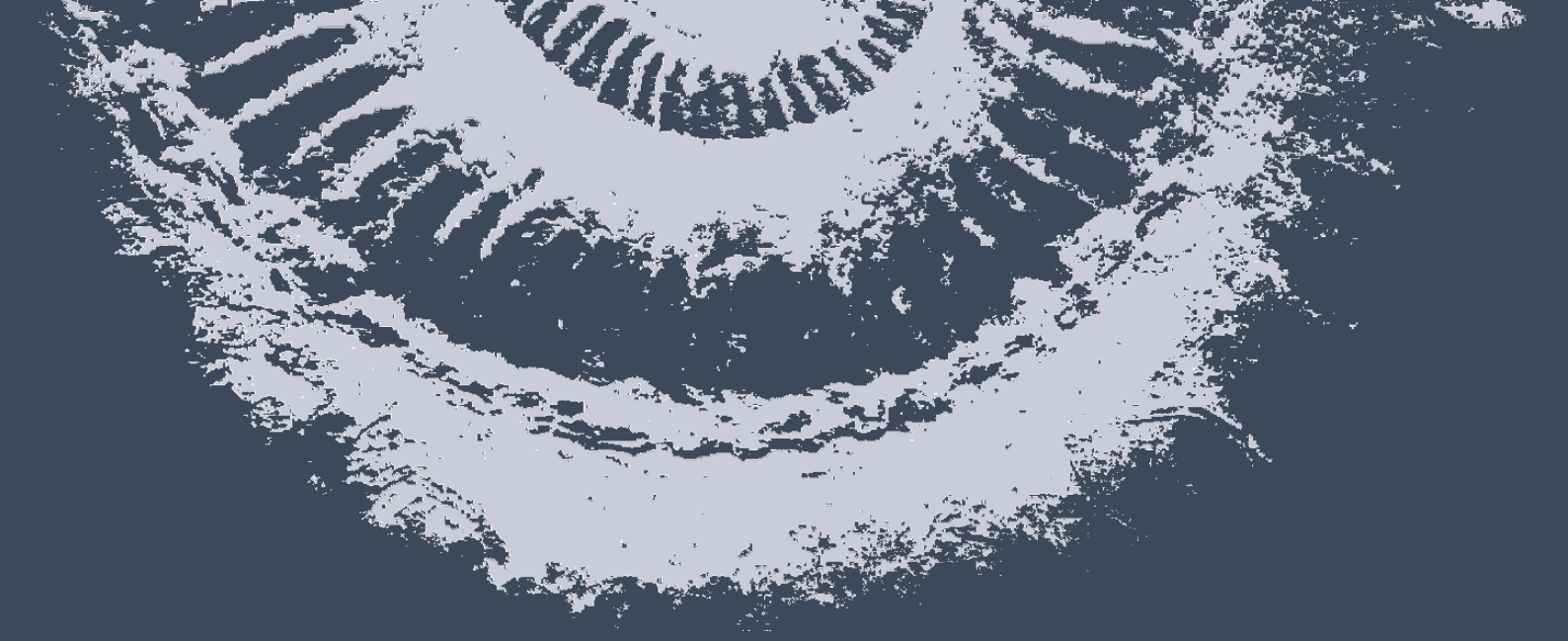


IOpener®

Immunotherapy guidance tests

PamGene

IOpener® - General Information

Introduction

Immunotherapy through the use of PD-1 and PD-L1 antibodies have revolutionized cancer therapy, with remarkable efficacy in the treatment of several cancers. Not all patients however respond to immunotherapy and some develop severe, potentially life-threatening toxicities. A diagnostic test which can be implemented clinically to predict immunotherapy response in advanced cancer patients can therefore be a supportive tool to the oncologist in selecting the best available treatment option for their patients.

PamGene has developed two blood-based IOpener® immunotherapy guidance tests for advanced stage melanoma and non-small cell lung cancer (NSCLC) patients. These tests provide a predictive score which should be used by the physician in conjunction with other laboratory and clinical findings as an aid to assess the likelihood of a patient's response to anti-PD-1 therapy. The tests have been registered as CE-IVDs in the European Union in May 2022 under the in-vitro diagnostic medical device Directive (98/79/EG) by the Dutch Ministry of Health, Welfare and Sport.

Test panel	Article code	IVDD registration number
IOpener®-*melanoma*	60100	NL-CA002-2022-70593
IOpener®-*NSCLC*	60110	NL-CA002-2022-70594

TEST PRINCIPLE:

The IOpener®-*melanoma* and IOpener®-*NSCLC* tests employ a Protein Tyrosine Kinase assay (PTK assay) to generate a kinase activity profile from the PBMC sample. Kinases in the sample actively phosphorylate peptide substrates on the PamChips microarray in the presence of ATP. A FITC-conjugated PY20 antibody is used to detect the peptide substrate phosphorylation. The test must be performed by professional users specifically trained by PamGene and according to PamGene's instructions and protocols in a dedicated laboratory. From the quantified kinase activity profile, a predictive score is calculated with the IOpener®-*melanoma* or IOpener®-*NSCLC* Analysis Software using a fixed pre-calibrated model.

REFERENCES:

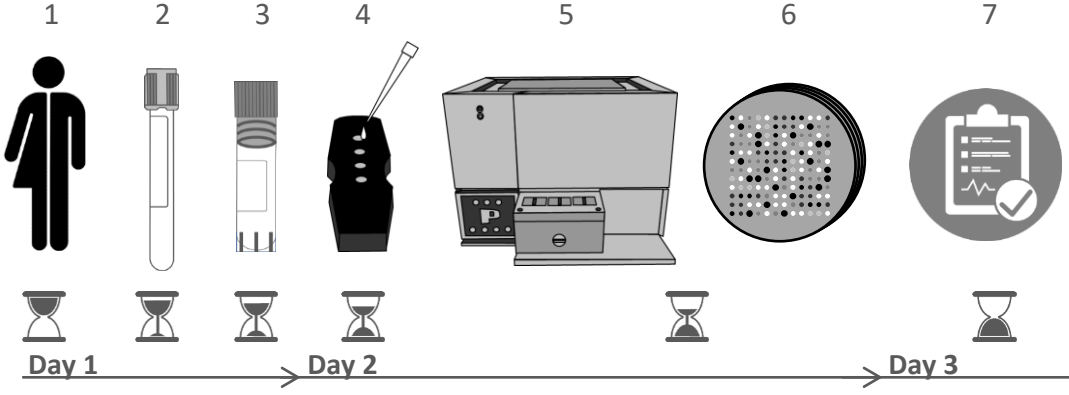
Hurkmans DP, Verdegaal EME, Hogan SA, et al. Blood-based kinase activity profiling: a potential predictor of response to immune checkpoint inhibition in metastatic cancer. J Immunother Cancer (2020)

De Joode, K. et al. Tyrosine kinase activity profiling as a predictive biomarker for clinical benefit to immune checkpoint inhibition in advanced melanoma and NSCLC. Ann. Oncol. 31, S725–S734 (2020).

De Joode, K. et al. The IOpener study: Tyrosine kinase activity in peripheral lymphocytes to predict durable response to immune checkpoint inhibition in patients with advanced melanoma. Ann. Oncol. 33, S356–S409 (2022).

IOpener® Workflow

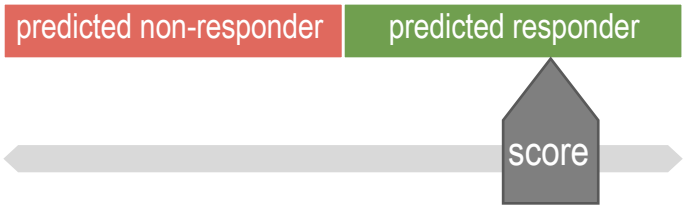
1 - Patient appointment
2 - Heparin blood-sample collected
3 - PBMC immune cell isolation
4 - Cell lysate of PBMCs loaded onto PamChip microarrays
5 - PamChips loaded in PamStation
6 - PamChip microarray images recorded
7 - Report generated with IOpener® Analysis Software



IOpener®-*melanoma*

Intended Use

- The IOpener®-*melanoma* is a semi-quantitative in-vitro diagnostic device to predict the likelihood for response to therapy with PD-1 checkpoint inhibitors monotherapy or in combination with CTLA4 inhibitor for advanced stage melanoma patients.



- The IOpener®-*melanoma* test result is reported as a predictive score for prediction of Durable Response (≥ 40 = Responder, < 40 = Non Responder).

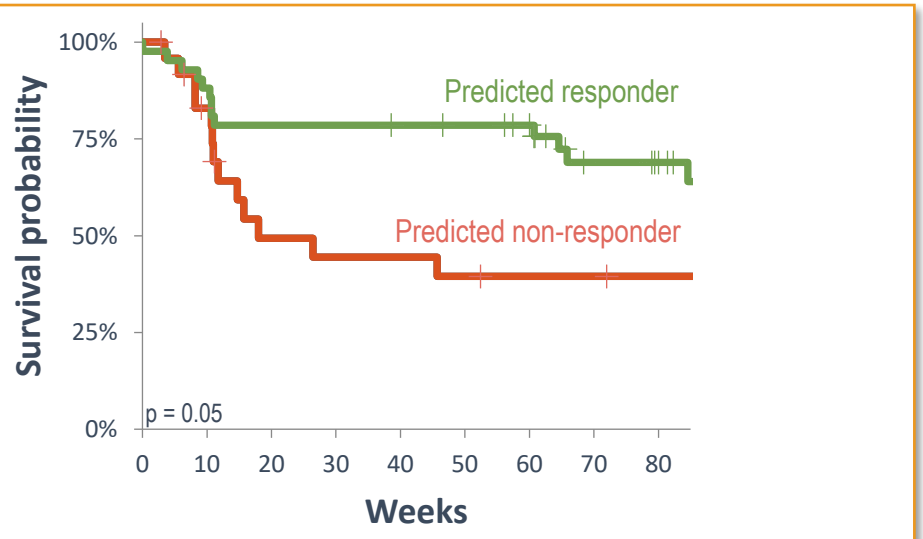
The test is based on measuring the kinase activity profile of peripheral blood mononuclear cells (PBMCs) obtained from a whole blood sample collected from a patient before start of the anti-PD-1 therapy.

The test result is provided as a predictive score which should be used by the physician in conjunction with other laboratory and clinical findings as an aid to assess the likelihood of a patient's response to anti-PD-1 therapy.

IOpener®-*melanoma* is intended for professional use only and is performed as a not-automated IVD assay service in a dedicated laboratory facility.

Clinical Validation

- The IOpener®-*melanoma* test was evaluated in a prospective multi-center study with the University Medical Center Utrecht, Isala Oncology Center, Amphia Hospital, Erasmus Universityand Leiden University Medical Center.
- Accuracy in independent validation cohort is 70-76 % for immune treatments. Hazard Ratio (HR) for the survival analysis ranges from 1.4 to 2.1.
- In conclusion the IOpener®-*melanoma* test is predictive for disease progression in melanoma patients treated with anti-PD1.



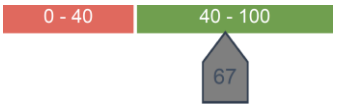
Note: The Kaplan Meier is based on the melanoma validation studies in combination with combined cohorts and a DLM model.

IOpener® Report (example)

IOpener®-melanoma report for test number: --

Patient ID	--	Physician ID	--
Blood Tube ID	--	E-mail adress	
IOpener©-LOT	--	Insitution Name	
		Institution Address	

IOpener®-melanoma testing results



In the group of treatment-naïve advanced melanoma patients undergoing first line immunotherapy with PD-1 checkpoint inhibitors for whom response was predicted, 73% (CI95 = 57-86%) showed a Durable Response[1] to the treatment. The median Progression Free Survival in this group was not reached (> 84 weeks, the lower bound of the 95% confidence interval).

Final result: predicted Response

IOpener®-melanoma was evaluated on a group of 61 treatment-naïve advanced melanoma patients who were treated with first line nivolumab, pembrolizumab, or nivolumab in combination with ipilimumab. The sensitivity for selecting patients who showed a Durable Response[1] was 81% (CI95: 65-92%). The specificity was 54% (CI95: 33-74%).

[1] Durable Response: A patient is a durable responder when a Partial Response (PR) or Complete Response (CR) occurs in the first year after start of the PD-1 checkpoint inhibitor treatment and lasts at least 6 months.

IOpener®-melanoma intended use

IOpener®-melanoma is a semi-quantitative in-vitro diagnostic device to predict the likelihood for response to therapy with PD-1 checkpoint inhibitors (e.g., pembrolizumab, nivolumab, nivolumab in combination with ipilimumab) for treatment-naïve advanced stage melanoma patients. The test is based on measuring the kinase activity profile of peripheral blood mononuclear cells (PBMCs) obtained from a whole blood sample collected from a patient before start of the PD-1 therapy. The test result is provided as a predictive score which should be used by the physician in conjunction with other laboratory and clinical findings as an aid to assess the likelihood of a patients' response to PD-1 therapy. IOpener-melanoma is intended for professional use only and is performed as a not-automated IVD assay service in a dedicated laboratory facility.

Disclaimers and Feedback

- This IOpener®-melanoma report is addressed to the treating physician at the institution and address listed above.
- The data on which this report is based is generated from IOpener® tests that are performed under Quality Management System of PamGene International B.V. and have passed all internal quality controls.
- The data on which this report is based is generated from IOpener® tests that are performed under Quality Management System of PamGene International B.V. and have passed all internal quality controls.
- Important information and warnings for the IOpener®-melanoma test can be found in the Instructions for Use of IOpener®-melanoma.
- The result in this report is based solely on the results of the IOpener®-melanoma assay performed on the PBMCs isolated from the heparin- blood tube collected from the patient. Therefore, the result in this report should therefore always be interpreted in conjunction with other laboratory and clinical findings by the treating physician as indicated in the intended use.
- For feedback, complaints or questions about the contents of this report please contact IOpener-PMS@pamgene.com. If you have received this report in error please call PamGene at the telephone number below.

Sign off

Travel Card ID: --
Release Date and Time: --
QA department
PamGene International B.V.

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About PamGene:

PamGene International B.V. has recently intensified its efforts to develop and commercialize a blood-based immunotherapy guidance test, the IOpener®, to improve patient outcomes. The company's kinase-activity profiling technology and proprietary software algorithms support clinicians in their treatment decisions using the IOpener® assay. PamGene's robust and unique peptide microarray technology for multiplex kinase-activity profiling is also used to provide dedicated assay services for patient stratification for clinical trials, biomarker discovery, and gaining mechanistic insights needed to understand human diseases. PamGene is headquartered in 's-Hertogenbosch, the Netherlands.

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The Netherlands

IOpener®-*melanoma*

Instructions for use

REF	60100	English

INTENDED USE

IOpener-melanoma is a semi-quantitative in-vitro diagnostic device to predict the likelihood for response to therapy with PD-1 checkpoint inhibitors (e.g., pembrolizumab, nivolumab, nivolumab in combination with ipilimumab) for advanced stage melanoma patients.

The test is based on measuring the kinase activity profile of peripheral blood mononuclear cells (PBMCs) obtained from a whole blood sample collected from a patient before start of the anti-PD-1 therapy.

The test result is provided as a predictive score which should be used by the physician in conjunction with other laboratory and clinical findings as an aid to assess the likelihood of a patient's response to anti-PD-1 therapy. IOpener-melanoma is intended for professional use only and is performed as a not-automated IVD assay service in a dedicated laboratory facility.

COLLECTION HANDLING AND PREPARATION OF THE SPECIMEN

Whole blood collection, PBMC isolation, PBMC lysis and determination of protein content of PBMC samples must be performed by professional users specifically trained by PamGene and according to PamGene's instructions and protocols. The following specimen type has been tested for use with the IOpener-melanoma test: PBMCs isolated from venous whole blood containing Na-Heparin as the anticoagulant.

TEST PRINCIPLE

The IOpener-melanoma test employs a Protein Tyrosine Kinase assay (PTK assay) to generate a kinase activity profile from the PBMC sample. Kinases in the sample actively phosphorylate peptide substrates on the PamChips microarray in the presence of ATP. A FITC-conjugated PY20 antibody is used to detect the peptide substrate phosphorylation.

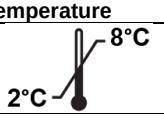
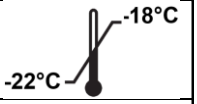
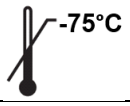
The test must be performed by professional users specifically trained by PamGene and according to PamGene's instructions and protocols in a dedicated laboratory. From the quantified kinase activity profile, a predictive score is calculated with the IOpener-melanoma Analysis Software using a fixed pre-calibrated model. The test result is reported as a predictive score between 0 and 100 for prediction of Durable Response (≧40 = Responder, < 40 = Non-Responder). The result is provided to the physician in a test report.

REAGENTS (DESCRIPTION)

The DAS PTK Reagent kit contains DL-Dithiothreitol (DTT), adenosine triphosphate (ATP), albumin bovine serum fraction V (BSA), PY20 conjugated to FITC, 10x Protein Kinase buffer containing Tris-HCl, $MgCl_2$, EDTA, Brij 35 and DTT, 10x PTK additive containing sodium phosphate dibasic and sodium phosphate monobasic. The Reference sample contains human PBMC lysate, mammalian protein extraction reagent (M-PER) and Halt Protease inhibitor Cocktail.

SOFTWARE REQUIREMENTS

BioNavigator data analysis platform.

Materials provided	Quantity	REF	Storage temperature
PamChips	4	32515	
DAS PTK Reagent kit	4	32121	
Reference sample	4	41011	
IOpener-melanoma Analysis Software	1	59100	Not applicable

Materials required but not provided	REF
PamStation 12	31500

QUALITY CONTROL

The IOpener-melanoma analysis software generates a Quality Control (QC) report for each test procedure. The results are checked and monitored for intra-run coefficient of variation (CV), concordance of the Reference sample with a reference profile, and applicability of the classification model for the measured kinase activity according to PamGene's instructions and protocols.

IN-USE STABILITY/CONTROL

Patient PBMC samples and Reference samples can be used for a maximum of 2 hours after thawing and must be kept at a temperature of 0-4°C until use.

After opening the sealed pouch PamChips can be used for up to 2 hours when stored at room temperature until use.

The DAS Reagent kit can be used for up to 6 hours when stored at a temperature of 0-4°C until use. The Reference sample must be used as a quality control for each test run. Use PamChips, DAS PTK Reagent kit and Reference sample only once.

INTERFERING SUBSTANCES

Interference testing was performed with a pool of three healthy donor PBMC lysate samples.

Substance	Maximal concentration in PBMC sample
Vemurafenib	2400 ng/ml
Heparin	6 U/ml
Ficoll	30 (% v/v)
Bilirubin	5 mg/dL
Hemoglobin	200 mg/dL

Number: 60100-002 Effective date: 2022-11-10

ANALYTICAL PERFORMANCE CHARACTERISTICS

IOpener-melanoma is a semi-quantitative test without a defined analyte. The LoD for kinase activity profiling was determined as the sample input for which the phosphorylation signal can be discriminated from the signal obtained with blank samples. The LoQ for kinase activity profiling was determined as the sample input corresponding to the lowest phosphorylation signal that can be quantified with a within-measurement CV = 20%. Measurement Range was determined as the range between the LoD and the saturation limit of the phosphorylation signal. Precision was determined as the Standard deviation (SD) of measurements.

Precision kinase activity profiling (within run, run-to-run and day-to-day variability)	SD = 0.22, CV 15%
Limit of Detection (LoD)	0.25 µg total protein/array
Limit of Quantification (LoQ)	0.50 µg total protein/array
Measurement Range (MR)	0.25 to 2.0 µg total protein/array
Sample Stability	Kinase activity of venous whole blood samples collected according to PamGene's procedures and stored at a temperature between 15-25ºC is stable for up to 30 hours between whole blood collection and PBMC isolation. PBMC isolation must be performed on the same day as venous whole blood collection.
Predictive Score Precision	SD = 2.4 (CI90 = score ± 4.1)

Precision and MR were determined using one healthy donor PBMC lysate sample. The LoD was determined using six healthy donor PBMC lysate samples. The LoQ was determined using a pool of three healthy donor PBMC lysate samples. The Sample Stability was determined using whole blood collected from four healthy donors. The Predictive Score Precision was determined using four patient PBMC lysate samples.

CLINICAL PERFORMANCE CHARACTERISTICS

IOpener-melanoma was evaluated on a group of 61 treatment-naïve advanced melanoma patients who were treated with first-line nivolumab, pembrolizumab, or nivolumab in combination with ipilimumab.

Accuracy	70% (CI95 = 57-81%)
Diagnostic Sensitivity	81% (CI95 = 65-92%)
Diagnostic Specificity	54% (CI95 = 33-74%)
Positive Predictive Value (PPV)	73% (CI95 = 57-86%)
Negative Predictive Value (NPV)	65% (CI95 = 41-85%)

In the group of treatment-naïve advanced melanoma patients undergoing first-line immunotherapy with anti-PD-1 checkpoint inhibitors for whom response was predicted, 73% (CI95 = 57-86%) showed a Durable Response to the treatment. The median Progression Free Survival in this group was not reached (> 84 weeks, the lower bound of the 95% confidence interval).
In the group of treatment-naïve advanced melanoma patients undergoing first-line immunotherapy with anti-PD-1 checkpoint inhibitors for whom Non-Response was predicted, 65% (CI95 = 41-85%) did NOT show Durable Response to the treatment. The median Progression Free Survival in this group was 18 weeks (> 12 weeks, the lower bound of the 95% confidence interval). A patient is a durable responder when a Partial Response (PR) or Complete Response (CR) occurs in the first year after start of the PD-1 checkpoint inhibitor treatment and lasts at least 6 months.

PRECAUTIONS/WARNINGS AND LIMITATIONS

- The predictive score that is provided as a result of the IOpener-melanoma test is based solely on the IOpener-melanoma kinase assay. The test result must always be judged in conjunction with the patient's medical history, other laboratory and clinical findings and used as an aid to assess the likelihood of a patients' response to anti-PD-1 therapy.
- The Clinical Performance Characteristics of IOpener-melanoma were determined for treatment-naïve advanced melanoma patients receiving immunotherapy as a first-line therapy. The test performance may be lower for patients who did not receive immunotherapy as a first-line therapy.
- For patients who have received prior therapy with kinase inhibitors there must be a period of at least two weeks between the last kinase inhibitor treatment and blood collection for the IOpener-melanoma test.
- The IOpener-melanoma test may provide incorrect results for patients with elevated (>5 mg/dL in plasma) bilirubin levels.
- The IOpener-melanoma test may provide incorrect results for patients who have received blood transfusion less than one week before collection of the blood sample.

BIBLIOGRAPHY

Hurkmans DP, Verdegaal EME, Hogan SA, et al. Blood-based kinase activity profiling: a potential predictor of response to immune checkpoint inhibition in metastatic cancer. *J Immunother Cancer*. 2020;8(2):e001607. doi:10.1136/jitc-2020-001607

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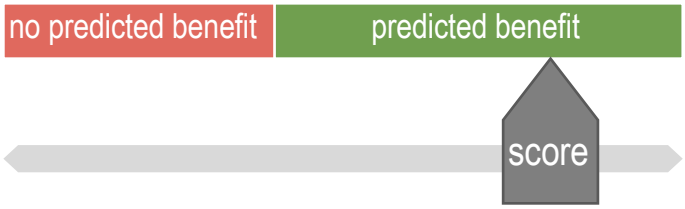
Netherlands

IOpener®-*NSCLC*

Intended Use

- The IOpener®-*NSCLC* is a semi-quantitative kinase activity profiling based in-vitro diagnostic device to predict the likelihood for response to therapy with PD-1 checkpoint inhibitor monotherapy or in combination with chemotherapy for advanced stage Non-Small Cell Lung cancer (NSCLC) patients.



- The IOpener®-*NSCLC* test result is reported as a predictive score between 0 and 100, for benefit of treatment with PD-1 checkpoint inhibitors, defined as no occurrence of Progressive Disease in the first 24 weeks after start of treatment with PD-1 checkpoint inhibitors (≥ 50 = Predicted Benefit, < 50 = No Predicted Benefit).

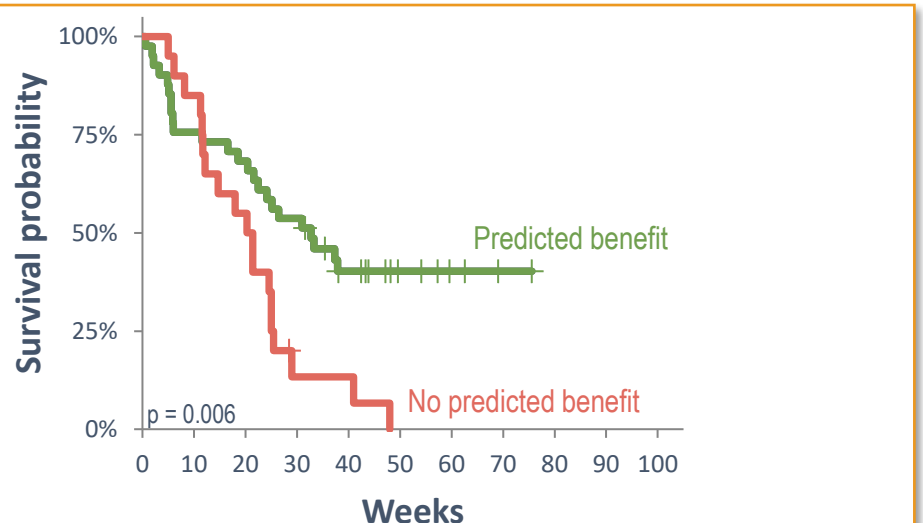
The test is based on measuring the kinase activity profile of peripheral blood mononuclear cells (PBMCs) obtained from a whole blood sample collected from a patient before start of the anti-PD-1 therapy.

The test result is provided as a predictive score which should be used by the physician in conjunction with other laboratory and clinical findings as an aid to assess the likelihood of a patient's response to anti-PD-1 therapy.

IOpener®-*NSCLC* is intended for professional use only and is performed as a not-automated IVD assay service in a dedicated laboratory facility

Clinical Validation

- The IOpener®-*NSCLC* was evaluated in a prospective multi-center study with the Elisabeth-TweeSteden Hospital Tilburg, University Medical Center Groningen, Diakonessenhuis Utrecht, Radboud University Medical Center Nijmegen, Isala Hospital Zwolle, Catharina Hospital Eindhoven, Amphia Hospital Breda, and Erasmus University Medical Center Rotterdam.
- Accuracy in independent validation cohort is 72% for patients with a Tumor Proportion Score (TPS) score <50%. Hazard Ratio (HR) for TPS score is 1.3 and HR is 2.3 when combined with IOpener.
- In conclusion the IOpener®-*NSCLC* test is predictive for disease progression in NSCLC patients treated with anti-PD1 who have a TPS score < 50%.



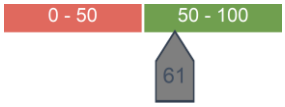
Note: The Kaplan Meier is based on a PD-L1 score combined with IOpener score obtained in the validation cohort. Patients with a TPS score <50% and a below 50 IOpener score have "No predicted benefit"

IOpener® Report (example)

IOpener®-NSCLC report for test number: --

Patient ID	--	Physician ID	--
Blood Tube ID	--	E-mail adress	
IOpener®-LOT	--	Insitution Name	
		Institution Address	

IOpener®-NSCLC testing results



For 63% (CI95: 35-85%) of patients in the group of patients with a TPS < 50% with "Predicted Benefit", Progressive Disease (PD) did not occur within 24 weeks after start of the PD-1 checkpoint inhibitor treatment. The median Progression Free Survival in this group was 33 weeks (≥ 25 weeks based on the lower bound of the 95% confidence interval).

Final result: Predicted benefit

IOpener®-NSCLC was evaluated on a group of 36 patients with NSCLC and a Tumor PD-L1 Score < 50% (TPS, the % of PD-L1 positive tumor cells), who were treated with pembrolizumab in combination with chemotherapy. The sensitivity for selecting patients who did not show progressive disease was 71% (CI95: 42-92%). The specificity was 73% (CI95: 50%-89%).

IOpener®-NSCLC intended use

IOpener®-NSCLC is a semi-quantitative kinase activity profiling based in-vitro diagnostic device to predict the likelihood for response to therapy with PD-1 checkpoint inhibitors (e.g. pembrolizumab, pembrolizumab in combination with chemotherapy) for advanced stage Non-Small Cell Lung cancer (NSCLC) patients. The test is based on measuring the kinase activity profile of peripheral blood mononuclear cells (PBMCs) obtained from a whole blood sample collected from a patient before start of the PD-1 therapy. The test result is provided as a predictive score which should be used by the physician in conjunction with other laboratory and clinical findings as an aid to assess the likelihood of a patient's response to anti-PD-1 therapy. IOpener-NSCLC is intended for professional use only and is performed as a not-automated IVD assay service in a dedicated laboratory facility.

Disclaimers and Feedback

- This IOpener®-NSCLC report is addressed to the attending physician at the institution and address listed above.
- The data on which this report is based is generated from IOpener® tests that are performed under Quality Management System of PamGene International B.V. and have passed all internal quality controls.
- The data on which this report is based is generated from IOpener® tests that are performed under Quality Management System of PamGene International B.V. and have passed all internal quality controls.
- Important information and warnings for the IOpener®-NSCLC test can be found in the Instructions for Use of IOpener®-NSCLC.
- The result in this report is based solely on the results of the IOpener®-NSCLC assay performed on the PBMCs isolated from the heparin- blood tube collected from the patient. Therefore, the result in this report should always be interpreted in conjunction with other laboratory and clinical findings by the attending physician as indicated in the intended use.
- For feedback, complaints or questions about the contents of this report please contact IOpener-PMS@pamgene.com. If you have received this report in error please call PamGene at the telephone number below.

Sign off

Travel Card ID: --
Release Date and Time: --
QA department
PamGene International B.V.

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Wolvenhoek 10 — 5211HH — 's-Hertogenbosch — the Netherlands
Phone +31 (0) 736 158 080 — info@pamgene.com — www.pamgene.com — ©2022

About PamGene:

PamGene International B.V. has recently intensified its efforts to develop and commercialize a blood-based immunotherapy guidance test, the IOpener®, to improve patient outcomes. The company's kinase-activity profiling technology and proprietary software algorithms support clinicians in their treatment decisions using the IOpener® assay. PamGene's robust and unique peptide microarray technology for multiplex kinase-activity profiling is also used to provide dedicated assay services for patient stratification for clinical trials, biomarker discovery, and gaining mechanistic insights needed to understand human diseases. PamGene is headquartered in 's-Hertogenbosch, the Netherlands.

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The Netherlands

IOpener®-*NSCLC*

Instructions for use

REF	60110	English

INTENDED USE

IOpener-NSCLC is a semi-quantitative kinase activity profiling based in-vitro diagnostic device to predict the likelihood for response to therapy with PD-1 checkpoint inhibitors (e.g. pembrolizumab, pembrolizumab in combination with chemotherapy) for advanced stage Non-Small Cell Lung cancer (NSCLC) patients. The test is based on measuring the kinase activity profile of peripheral blood mononuclear cells (PBMCs) obtained from a whole blood sample collected from a patient before start of the anti-PD-1 therapy.
The test result is provided as a predictive score which should be used by the physician in conjunction with other laboratory and clinical findings as an aid to assess the likelihood of a patient's response to anti-PD-1 therapy.
IOpener-NSCLC is intended for professional use only and is performed as a not-automated IVD assay service in a dedicated laboratory facility.

COLLECTION HANDLING AND PREPARATION OF THE SPECIMEN

Whole blood collection, PBMC isolation, PBMC lysis and determination of protein content of PBMC samples must be performed by professional users specifically trained by PamGene and according to PamGene's instructions and protocols. The following specimen type has been tested for use with the IOpener-NSCLC test: PBMCs isolated from venous whole blood containing Na-Heparin as the anticoagulant.

TEST PRINCIPLE

The IOpener-NSCLC test employs a Protein Tyrosine Kinase assay (PTK assay) to generate a kinase activity profile from the PBMC sample. Kinases in the sample actively phosphorylate peptide substrates on the PamChips microarray in the presence of ATP. A FITC-conjugated PY20 antibody is used to detect the peptide substrate phosphorylation.
The test must be performed by professional users specifically trained by PamGene and according to PamGene's instructions and protocols in a dedicated laboratory. From the quantified kinase activity profile, a predictive score is calculated with the IOpener-NSCLC Analysis Software using a fixed pre-calibrated model. The test result is reported as a predictive score between 0 and 100, for benefit of treatment with PD-1 checkpoint inhibitors, defined as no occurrence of Progressive Disease in the first 24 weeks after start of treatment with PD-1 checkpoint inhibitors (≧ 50 = Predicted Benefit, < 50 = No Predicted Benefit). The result is provided to the physician in a test report.

REAGENTS (DESCRIPTION)

The DAS PTK Reagent kit contains DL-Dithiothreitol (DTT), adenosine triphosphate (ATP), albumin bovine serum fraction V (BSA), PY20 conjugated to FITC, 10x Protein Kinase buffer containing Tris-HCl, $MgCl_2$, EDTA, Brij 35 and DTT, 10x PTK additive containing sodium phosphate dibasic and sodium phosphate monobasic. The Reference sample contains human PBMC lysate, mammalian protein extraction reagent (M-PER) and Halt Protease inhibitor Cocktail.

SOFTWARE REQUIREMENTS

BioNavigator data analysis platform.

Materials provided	Quantity	REF	Storage temperature
PamChips	4	32515	8°C / 2°C
DAS PTK Reagent kit	4	32121	-18°C / -22°C
Reference sample	4	41011	-75°C
IOpener-NSCLC Analysis Software	1	59110	Not applicable

Materials required but not provided	REF
PamStation 12	31500

QUALITY CONTROL

A Quality Control (QC) report is generated by the IOpener-NSCLC analysis software for each test procedure. The results are checked and monitored for intra-run CV, concordance with a reference profile, and applicability off the classification model for the measured kinase activity according to PamGene's instructions and protocols.

IN-USE STABILITY/CONTROL

Patient PBMC samples and Reference samples can be used for a maximum of 2 hours after thawing and must be kept at a temperature of 0-4°C until use.
After opening the sealed pouch PamChips can be used for up to 2 hours when stored at room temperature until use.
The DAS Reagent kit can be used for up to 6 hours when stored at a temperature of 0-4°C until use. The Reference sample must be used as a quality control for each test run. Use PamChips, DAS PTK Reagent kit and Reference sample only once.

INTERFERING SUBSTANCES

Interference testing was performed with a pool of three healthy donor PBMC samples.

Substance	Maximal concentration in PBMC sample
Vemurafenib	2400 ng/ml
Heparin	6 U/ml
Ficoll	30 (% v/v)
Bilirubin	5 mg/dL
Hemoglobin	200 mg/dL

Number: 60110-002 Effective date: 2022-11-10

ANALYTICAL PERFORMANCE CHARACTERISTICS

IOpener-NSCLC is a semi-quantitative test without a defined analyte. The LoD for kinase activity profiling was determined as the sample input for which the phosphorylation signal can be discriminated from the signal obtained with blank samples. The LoQ for kinase activity profiling was determined as the sample input corresponding to the lowest phosphorylation signal that can be quantified with a within-measurement CV = 20%.
Measurement Range was determined as the range between the LoD and the saturation limit of the phosphorylation signal.

Precision kinase activity profiling (within run, run-to-run and day-to-day variability)	SD = 0.22, CV 15%
Limit of Detection (LoD)	0.25 µg total protein/array
Limit of Quantification (LoQ)	0.50 µg total protein/array
Measurement Range (MR)	0.25 to 2.0 µg total protein/array
Sample Stability	Kinase activity of venous whole blood samples collected according to PamGene's procedures and stored at a temperature between 15-25ºC is stable for up to 30 hours between whole blood collection and PBMC isolation. PBMC isolation must be performed on the same day as venous whole blood collection.
Predictive Score Precision	SD = 1.5 (CI90 = score ± 2.5)

Precision was determined as the Standard deviation (SD) of measurements. Precision and MR was determined using one healthy donor PBMC sample. The LoD was determined using six healthy donor PBMC samples. The LoQ was determined using a pool of three healthy donor PBMC samples. The Sample Stability was determined using whole blood collected from four healthy donors. The Predictive Score Precision was determined using four patient PBMC samples.

CLINICAL PERFORMANCE CHARACTERISTICS

IOpener-NSCLC was evaluated on a group of 36 patients with NSCLC and a Tumor PD-L1 Score < 50% (TPS, the % of PD-L1 positive tumor cells), who were treated with pembrolizumab in combination with chemotherapy.

Accuracy	72% (CI95 = 55-86%)
Diagnostic Sensitivity	71% (CI95 = 42-92%)
Diagnostic Specificity	73% (CI95 = 50-89%)
Positive Predictive Value (PPV)	63% (CI95 = 35-85%
Negative Predictive Value (NPV)	80% (CI95 = 59-94%)

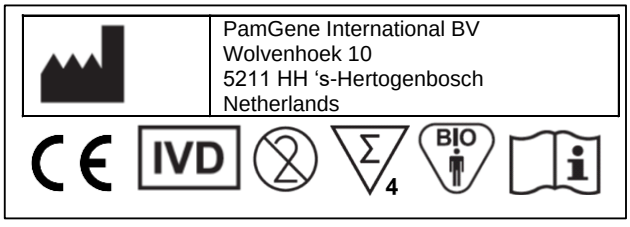
For 63% (CI95: 35-85%) of patients in the group of patients with a TPS < 50% with "Predicted Benefit", Progressive Disease (PD) did not occur within 24 weeks after start of the PD-1 checkpoint inhibitor treatment. The median Progression Free Survival in this group was 33 weeks (= 25 weeks based on the lower bound of the 95% confidence interval).
For 80% (CI95: 56-94%) of patients in the group of patients with a TPS < 50% with "No Predicted Benefit", Progressive Disease (PD) did occur within 24 weeks after start of the ICI treatment. The median Progression Free Survival in this group was 21 weeks (CI95: 12-25 weeks).

PRECAUTIONS/WARNINGS AND LIMITATIONS

- The predictive score that is provided as a result of the IOpener-NSCLC test is based solely on the IOpener-NSCLC kinase assay. The test result must always be judged in conjunction with the patient's medical history, other laboratory and clinical findings and used as an aid to assess the likelihood of a patients' response to anti-PD1 therapy.
- The Clinical Performance Characteristics of IOpener-NSCLC were determined for patients with a TPS < 50%. The performance of the test may be lower for patients with a TPS score = 50%.
- For patients who have received prior therapy with kinase inhibitors there must be a period of at least two weeks between the last kinase inhibitor treatment and blood collection for the IOpener-NSCLC test.
- The IOpener-NSCLC test may provide incorrect results for patients with elevated (>5 mg/dL in plasma) bilirubin levels.
- The IOpener-NSCLC test may provide incorrect results for patients who have received blood transfusion less than one week before collection of the blood sample.

BIBLIOGRAPHY

Hurkmans DP, Verdegaal EME, Hogan SA, et al. Blood-based kinase activity profiling: a potential predictor of response to immune checkpoint inhibition in metastatic cancer. *J Immunother Cancer*. 2020;8(2):e001607. doi:10.1136/jitc-2020-001607



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Information and Ordering

Ordering and Logistics

Contact IOpener@pamgene.com

PamGene provides a sample shipment kit to the hospital

Patient blood sample obtained and shipped

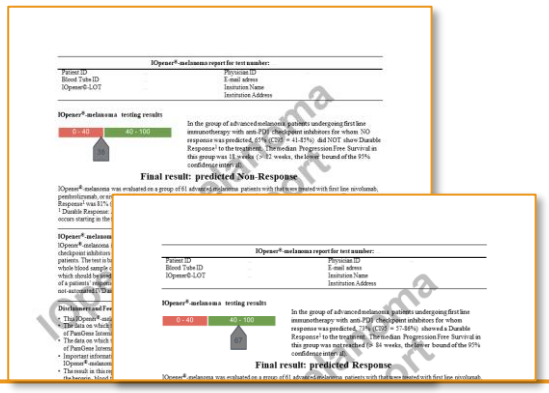
IOpener® report provided to physician

Sample-kit

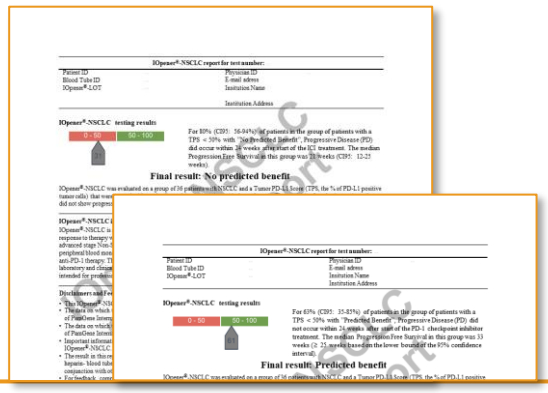
IOpener® Score & Report

From the quantified kinase activity profile, a predictive score is calculated with the IOpener® Analysis Software using a fixed pre-calibrated model.

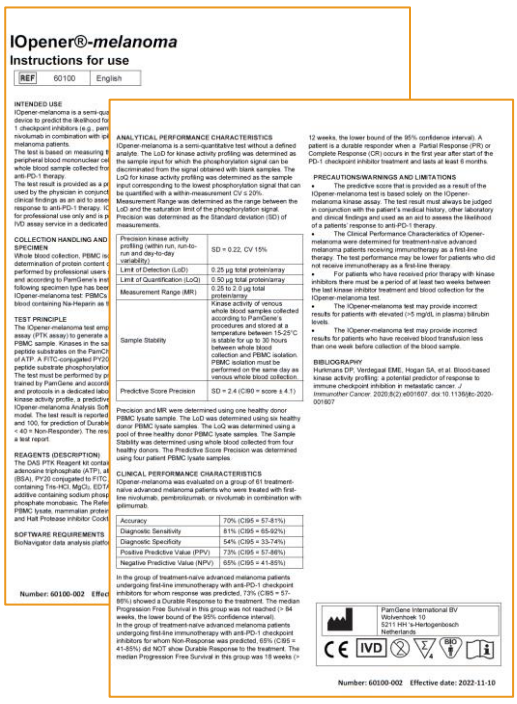
IOpener®-*melanoma* Report

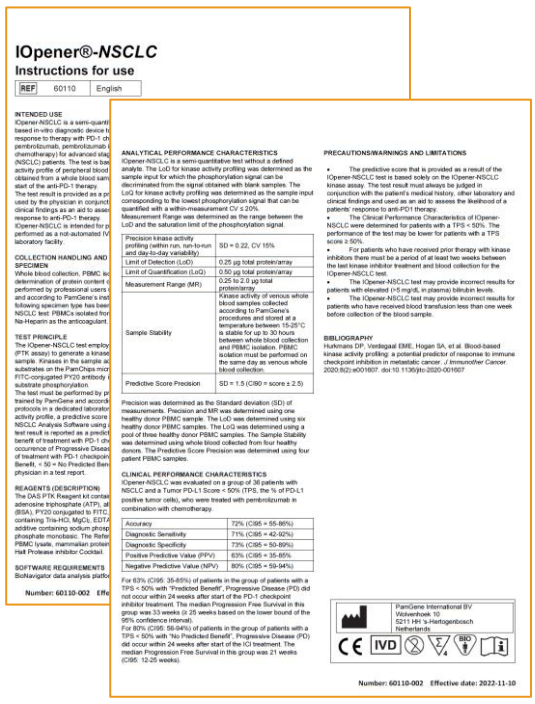


IOpener®-*NSCLC* Report



Details provided in the Instructions-For-Use





About IOpener®:

A blood-based biopsy immuno-therapy treatment guidance test

The IOpener® is a blood-based diagnostic test that supports immunotherapy guidance. The test measures the kinase activity in peripheral blood mononuclear cells isolated from a single tube of blood from a patient to determine the likelihood of responding to immunotherapy. The test is based on ample experience with the technology for measuring and interpreting kinase activity to support academic and clinical research. The IOpener®-*NSCLC* and IOpener®-*melanoma* are CE-IVD registered devices that is developed and manufactured in PamGene's ISO 13485:2016 certified facility.

About PamGene:

PamGene International B.V. has recently intensified its efforts to develop and commercialize a blood-based immunotherapy guidance test, the IOpener®, to improve patient outcomes. The company's kinase-activity profiling technology and proprietary software algorithms support clinicians in their treatment decisions using the IOpener® assay. PamGene's robust and unique peptide microarray technology for multiplex kinase-activity profiling is also used to provide dedicated assay services for patient stratification for clinical trials, biomarker discovery, and gaining mechanistic insights needed to understand human diseases. PamGene is headquartered in 's-Hertogenbosch, the Netherlands.

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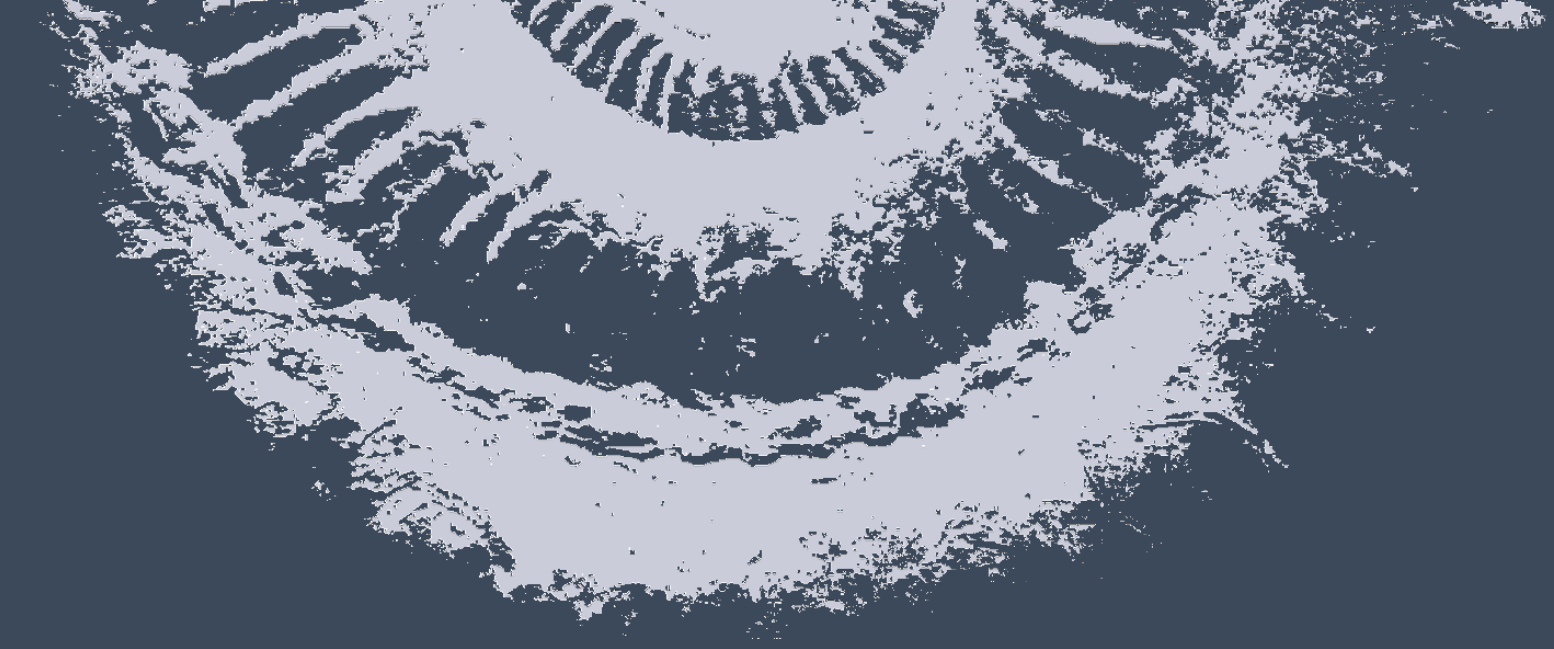

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