection.

* Corresponding author at: Division of Pediatric Oncology/Hematology, Department of Pediatrics, Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands. Tel.: +31 50 3614213; fax: +31 50 3614235.
*E-mail address:* e.s.j.m.de.bont@bkk.umcg.nl (E.S.J.M. de Bont).


doi:10.1016/j.critrevonc.2011.05.002

Recent advances in the development and application of array-based peptide-substrate kinase activity screens show great promise in overcoming the discrepancy between the evaluation of aberrant cell signaling in specific malignancies or even individual patients and the currently available ensemble of highly specific targeted treatment strategies. These developments have the potential to result in a more effective selection of kinase inhibitors and thus optimize mechanism-based patient-specific therapeutic strategies. Given the results from current research on the tumor kinome, generating network views on aberrant tumor cell signaling is critical to meet this challenge.


*Keywords:* Systems biology; PamChip; PepChip; Kinome; Kinase; Kinase activity screening; Proteomics; Cancer

## 1. Introduction

Cancer is defined as an uncontrolled proliferation of clonally derived cells. This malignant transformation is characterized by changes in the expression and activity of key mediators of signal transduction coordinating proliferation, migration and cell death [1]. The tremendous significance of changes in cell signaling activity in the malignant transformation of cells has only been fully recognized in the last decade [2–4]. The identification of disease-related signal transduction effectors culminated into the acknowledgement of protein kinases as one of the most important classes of potential drug targets to date [5]. Significant advances in the development of small molecule inhibitors to counteract aberrant cell signaling promoting tumor cell proliferation have been made since then. Kinase signaling, because of the excellent specific drugability of the ATP-binding pocket, as well as the general importance in virtually all signal transduction pathways, holds great promise here. The first breakthrough was the approval of the Abl kinase inhibitor Imatinib for treatment of BCR-Abl positive chronic myeloid leukemia [6]. Application of this inhibitor is at present part of first-line treatment for chronic myeloid leukemia and has improved patient outcome dramatically.

Despite the successful application of kinase inhibitors for the treatment of specific malignancies, tumor resistance due to acquired mutations in downstream effectors of the targeted molecule and toxicities as a result of limited agent specificity remain important challenges to overcome [7–13]. Insufficient ways to predict the efficacy of inhibiting specific kinase activity gives rise to a discrepancy between the evaluation of aberrant cell signaling and the currently available ensemble of highly specific targeted treatment strategies. Hence, providing better ways to predict the efficacy of kinase-targeted small molecule inhibitors in the tumor- or patient-specific situation will be one of the most important goals of cancer research in the upcoming decade. Accurate identification of tumor type-specific or even patient-specific aberrations in cell signaling activity that are crucial for tumor progression, is required. In this review we provide an overview of recent developments in kinase activity screening concepts focused on achieving that goal. An introduction concerning aberrant protein kinase activity as the essence of the oncogenic phenotype will be followed by a discussion of the gap in knowledge on anomalous cell signaling in specific malignancies that is becoming ever more evident, thereby severely hampering the implementation of kinome-targeted cancer therapies. After a description of the most apparent challenges that have emerged in recent research, we will address the most encouraging strategies aimed at dissecting the cancer-specific signal transduction network. Promising research focused on allowing us to obtain a better grip on the selection of effective and specific kinome-targeted cancer treatment strategies will be evaluated. Future investigational topics will be discussed.

## 2. Molecular biology of cancer cells: targeting signaling kinases

Up until the last decade, cancer research essentially relied on the expression of messenger RNA and, to a lesser extent, protein to elucidate changes in cell signaling [14]. Microarray applications have made it possible to simultaneously study the expression of a tremendous number of genes, thus providing a network view on gene expression. The concept of personal genomics in individualized medicine is aiming at the assessment of risk and the selection of therapeutic approaches based on the individual genetic signature [15–17]. Successful identification of gene expression phenotypes mirroring a clinically relevant genotype has been reported for numerous malignancies. In medulloblastoma, gene expression profiling resulted in the identification of five (recently re-defined as four) medulloblastoma subtypes with a distinct gene expression profile [18–20]. However, a link with patient outcome has not been established yet. In breast cancer gene expression signatures have been proven useful in the prediction of clinical outcome [21–24]. A gene expression profiling approach recently made it to clinical practice in the risk stratification of patients to evaluate the application of adjuvant systemic therapy in breast cancer treatment [25–27]. Furthermore, Her2 and estrogen receptor expression levels in addition to BRCA mutational status are well established prognostic markers for breast cancer treatment. Nonetheless, the consistency of the gene expression profiles deals with a tremendous interpatient variability preventing stable patient stratification [28,29]. Moreover, gene expression only partially reflects the expression and activity of cell signaling mediators.

Not only gene and protein expression levels but rather the posttranslational modification of proteins is the determining factor in the eventual phenotype [30]. During or after translation modification of proteins can occur on multiple