A promising approach towards personalized targeted therapy for pancreatic cancer using an innovative kinase activity profiling assay

Savithri Rangarajan¹, Ignace H. de Hingh², Geert-Jan Creemers², Adriana van den Brule³, Jan van der Hoeven¹

Introduction

Targeted therapy with protein tyrosine kinase inhibitors (PTKIs) has emerged as a beneficial treatment option for various cancers. Since only very few patients respond to treatment it would be advantageous to develop technology for patient selection that could predict patient response to PTKIs. By kinase activity profiling, we aimed to differentiate PTKI responses among pancreatic tumours, sourced both directly from patients and human xenografts models fairly predictive of drug efficacy in humans.

Methods

➢ Assay: The innovative phosphorylation-based kinase activity profiling assay of the PamGene platform uses porous flow-through PamChip arrays comprising of 144 tyrosine-containing peptides derived from known human kinase phosphorylation sites (Figure 1; www.pamgene.com).

➢ Xenograft model: Kinase activity profiles were generated from protein lysates of 18 human pancreatic tumour xenografts (from Oncotest GmbH, Germany; www.oncotest.de). Select lysates were exposed ex vivo to various PTKIs, including erlotinib, dasatinib, sunitinib and sorafenib, at EC50 concentrations determined on-chip using a pooled reference lysate. In vivo efficacy studies were performed at Oncotest, for select xenografts that were differentially inhibited in the PamChip assay by erlotinib (70 mg/kg/d) and dasatinib (70 mg/kg; Data not shown).

➢ Patient tumours: Tumours snap-frozen directly after surgery² were used to prepare protein lysates for which kinase activity profiles were generated by ex vivo treatments with various PTKIs.

➢ Data analysis: PamGene’s EVOLVE and BioNavigator (BN) software were used to quantify signals and analyse data, and also Excel, GeneSpring, R-plugs-ins with BN etc.

Results and discussions

Representative results are presented and discussed in Figures 2 to 4. Inhibition ratios of the PTKIs could be used to rank the ex vivo sensitivity of different tumours, possibly predictive of the in vivo outcome. To enable correlation with these ex vivo signatures, in vivo efficacy studies with select corresponding Xenograft mouse models were performed. Inhibition of kinase activity ex vivo by erlotinib correlated with in vivo data: PAXF 546 and PAXF 1869 showed highest inhibition in the PamChip assay relating to the highest reduction in median relative tumour volume in vivo. Extent of tumour growth inhibition in vivo are similar to the ranking patterns ex vivo. PAXF 1876, which also expresses very high p-AKT (Oncotest historic data), is least inhibited ex vivo but responds to treatment at a later stage in vivo. Results are under further investigation, with insights into molecular mechanisms, development of resistance, and prediction models. Dasatinib which is highly potent and non-selective in the PamChip assay is under further investigation.

Conclusions

Kinase activity profiles of pancreatic tumours exposed to various kinase inhibitors ex vivo could identify differences in sensitivity to different PTKIs. The ex vivo profiles correlated well with in vivo responses in tumour-bearing mice, for erlotinib. Though it is not entirely clear if treatment outcome for all or only PTKIs that elicit differential kinase activity inhibition profiles can be predicted, the method paves the way for patient selection and to enable prediction of treatment outcome individually without extensive activity inhibition profiles can be predicted, the method paves the way for patient selection.

Acknowledgments

We would like to thank PamGene International B.V., NL, for their support and collaboration, and Prof. Dr. Bob Pinedo, NL, for his guidance.

Email: savithri.rangarajan@vitromics.nl

References

¹ Vitromics Healthcare Services B.V., ’s-Hertogenbosch, ² Catharina Ziekenhuis, Eindhoven, ³ P.A.M.M. (Regional Institute of Pathology), Eindhoven

Figure 1. PamGene technology for kinase activity profiling

The novelty of the assay is the generation of a kinetic read-out of ATP-dependent kinase activity profiles in real time.

Figure 2. Kinase activity inhibition profiles for PTKIs in human pancreatic tumours

The same PCA condition plot coloured by PTKI or lysate source shows variance in kinase inhibition profiles of the PTKIs rather than lysate source. Kinase activity profiles (inhibition ratios) were comparable between human pancreatic tumours that were from xenografts or patient-derived, promising for inter-model extrapolation.

Figure 3. % inhibition in patient tumours treated ex vivo with erlotinib (10 μM)

Figure 4. Correlation of erlotinib ex vivo kinase activity profiles of xenograft lysates with in vivo efficacy studies in mice bearing the same xenografts

a. Inhibition ratios (Erlotinib 40 μM/ 1% Dmso, expressed as % inhibition) in select xenograft lysates treated ex vivo in the PamChip assay showing the number of peptide phosphorylation sites significantly inhibited (T-test p value 0.05) in the PamChip assay

b. In vivo efficacy studies (Route P.O.: Erlotinib 70 mg/kg/day; vehicle 0.3% carboxymethyl cellulose, 0.1% Tween 80, 10 ml/kg/day), showing relative tumour volumes and body weights in select xenograft lysates implanted in nude mice.

Induction time between tumour implantation and randomization were 29, 56, 19, 22, 27 and 27 days in the order shown. Group sizes were 7 and 4 mice in the treatment and control groups respectively.

For dasatinib, all the 6 xenografts showed potent and non selective inhibition in the ex vivo PamChip assays and differential kinase activity inhibition profiles could not be established. In the in vivo efficacy studies, we observed differences in response profiles; PAX 546 and PAX 346 were "responsive" but less than erlotinib; PAXF 1876 and 1881 responded better than erlotinib; PAXF 1900 responded similar to erlotinib, and PAXF 1890 did not respond to dasatinib treatment. Currently investigation is in progress to further evaluate the dasatinib profiles both ex vivo and in vivo.
Healthcare Services

Targeted therapy with protein tyrosine kinase inhibitors (PTKIs) has emerged as a beneficial treatment option for various cancers. However, only very few patients respond to treatment and time is a critical factor in pancreatic cancer therapy since it progresses rapidly. Therefore, it would be advantageous to develop technology for patient selection that could predict patient response to PTKIs quickly without extensive analysis of mutation status and numerous biomarker assays. The goal of the current study is to differentiate PTKI responses among pancreatic tumours, sourced both from direct patient tumours and human xenograft models that retain important biological features of the tumour of origin and can be highly predictive of drug efficacy in humans. The innovative phosphorylation-based kinase activity profiling assay of the PamGene platform uses porous flow-through PamChip arrays comprising of 144 tyrosine-containing peptides derived from known human kinase phosphorylation sites. The novelty of the assay is that kinase activity in tumour lysates can be measured in a real-time kinetic read-out by detection of peptide phosphorylation. Kinase activity profiles were generated for lysates of pancreatic tumours that were exposed ex vivo to various PTKIs including erlotinib, dasatinib, sunitinib, sorafenib, crizotinib and bosutinib. Our data shows that phosphorylation of well-known downstream targets of the PTKIs was inhibited, for example, the Src and ephrin family substrates by dasatinib, also the most inhibitory in the assay. No significant differential Kinase activity was evident in the absence of PTKIs. Inhibition ratios of the PTKIs could be used to rank the ex vivo sensitivity of different tumours, possibly predictive of the in vivo outcome. To enable correlation with these ex vivo signatures, in vivo efficacy studies with corresponding xenograft mouse models are in progress and preliminary results indicate corroboration. Initiatives are also underway for clinical intervention trials to select targeted treatment for patients for whom no standard treatment is available, based on ex vivo kinase activity inhibition profiles. Patient follow-up would indicate prediction accuracy. Conclusion: Kinase activity profiles of pancreatic tumours exposed to various kinase inhibitors ex vivo could identify differences in sensitivity to different PTKIs. The method paves the way to enable prediction of treatment outcome individually and could potentially be used to guide therapeutic decisions in the selection of the most effective kinase inhibitor(s).

S. Rangarajan¹, I.H.T. de Hingh², G.J. Creemers², A.J. van den Brule³, J.C.M. van der Hoeven¹

¹ VitrOomics Healthcare Services B.V., 's-Hertogenbosch, ² Catharina Ziekenhuis, Eindhoven, ³ P.A.M.M. (Regional Institute of Pathology), Eindhoven

For further information, please contact Dr. Savithri Rangarajan, VitrOomics Healthcare Services, PO Box 3061, 5203 DB 's-Hertogenbosch, The Netherlands Telephone: +31 73 627 4520 Fax: +31 73 627 4525 Email: savithri.rangarajan@vitromics.nl