OneCell Diagnostics
OncoPredikt HRD
Predicting Genomic Signatures

GENETICS INSTITUTE OF AMERICA

OncoPredikt HRD

AI-enabled prediction of Homologous Recombination Deficiency (HRD)

Screen.
Predict.
Treat.

AI enabled rapid, precise and cost effective HRD testing

Identifying patients that respond well to Immunotherapy, PARP Inhibitors, platinum therapies

Avoid the potential adverse effects

Introduction

Homologous recombination deficient (HRD) tumours are highly responsive to platinum-based chemotherapy and poly (ADP-ribose) polymerase inhibitor (PARPi) therapy. Various PARPi inhibitors have been FDA approved to treat breast, ovarian, pancreatic and prostate cancers while growing evidence also suggest its relevance in gastric, endometrial, colon, bladder, lung, as well as other solid tumors.

HRD testing is currently performed using diagnostic laboratory tests which rely heavily on Next Generation sequencing (NGS) method. Though definitive, their time-consuming and expensive nature may leave a large cohort of patients frustrated if the test results rule them out for PARPi therapies.

In our mission to provide affordable precision therapy to patients, we have developed a rapid, precise and cost-effective AI/ML enabled image based HRD testing tool 'OncoPredikt-HRD' which can screen for patients who are likely to be HRD positive and benefit from PARPi therapies and should, therefore, undergo NGS testing.

Significance of HRD for PARPi therapy

Homologous Recombination Deficiency (HRD) generally occurs when genes involved in the HRR pathway are altered or disrupted and the cells are unable to carry out DNA repair. In tumor cells where backup DNA repair mechanisms are impaired, PARP inhibitors can be used to inhibit action of PARP enzymes which mediate DNA repair of tumor cells making HRD an important biomarker to identify patients who may benefit from PARPi therapy.

What is the rationale for identifying HRD tumor patients?

Traditionally, HRD status has been mainly linked mutations in BRCA1 and BRCA2 genes. Several studies have now established other key biomarkers in determining HRD status (Figure 1).

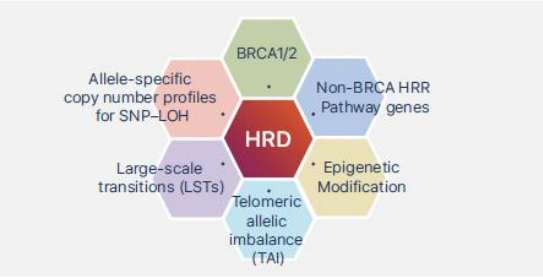

Figure 1: Relevant Biomarkers for HRD status identification and PARPi treatmenteligibility.

BRCA1 and BRCA2 alterations

Many germline and somatic contributors to HRD have been identified and extensively studied in the recent past. Germline BRCA-1 and BRCA-2 alterations are key members of the HR DNA repair pathway and majority of HRD tumors occur in these cases, however, somatic BRCA-1 and BRCA-2 mutations as well as other mutations in the HR pathway can also be indicative of HRD.

Genomic Instability

HRD also gives rise to specific patterns of mutations, Indels, Copy number variations and structural rearrangements. The larger genomic alterations give rise to Genomic Instability and comprises of Loss of heterozygosity, Telomeric allelic imbalance and large scale state transitions all of which are also predictive of HRD.

Loss of Heterozygosity

Loss of Heterozygosity (LOH) is the loss of one of the two alleles at a heterozygous locus. HRD impairs normal DNA damage repair which results in loss or duplication of chromosomal regions and causes genomic loss of heterozygosity (LOH). Therefore, LOH is a relevant biomarker of HRD.

Telomeric allelic imbalance

Telomeric allelic imbalance, another known predictor of HRD, is caused by unequal contribution of paternal and maternal allele sequences regardless of whether the copy number has changed and is defined as the number of allelic imbalance regions which extend to the telomere but do not cross the centromere. They are significantly present in tumors with BRCA1 and BRCA2 deficiency as well as tumors which are sensitive to platinum chemotherapy.

Large scale transitions

Large scale transitions, another predictor of HRD, is the number of chromosomal break points that generate fragments of ≥10Mb. Their presence has been established in breast cancer and cell lines with BRCA mutations.

Epigenetic modifications

Epigenetic modifications are also being studied as an important contributor to HRD. For example, interactions with different proteins in the DNA repair pathway or promoter methylation can lead to silencing of BRCA1 and BRCA2 genes and lead to HRD.

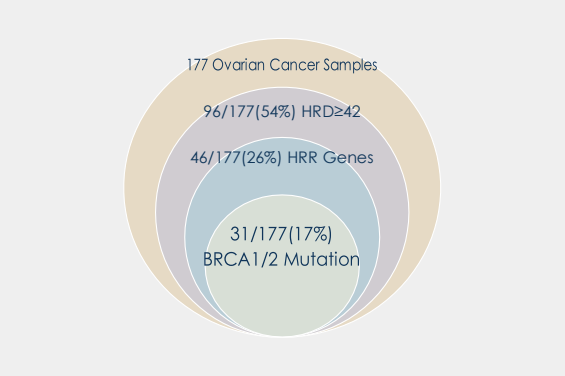

Figure 2: Diagrammatic representation showing the distribution of each category used as basis for HRD selection. Note here that our training model for building the AI enabled tool was a comprehensive panel containing multiple genomic signatures.

Challenges associated with HRD prediction using NGS testing

HRD testing, currently performed using next generation sequencing, poses the following drawbacks:

- It can take 2-4 weeks for results.
- It has a high failure rate.
- It requires significant amount of tissue.
- It is costly.
- There may be sample QC rejection due to low DNA quantity in many cases.
- It involves multitechnique processes such as NGS + MLPA + Chromosomal Microarray.

All above mentioned reasons make prediction of HRD inconvenient and less reliable.

Solution: OncoPredikt-HRD 'An AI Enabled HRD Testing Tool'

As a solution to the long drawn and inefficient process of HRD testing, we have developed and tested the ability of an AI enabled platform 'OncoPredikt-HRD' to predict HRD status. The tool can predict HRD status using whole slide imaging analysis of the diagnostic H&E slide (Figure 3) to identify all key biomarkers involved including BRCA1 and BRCA2 mutations, other mutations e.g. HRR genes, genomic instability markers including LOH, TAI and LST as well as epigenetic modifications.

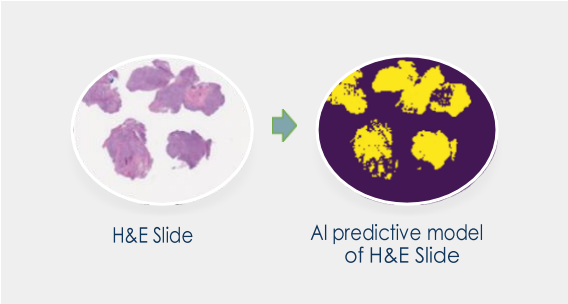

Figure 3: Diagram representing the AI enabled tool 'OncoPredikt-HRD' to predict key genomic signatures from whole-slide images.

This platform, OncoPredikt-HRD is rapid, precise, and cost effective. The AI model was trained on 120 H&E slides that were used to identify tumour prior to manual microdissection for HRD assessment by NGS.

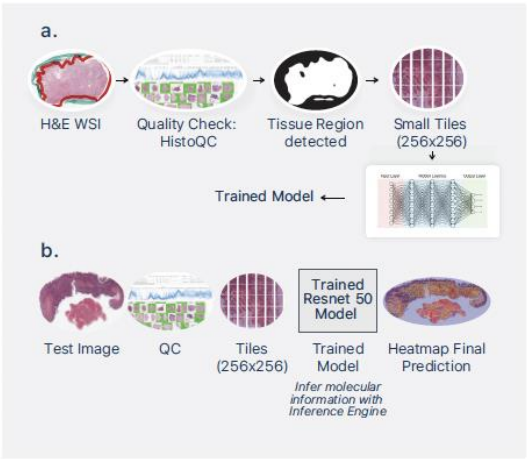

Figure 4: Training model for HRD prediction (a), Inference engine for HRD prediction (b)

Figure 4 describes the process flow for HRD prediction using OncoPredikt-HRD. Following quality checks on the H&E slide to ensure good quality data (checks for cellular morphology, removal of non-cellular entities etc.), the region of interest in the tissue was identified and the image was then fragmented into multiple smaller segments (256x256 smaller tiles).
Histopathological features were extracted, followed by feature mapping to predict HRD status based on the results of NGS testing (Figure 4a). ResNet AI algorithm was trained to segment, annotate and predict HRD status. The trained model was able to accurately infer molecular information with the inference engine (Figure 4b).

Clinical Testing of OncoPredikt-HRD AE Model

The AI engine was first trained on TCGA datasets (1892 images). WSI were randomly split into training and validation sets designated either HRD or HRP based on, BRCA1/2, HRR genes and previously generated aggregate combined HRD score from:

- Loss of heterozygosity (LOH)
- Large scale genomic instability (LST)
- Telomeric allelic imbalance (TAI)

120 Whole Slide Images (WSI) which were already tested for HRD status by Ambry Genetics, USA (already identified tumor prior to manual microdissection for HRD Assessment by NGS) were used for training, validation and testing.
Histopathological features were extracted, followed by feature mapping to predict HRD status based on the results of NGS testing.

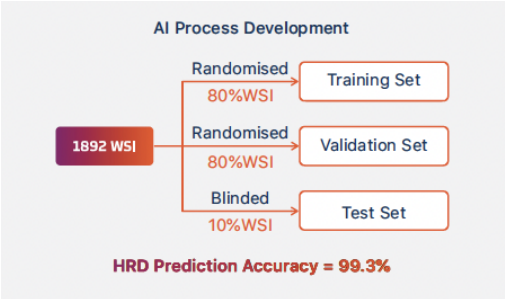

Figure 5: Clinical testing of the OncoPredikt tool demonstrated a 99.3% HRD Prediction accuracy in single blinded clinical samples.

ResNet AI algorithm was trained to segment, annotate and predict HRD status. 80% of the randomized dataset was used for training while 10% of the randomized dataset was used for validation studies of the AI model. The final 10% data was used for the test set wherein using single blinded clinical samples, OncoPredikt-HRD tool detected HRD positive samples with 99.3% accuracy along with 100% sensitivity and 99% specificity (Figure 5).

ROC/AUC curves for the samples demonstrated robustness of our model wherein a high true positive rate and negligible false positives were observed with a prediction time of a few minutes (Figure 6).

Figure 6: Receiver operating characteristic ROC/AUC curves for HRD

Patch-level predictions of HRD status demonstrated intra-tumor heterogeneity within the H&E slides. Visual inspection of the heatmap suggested the presence of patches
With high predictive ability of HRD status and this outperformed an average HRD score for slides with heterogeneity.

Conclusions

AI-enabled prediction of HRD status can be accurately performed on diagnostic H&E slides potentially yielding results quickly and affordably, even when limited tissue is available for testing, thus, providing a reliable and accurate screening tool to classify HRD status. Using our clinically and analytically validated OncoPredikt-HRD AE based NGS enabler tool can enable clinicians to predict HRD status and identify patient candidates likely to benefit from PARPi therapy before they undergo expensive and time- consuming NGS testing.