### Introduction

Treatment of patients with metastasized renal cell carcinoma (RCC) with Tyrosine Kinase Inhibitors (TKI) leads to disease stabilization and disease response in a substantial percentage of patients. The increase in progression-free survival is considerable, but the effect on overall survival is less clear. Additionally, many patients experience mild to severe toxicity, which may even lead to dose reduction. In view of the heterogeneous response it would be beneficial to stratify patients to delineate e.g. responder versus non-responder patients. To achieve this goal of subclassification of patients based on testing tissue material obtained before they will be treated with the TKI, response biomarkers need to be identified. The biomarker method used here is based on kinase activity profiling which is at the biological level these drugs act (fig 1, 2 and 3).

### Materials and Methods

Tissue was collected immediately after surgery, snap-frozen and stored at -80°C until use. Approximately 50-100 mg tissue was lyzed and tyrosine kinase activity of paired normal kidney tissue and RCC tissue was measured on PamChip® Peptide micro-arrays (fig 4) using 5-7 µg of total protein per test, containing 144 peptides derived from known phosphorylation sites in Protein Tyrosine Kinase substrates.

### Results

RCC showed unique, individually distinct tyrosine kinase activity patterns (fig 5a), with 4/5 RCC demonstrating much higher kinase activity then the parallel normal kidney tissue. Phosphorylation of multiple peptides, e.g. derived from platelet-derived growth factor B (PDGFB) were identified, indicating that this approach could guide the selection of TKI treatment.

### Conclusions and Discussion

Our results show that tyrosine kinase activities of individual RCC specimens can be measured accurately with this chip technology. Importantly, amongst peptides that differed between RCC and normal kidney, tyrosine kinase protein targets of which phosphorylation is known to be upregulated in RCC (e.g. PDGFB) were identified, indicating that this approach could identify appropriate target proteins. Despite the difference in individual tyrosine kinase activity, it appears that stratification of RCC with different kinase activity can be achieved. This suggests that it may be possible to stratify RCC patients based on their tyrosine kinase activity profile. Such stratification might be helpful in guiding treatment strategies.

We are currently studying the effects of TKI on the tyrosine kinase activity in vitro (fig 5b and c), which might allow us to guide the selection of TKI treatment.

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