MammaPrint 70-gene test using targeted RNA sequencing
Lorenza Mittempergher1, Jacob B Spangler2, Mireille Hi Snel3, Leonie JMJ Delahaye1, Anke T Witteveen1, Nick Chen2, Bob Chan2, Diederik Wehkamp2, René Bernards1,4, Annuska M Glas1

Department of Research and Development, Agendia NV, Amsterdam, the Netherlands; Department of Product Support, Agendia Inc, Irvine, California, US; Department of Molecular Carcinogenesis, The Netherlands Cancer Institute NKI-AVI, Amsterdam, the Netherlands.

BACKGROUND

MammaPrint 70-gene test [1]• In vitro diagnostic microarray-based test to assess a patient’s risk for distinct metastasis within 5 years• Low Risk and High Risk results with no intermediate or indeterminate category• FDA 510(k) clearances and CE marking for fresh and FFPE tissues on microarray

BluePrint 80-gene test [2]• In vitro diagnostic microarray-based test to assess breast cancer molecular subtypes (Luminal-type, HER2-type, Basal-type)

Next Generation RNA-Seq technology [3,4]• Is becoming a standard method for transcriptome analysis• Low background signal with a large dynamic range of expression levels• Multiple ongoing efforts to establish benchmark standards for technical and analytical best practices• Potential to revolutionize clinical testing

Advantages of MammaPrint 70-gene test on RNA-Seq platform• Decentralized setting: “in-house solution” to hospitals without compromising the level of clinical utility• Easier reimbursment process thanks to local processing of the sample• Involvement of countries with ethical restriction for the exchange of patient material

METHODS

Development of the MammaPrint and BluePrint diagnostic test from the microarray to the RNA-seq platform using NGS targeted RNA sequencing technology (RNA-Seq).

- Total RNA isolated using Qiagen RNeasy FFPE kit
- 85 FFPE samples processed with both on Microarray and RNA-Seq technologies
- 43 FFPE samples underwent two independent RNA isolations and processed with RNA-Seq technology
- 1 FFPE control samples measured over time and sequenced in 14 consecutive runs
- Gene counts (reads) for NGS normalized using Counts per Million (CPM) method

RESULTS

- MammaPrint 70-gene and BluePrint 80-gene signatures successfully mapped to the RNA-Seq genes
- On average 1.2 million reads assigned to gene per sample (15 samples on average per run)
- 96.3% reads were mapped to genes (hg19 build 37) with 74.8% reads on-target
- High correlation between the MammaPrint index calculated using the RNA-Seq data and the correspondent Microarray data (Pearson’s correlation=0.98) in 85 FFPE samples (Figure 1)
- High correlation between the BluePrint indices calculated using the RNA-Seq data and the correspondent Microarray data (Luminal Pearson’s correlation=0.98, Basal Pearson’s correlation =0.98, HER2 Pearson’s correlation =0.94) in 85 FFPE samples
- High correlation between RNA-Seq MammaPrint indices derived from two independent RNA isolations (Pearson’s correlation=0.99) for intratumor heterogeneity assessment (Figure 2)
- High index reproducibility of 14 consecutive assessments of 1 FFPE control samples (standard deviation ± 0.03) (Figure 3)

CONCLUSION

Preliminary analyses show that FFPE MammaPrint and BluePrint gene signature readouts generated from Targeted RNA-Seq technology, are highly comparable to the microarray diagnostic test readouts in a series of 85 FFPE early-brest cancer samples. Further work assessing the stability and reproducibility are ongoing

REFERENCES


Contact: Lorenza.Mittempergher@agendia.com, Annuska.Glas@agendia.com