



epicup

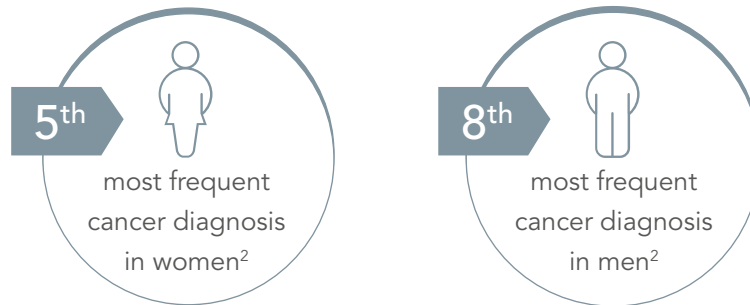
WHEN THE UNKNOWN BECOMES KNOWN

The first and only epigenetic test based on the DNA methylation profile for providing diagnostic guidance in Cancer of Unknown Primary

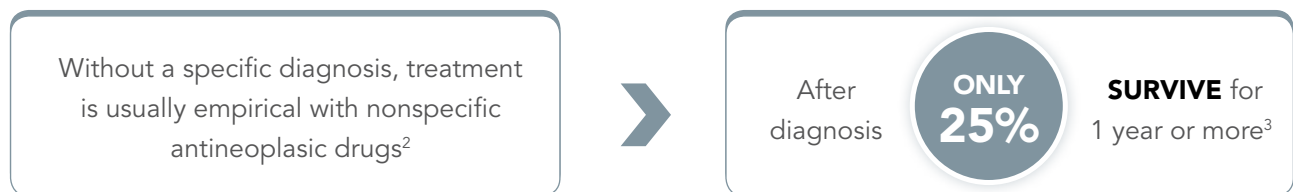
UNKNOWN

CUP*: THE 4th MOST COMMON CAUSE OF CANCER-RELATED DEATH¹

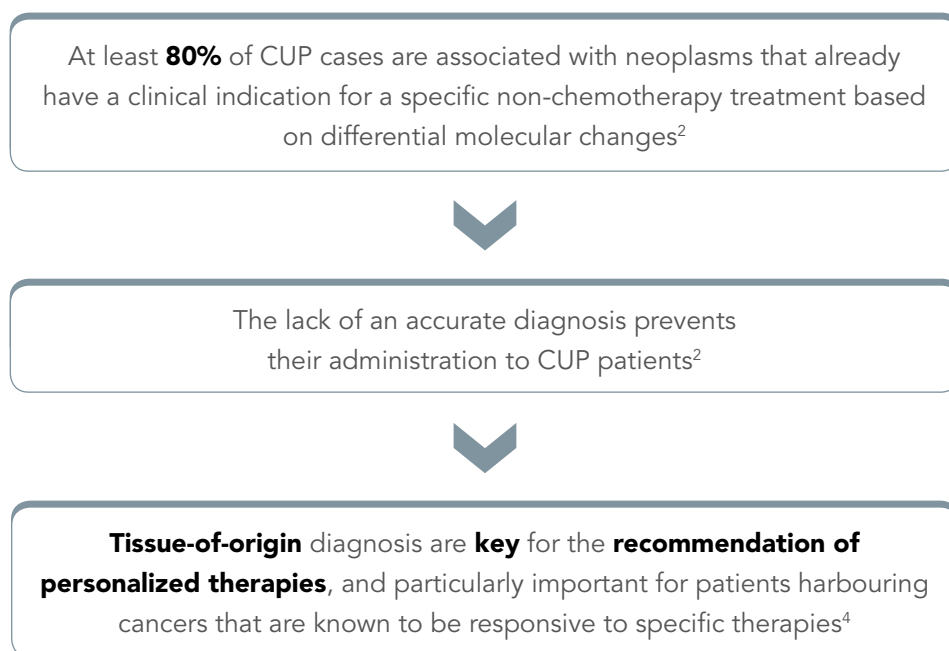
CUP is the



Life expectancy between 6 and 9 months²



80% of CUP cases could have a specific treatment²



* CUP = Cancer of Unknown Primary



€³epicup THE FIRST AND ONLY DIAGNOSTIC TEST TO PREDICT THE TUMOUR TYPE BASED ON DNA METHYLATION PROFILE

- **Molecular cancer-classifier assays** complement immunohistochemistry (IHC) and, when used in concert with assessments of clinical features, histopathology and IHC*, **enable a tissue-of-origin diagnosis in more than 90% of patients**⁴

DNA methylation is a marker that being present in the CUP, retains the signature of the primary origin³

€³epicup **is based on DNA**, a material that is stable over time and not very reactive to change due to minimal external factors, unlike RNA expression levels³

More than 10,000 tumour samples were used to develop and validate €³epicup⁵:

4,892

primary and metastatic tumour samples of known origin to establish a classifier of cancer type³

5,589

primary and metastatic tumour samples of known origin to validate the test³

The tumour type classifier showed³:



99,9%

specificity



96,5%

positive predictive value



96,7%

sensitivity



99,9%

negative predictive value



99,8%

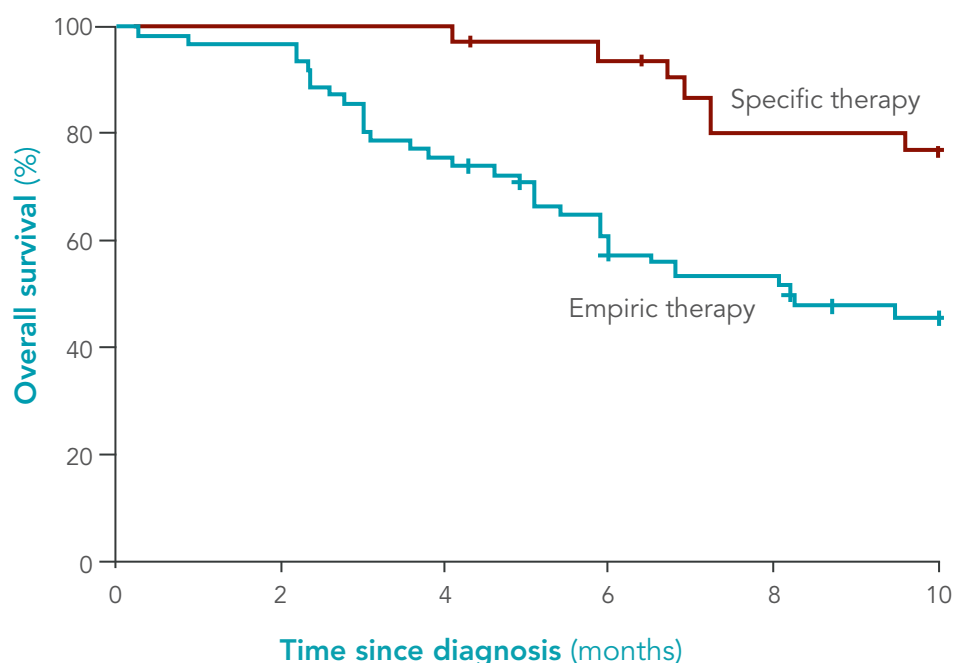
global accuracy

* IHC = Immunohistochemistry

IMPROVED OVERALL SURVIVAL SHOWED IN CUP PATIENTS WHO RECEIVED A TUMOUR TYPE-SPECIFIC THERAPY³

- epicup was applied to predict the tumour type of **216 well-characterised cases of CUP*** (retrospective analysis)³
- It predicted a primary cancer of origin in **87%** of cases³
- Patients with epicup diagnoses who received a tumour-specific therapy* **showed improved median survival** compared with patients who received empiric therapy: **13,6 months vs. 6 months**³

Outcome of patients with cancer of unknown primary who receive a site-specific treatment that matches the epicup prediction



Number at risk
(censored)

Specific therapy	31 (0)	31 (0)	31 (0)	29 (2)	25 (2)	24 (24)
Empiric therapy	61 (0)	59 (0)	46 (2)	38 (8)	34 (8)	30 (30)

* These patients were not given site-specific therapy based on the results of the Epicup essay³



EFFICACY TO PREDICT 38 TUMOUR TYPES, CORRESPONDING TO THE MOST COMMON HUMAN CANCERS³

Cancer types included in the diagnostic algorithm of epicup³

Acute lymphoblastic leukemia	Ovary carcinoma
Acute myeloid leukaemia	Pancreatic carcinoma
Adrenocortical carcinoma	Pheochromocytoma
Bladder urothelial carcinoma	Prostate carcinoma
Brain lower-grade glioma	Rectum adenocarcinoma
Breast carcinoma	Renal tumour chromophobe
Cervical carcinoma	Renal tumour clear cell
Chronic lymphocytic leukemia	Renal tumour papillary
Colon carcinoma	Retinoblastoma
Cutaneous lymphoma	Sarcoma
Endometrial carcinoma	Skin cutaneous melanoma
Head and neck squamous cell carcinoma	Small cell lung cancer
Hepatocellular carcinoma	Stomach carcinoma
Lymphoid neoplasm diffuse large-B-cell lymphoma	Testis non-seminoma carcinoma
Meningioma	Testis seminoma carcinoma
Mesothelioma	Thymoma
Non-small cell lung cancer	Thyroid carcinoma
Neuroendocrine carcinoma	Uveal melanoma

epicup

REPORT CONTENT

WHEN THE UNKNOWN BECOMES KNOWN

Intended use

EPICUP is an epigenetic diagnostic test based on DNA methylation profiles that helps identify the primary tumor in patients with cancers of unknown primary.

EPICUP is not a substitute for clinical, radiological or histopathological procedures. Its results should be taken within the context of the aforementioned procedures.

Validation study

EPICUP has been developed and clinically validated using over 10,000 oncology patient samples, achieving overall sensitivity and specificity results of 96.7% and 99.9%, respectively.^{1,2,3}

Understanding the results

Main prediction

This indicates the tumor type within the prediction area that achieved the highest similarity score.

The similarity shows the closeness of the sample analyzed to the prediction.

Haematoxylin and eosin staining (H&E)

This indicates the tumor sample area used to carry out the test.

Cannot be ruled out

This indicates the tumor types within the prediction area with lower scores than the main prediction.

Ruled out

This indicates the tumor types within the exclusion area ordered from the highest to the lowest score values.

For a more detailed explanation please see the other side of the report.

EPICUP was carried out by:

PEBC

Cancer Epigenetics and Biology Programme

Buylde Biomedical Research Institute

CE

Certification UNE-EN ISO 15485

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Request information

Ferrer ID:	Hospital ID:
Kit/bell ID:	FFPE block ID:
Date of birth:	Sex:
Date sample collected:	Date sample received:
Site of biopsy:	Date report issued:

EPICUP RESULT

<p>Main Prediction</p> <p>Hepatocellular Carcinoma</p>	<p>H&E</p> <p>Tumor cells 60% Necrosis 10%</p>			
<p>Cannot be ruled out</p> <table> <tr> <td>Carcinoma of the pancreas</td> <td>Carcinoma of the stomach</td> </tr> </table>		Carcinoma of the pancreas	Carcinoma of the stomach	
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<p>Ruled out with 99.9% specificity</p> <table> <tr> <td> <p>Adenocarcinoma of the colon</p> <p>Adenocarcinoma of the rectum</p> <p>Non-small cell lung cancer (squamous and adenocarcinoma)</p> <p>Carcinoma of the cervix (squamous and adenocarcinoma)</p> <p>Carcinoma of the thyroid (papillary and follicular)</p> <p>Uterine leiomyosarcoma</p> <p>Uterine sarcoma</p> <p>Adenocarcinoma of the endometrium (serous, papillary and endometrioid)</p> <p>Carcinoma of the esophagus (squamous and adenocarcinoma)</p> <p>Carcinoma of the stomach (intestinal and diffuse)</p> </td> <td> <p>Carcinoma of the breast</p> <p>Carcinoma of the ovaries (serous and papillary)</p> <p>Carcinoma of the pancreas</p> <p>Carcinoma of the prostate</p> <p>Small cell lung carcinoma</p> <p>Squamous carcinoma of the head and neck</p> <p>Hepatocellular carcinoma</p> <p>Chromophobe renal carcinoma</p> <p>Clear cell renal carcinoma</p> <p>Papillary renal carcinoma</p> <p>Pheochromocytoma</p> <p>Glioma</p> <p>Acute lymphoblastic leukemia</p> </td> <td> <p>Chronic lymphocytic leukemia</p> <p>Acute myeloid leukemia</p> <p>Cutaneous lymphoma</p> <p>Diffuse large B-cell lymphoma</p> <p>Uveal melanoma</p> <p>Meningioma</p> <p>Mesothelioma</p> <p>Multiple myeloma</p> <p>Neuroblastoma</p> <p>Rhabdomyosarcoma</p> <p>Sarcoma</p> <p>Seroma</p> <p>Thyoma</p> <p>Non-seminomatous germ cell tumor</p> </td> </tr> </table>		<p>Adenocarcinoma of the colon</p> <p>Adenocarcinoma of the rectum</p> <p>Non-small cell lung cancer (squamous and adenocarcinoma)</p> <p>Carcinoma of the cervix (squamous and adenocarcinoma)</p> <p>Carcinoma of the thyroid (papillary and follicular)</p> <p>Uterine leiomyosarcoma</p> <p>Uterine sarcoma</p> <p>Adenocarcinoma of the endometrium (serous, papillary and endometrioid)</p> <p>Carcinoma of the esophagus (squamous and adenocarcinoma)</p> <p>Carcinoma of the stomach (intestinal and diffuse)</p>	<p>Carcinoma of the breast</p> <p>Carcinoma of the ovaries (serous and papillary)</p> <p>Carcinoma of the pancreas</p> <p>Carcinoma of the prostate</p> <p>Small cell lung carcinoma</p> <p>Squamous carcinoma of the head and neck</p> <p>Hepatocellular carcinoma</p> <p>Chromophobe renal carcinoma</p> <p>Clear cell renal carcinoma</p> <p>Papillary renal carcinoma</p> <p>Pheochromocytoma</p> <p>Glioma</p> <p>Acute lymphoblastic leukemia</p>	<p>Chronic lymphocytic leukemia</p> <p>Acute myeloid leukemia</p> <p>Cutaneous lymphoma</p> <p>Diffuse large B-cell lymphoma</p> <p>Uveal melanoma</p> <p>Meningioma</p> <p>Mesothelioma</p> <p>Multiple myeloma</p> <p>Neuroblastoma</p> <p>Rhabdomyosarcoma</p> <p>Sarcoma</p> <p>Seroma</p> <p>Thyoma</p> <p>Non-seminomatous germ cell tumor</p>
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Request information and patient details

Main prediction:

Tumor type within the prediction area that achieved the highest similarity score

Tumor sample area used to carry out the test

Other tumor types that cannot be ruled out:

Tumor types within the prediction area with lower scores than de main prediction

Tumor types that can be ruled out:

Tumor types within the exclusion area

WHEN THE UNKNOWN BECOMES KNOWN

Laboratory comments

The primary tumor prediction for the sample analyzed is consistent with **Hepatocellular Carcinoma**, with a similarity score (SS) of 65%. In the validation study completed by EPICUP, **Hepatocellular Carcinoma** obtained a Positive Predictive Value of **89.5%**.

The remaining tumor types are ruled out with 99.9% specificity.

Understanding EPICUP

The Similarity Score (SS) shows the closeness of the sample analyzed to the prediction obtained. In the development of EPICUP, a cut-off was established to delimit two areas: i) the prediction area, including SS values greater than or equal to 12 and ii) the exclusion area, including SS values lower than 12. Thus, for the 38 tumor types included in the database, the overall sensitivity, specificity, positive predictive value and negative predictive value are 96.7%, 99.9%, 96.5% and 99.9%, respectively.^{1,2,3}

When a sample obtains more than one result in the prediction area, the highest SS value becomes the "main prediction" while the remaining predictions are included in the "cannot be ruled out" section. Likewise, those tumor types with a SS lower than 12 are ruled out with 99.9% specificity.

Positive

Understanding EPICUP

The Similarity Score (SS) shows the closeness of the sample analyzed to the prediction obtained. In the development of EPICUP, a cut-off was established to delimit two areas: i) the prediction area, including SS values greater than or equal to 12 and ii) the exclusion area, including SS values lower than 12. Thus, for the 38 tumor types included in the database, the overall sensitivity, specificity, positive predictive value and negative predictive value are 96.7%, 99.9%, 96.5% and 99.9%, respectively.^{1,2,3}

When a sample obtains more than one result in the prediction area, the highest SS value becomes the "main prediction" while the remaining predictions are included in the "cannot be ruled out" section. Likewise, those tumor types with a SS lower than 12 are ruled out with 99.9% specificity.

Positive predictive value is the proportion of patients where the prediction matches the primary tumor.³

EPICUP is an epigenetics-based test recommended to help as a guide during the tumor-classification process. The aim is not to diagnose the origin of tumors that cannot be diagnosed using current clinical procedures, nor is it to sub-classify or alter classifications obtained via said procedures. EPICUP does not aim to predict the course of the disease, survival rate or efficacy of any treatment, nor to distinguish primary tumors from metastatic tumors. Tumor types that are not included in the database may have similar methylation profiles to the tumors that are included. Consequently, the test results cannot be used to distinguish among the tumors included in the EPICUP test and those that are not. EPICUP is not a substitute for clinical, radiological or histopathological procedures. EPICUP results should be taken within the context of the aforementioned procedures.

Bibliography

1. Morin V, et al. (2015) The EPICUP test: a new tool for the diagnosis of primary tumors of unknown origin. *Ann Oncol* 26:1111-1116.
2. Morin V, et al. (2016) The EPICUP test: a new tool for the diagnosis of primary tumors of unknown origin. *Ann Oncol* 27:1111-1116.
3. Data from the EPICUP validation study.

Similarity score and positive predictive value of the main prediction, and similarity score of other tumor types within the prediction area



THE UNKNOWN BECOMES KNOWN

- The first and only epigenetic test based on the DNA methylation profile for diagnostic guidance in CUP³
- More than 10,000 tumour samples used for development and validation³
- High accuracy to predict the primary tumour type^{3,5}
- 38 tumour types included in the algorithm³

➤ epicup can be used to provide a diagnostic guidance in cancers of unknown primary or uncertain primary³

With an accurate diagnostic guidance patients can receive a less toxic and more site-directed therapy³

Developed by



Fundación Instituto de Investigación
Biomédica de Bellvitge.



Certificación
UNE-EN-ISO13485