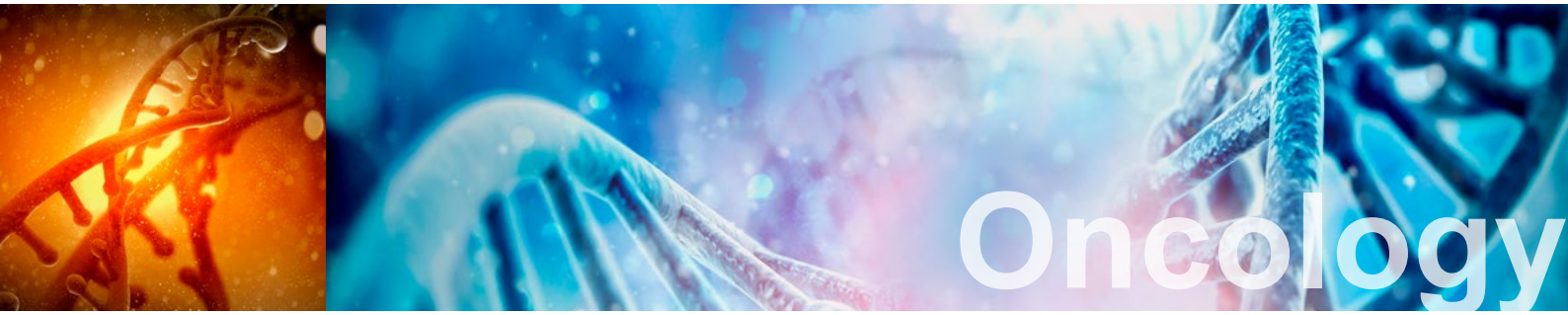
eurofins | Biomnis



Other solid tumours

- Melanoma
- Soft tissue tumours and sarcomas
- Central Nervous System (CNS) tumours
- Urological tumours
- Neuroblastomas
- Lymphomas

Melanoma

Skin melanoma is the 6th most common cause of cancer in women and the 8th most common in men. Its metastatic potential directly impacts patient survival and it is therefore essential to have an effective therapeutic arsenal. Targeted anti-BRAF therapy has revolutionised the management of metastatic BRAF-V600 mutated melanomas. A new immunotherapy-based treatment also seems to bring real benefits in the care of these patients, whether in combination with a targeted therapy or not.

"MELA" NGS panel	BRAF – NRAS – CKIT
Pan-Organ panel	AKT1 - ALK - BRAF - CTNNB1 - DDR2 - EGFR - HER2 - HER4 - FBXW7 - FGFR1 - FGFR2 - FGFR3 - KIT - KRAS - MAP2K1- MET - NOTCH1 - NRAS - PDGFRA - PIK3CA - PTEN - SMAD4 - STK11 - TP53

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"MELA" NGS panel

BRAF BRAF mutations are common in melanomas, and about 50% of patients with metastatic melanoma have a BRAF-V600 mutation. The combination of a BRAF inhibitor and a MEK inhibitor is revolutionising the therapeutic management of this group of patients. Other BRAF mutations have also been described and it is worth screening for these in connection with theranostic approaches.

NRAS NRAS mutations are described in nodular (with poor prognosis) and mucosal forms. Exon 3 mutations are believed to be associated with a poor prognosis. Immunotherapy remains the first-line treatment in the presence of an NRAS mutation (with wild-type BRAF) in preference to MEK inhibitors.

KIT KIT mutations are mainly found in acro-lentiginous melanomas. Unlike GIST, no targeted therapy with a marketing authorisation is available to date, but trials are underway with TKIs.

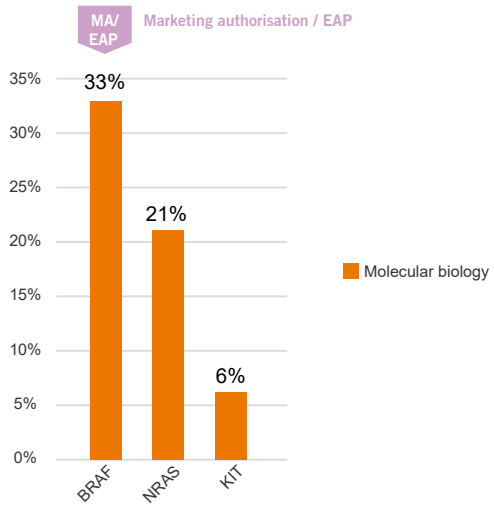
NRAS and KIT mutations are not yet actionable mutations, but screening for them is recommended in clinical trials in the context of wild-type BRAF melanomas.

Gene rearrangements (ALK, ROS1, NTRK) have been reported in spitzoid melanoma. Screening for them is of minor interest given the favourable prognosis of these tumours and their low metastatic potential.

The pan-organ NGS panel* is likely to prove useful in clinical research.

NB: on the basis of the results of the PD-L1 IHC it is possible to offer immunotherapy, in particular for wild-type BRAF cases. This offer will be complemented by the TMB analysis in the near future.

Frequency of molecular abnormalities in melanoma and available targeted therapy(-ies) (Marketing authorisation/EAP)



In uveal melanoma, monosomy 3 is associated with a poor prognosis and a metastatic potential.

Targeted FISH test (uveal melanoma)	status of chromosome 3

*__Comment:__ we do not offer screening for NF1, GNA11 and GNAQ mutations

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Bone and soft tissue tumours

More than 50% of sarcomas are associated with molecular abnormalities. These mainly consist of chromosomal translocations (e.g. rearrangements of EWSR, SS18, FOXO1A ...) or gene amplifications (e.g. MDM2). These are strong diagnostic markers, but they may also have a prognostic value. They therefore complement the morphological and immunohisto-chemical criteria established by the clinical pathologist. Our laboratory offers targeted FISH testing.

FISH unit tests – Sarcoma	EWSR1 - MDM2 - DDIT3 (CHOP) - SS18 (SYT) - ALK - FOXO1A (FKHR) - ETV6 - NTRK3

The most common rearrangements in sarcomas are:

Tumours	Molecular abnormalities analysed by FISH	Chromosome rearrangements	Fusion genes
Synovial sarcoma	Rearrangement of SS18 (SYT)	t(X;18)(p11;q11)	SS18(SYT)-SSX1 SS18(SYT)-SSX2 SS18(SYT)-SSX4
Alveolar rhabdomyosarcoma	Rearrangement of FOXO1A (FKHR)	t(2;13)(q35;q14) t(1;13)(p36;q14) and variants	PAX3-FOXO1A(FHKR) PAX7-FOXO1A(FHKR)
Myxoid liposarcoma	Rearrangement of DDIT3 (CHOP)	t(12;16)(q13;p11) t(12;22)(q13;q12)	TLS(FUS)-DDIT3(CHOP) EWSR1-DDIT3(CHOP)
Differentiated/ dedifferentiated liposarcoma	Amplification of MDM2		
Ewing sarcoma	Rearrangement of EWSR1	t(11;22)(q24;q12) t(21;22)(q22;q12) and variants	EWSR1-FLI1 EWSR1-ERG
Clear cell sarcoma		t(12;22)(q13;q12) t(2;22)(q32;q12)	ATF1-EWSR1 EWSR1-CREB1
Desmoplastic small round cell tumour		t(11;22)(p13;q12)	WT1-EWSR1
Extraskeletal myxoid chondrosarcoma		t(9;22)(q22;q12)	EWSR1-TEC
Angiomatoid fibrous histiocytoma		t(2;22)(q34;q12) t(12;22)(q13;q12)	EWSR1-CREB1 EWSR1-ATF1
Inflammatory myofibroblastic tumour	Rearrangement of ALK	Rearrangements in 2p23	
Infantile fibrosarcoma	Rearrangement of ETV6 or of NTRK3	t(12;15)(p13;q25)	ETV6-NTRK3

Comment: for GISTs, please consult the Oncology - Digestive System Sheet (ref. DS84-INTGB).

Tumours of the Central Nervous System (CNS): gliomas and medulloblastomas

Gliomas

The WHO 2016 classification applied the concept of “integrated diagnosis” to gliomas by combining clinical, histological and molecular data. It has thus been possible to define homogeneous diagnostic groups in terms of prognosis and predictive value for a treatment. 3 techniques are used to create this integrated diagnosis:

- IHC: expression of IDH (IDH1-R132H), ATRX, H3K27M
- FISH: 1p/19 codeletion, EGFR amplification, 7p loss, 10q gain
- Molecular biology: IDH1-IDH2 mutation, Histone H3 mutation, TERT promoter mutation

IDH status determined by means of IHC is the basis of the WHO classification of gliomas: i.e. mutated IDH tumours versus wild-type IDH tumours. IDH1 mutations account for approximately 95% of cases and of these IDH1 mutations, the R132H mutation accounts for more than 90% of cases. IDH sequencing should be performed in cases of IDH1-R132H immuno-histochemical negativity. Mutated IDH status is associated with a favourable prognosis in grade II, III and IV gliomas.

FISH unit test	1p/19q – p16 - EGFR status
MGMT methylation	

FISH unit test:

1p 19q

1p/19q codeletion corresponds to the complete loss of 1p and 19q due to an unbalanced translocation mechanism t(1;19)(q10;p10).

It has:

- **a diagnostic role** (presence of an IDH mutation): its presence confirms the diagnosis of oligodendroglioma. Two WHO units are described: “IDH-mutated/1p19q co-deleted oligodendroglioma” and “IDH-mutated/1p19q co-deleted anaplastic oligodendroglioma” but absence of the mutation does not rule out a diagnosis of oligodendroglioma (NOS).
- **a prognostic role:** it is associated with a good prognostic factor in grade II gliomas. On the other hand, isolated 1p loss is associated with an unfavourable prognosis.
- **a therapeutic role:** patients with grade III glioma may benefit from treatment with PCV and radiation therapy. It is therefore associated with chemosensitivity.

p16

Screening for the p16 deletion (homozygous or heterozygous) in 9p21 with FISH is a diagnostic aid. It points to the presence of a higher grade glioma (grade III) versus a grade II.

EGFR

In relation to a tumour with an astrocytoma histology, testing for EGFR amplification may help in the diagnosis of the WHO entity “Non-mutated IDH glioblastoma”. 7p gain or 10q loss may also be observed in this context.

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MGMT methylation

The methylated or unmethylated status of MGMT is not of diagnostic interest according to WHO 2016. The MGMT protein is involved in the DNA repair system. Methylation of MGMT is a predictive marker of response to alkylating agents and an independent favourable prognostic marker in glioblastoma.

Comment: in molecular biology, we only offer screening in the lab for MGMT methylation in the context of gliomas.

Medulloblastomas

FISH unit test	C-MYC and N-MYC

The amplification of C-MYC or N-MYC is associated with a poor prognostic factor.

Urological tumours: bladder and prostate

Bladder cancer

Bladder cancer is the seventh most common cancer in France and affects mainly men. It is the second most common urological cancer after prostate cancer. The available treatments are surgery, chemotherapy, immunotherapy and radiation therapy.

Non-invasive molecular test for bladder cancer monitoring
MSI test

Non-invasive molecular test for bladder cancer monitoring (Xpert Bladder Cancer Monitor-Cepheid test)

In bladder tumors, the "superficial" forms or NMIBC (Non-Muscle-Invasive Bladder Cancer) represent 80% of cases. The management of these NMIBC tumors is different from that of muscle-invasive bladder tumors. Recurrence (50% of cases) and progression to MIBC (15% of cases) characterize these NMIBC tumors. Monitoring of patients is therefore essential. This follow-up is based on the combination of cytoscopy and urinary cytology. Cytoscopy is an invasive and operator-dependent procedure, with risks of local trauma and infection. Urine cytology is cytologist-dependent and has an average sensitivity (20 to 40% for the low-risk group).

The Xpert Bladder Cancer Molecular Test (Cepheid) is a non-invasive test that can be used to monitor patients with NMIBC bladder cancer to prevent tumor recurrence. The test measures the RT-PCR expression of 5 target mRNAs in a urine sample using a closed cartridge. The 5 mRNAs measured are: ABL1, ANXA10, UPK1B, CRH and IGF2. ANXA10, UPK1B, CRH and IGF2 are tumor molecular biomarkers. The ABL1 gene is used as a control gene. The test gives a POSITIVE (likely presence of tumor recurrence) or NEGATIVE (likely absence of tumor recurrence) result based on the results of a linear discrimination analysis algorithm that uses the threshold cycle (Ct) results of the 5 target mRNAs. The Xpert Bladder Cancer Monitor has a sensitivity of 75%, specificity of 81%, NPV of 94% and PPV of 45%.

MSI test

(*cf.* Oncology – Digestive systeme sheet (Ref. DS84-INTGB)

The indications for the MSI test in bladder tumours are:

- Theranostic stratification using Immunotherapy (PD-1/PDL-1 axis) in advanced or metastatic forms with an MSI+ test
- Pre-screening for Lynch syndrome

Prostate cancer

Prostate cancer is the leading cause of cancer in men and the third most common cause of death. The first-line treatment for metastatic cancer is hormonal therapy. In case of resistance, chemotherapy may be offered. PARP inhibitors appear to offer a new therapeutic alternative. Immunotherapy is still in the trial stage.

Somatic BRCA1 and BRCA2 mutations

(*cf.* Oncology - Gynaecology sheet (Ref. DS85-INTGB)

BRCA1 or BRCA2 mutations (with the latter being more frequent) are reported in around 5% of prostate cancers. PARP inhibitors are set to offer a new class of targeted therapy for metastatic prostate cancers that no longer respond to hormonal therapy.

Note: As with breast and ovarian cancer, oncogenetic consultations are a key aspect of individual and family care. In France, a number of different genetic oncology courses of treatment for the prescription of a PARP inhibitor, dependent on the presence of a germline or somatic mutation, are offered by the National Cancer Institute (INCa)

MSI test

(*cf.* Oncology – Digestive system sheet (Ref. DS84-INTGB)

The indications for the MSI test in prostate cancer are:

- Theranostic stratification using immunotherapy (PD-1/PDL-1 axis) in advanced or metastatic forms with an MSI+ test
- Pre-screening for Lynch syndrome

Neuroblastomas

Neuroblastomas are the most common extracerebral solid malignancy in young children. They are characterised by great clinical variability (spontaneous regression or fatal course). The treatment is adapted according to the age of the child, the stage of the tumour, the possibility of surgery and whether or not the status of the N-MYC oncogene is amplified.

FISH unit tests	N-MYC

The amplification of N-MYC is associated with a poor prognostic factor.

Lymphomas

Eurofins Biomnis offers a comprehensive list of cytogenetic (karyotype and FISH) and molecular biology analyses for optimum care of patients with lymphoma.

Karyotype (DSP30-IL2 stimulated culture)	
Haematological FISH	LLC (TP53-ATM-13q-CEP12) - IGH-CCND1 - IGH-BCL2 - CMYC - BCL6 - IGHDC
B-cell clonality	
Rearrangement of IG-VH	
Mutation MYD88 / CXCR4	
T-cell clonality	
TP53 mutations	
BRAF mutation	

Find all of our tests on our "Hematologic malignancies" test request form (Ref. B8-INTGB) as well as on our information materials at **www.eurofins-biomnis.com** > Specialities > Solid tumours and malignant haemopathies.

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The routine use of the NGS technique in oncology has given rise to molecular classifications for diagnostic, prognostic or theranostic purposes.

In this regard, our laboratory offers:

1. A pan-organ NGS panel

Gene	NM_	EXONS	PAN-ORGAN
AKT1	NM_005163.2	3	●
ALK	NM_004304.4	21, 22, 23, 25	●
BRAF	NM_004333.5	11, 15	●
CTNNB1	NM_001904	3	●
DDR2	NM_006182	5, 8, 12-15, 17	●
EGFR	NM_005228	12, 18-21	●
ERBB2	NM_004448.3	19-21	●
ERBB4	NM_005235	3, 4, 6-9, 15, 23	●
FBXW7	NM_033632,3	5, 8-11	●
FGFR1	NM_023110	3, 4	●
FGFR2	NM_000141	7, 9, 12	●
FGFR3	NM_000142	7, 9, 14, 16, 18	●
KIT	NM_000222	8, 9, 11, 13, 17	●
KRAS	NM_004985	2, 3, 4	●
MAP2K1	NM_002755	2	●
MET	NM_001127500	2, 14, 16, 19	●
NOTCH1	NM_017617	26	●
NRAS	NM_002524	2, 3, 4	●
PDGFRA	NM_006206	12, 14, 18	●
PIK3CA	NM_006218	2, 8, 10, 14, 21	●
PTEN	NM_000314.6	1, 3, 6-8	●
SMAD4	NM_005359	3, 5, 6, 8, 9-12	●
STK11	NM_000455	1, 4, 6, 8	●
TP53	NM_000546	2, 4-10	●

2. The evaluation of tumour mutational burden (TMB) as a predictive test for response to immunotherapy is also available from Eurofins Biomnis.

The ESMO 2020 recommendations recommend performing the first-line TMB test for the following tumours: cervical cancer, neuroendocrine tumours, salivary tumours, cancer of the vulva and cancer of the thyroid.

These 2 tests can be useful in CPR.

In conclusion, the molecular biology and FISH approaches for solid tumours are constantly developing. This document was written according to the state of knowledge in 2020.

Note: Alongside FISH and molecular analyses, Eurofins Biomnis can also test other biological parameters:

- Radioimmunological assays such as hCG dimers (Alpha + Beta) and hCG - free beta chain - subunit (testicles) and Chromogranin A (neuroblastoma),
- Tumour markers such as [-2] pro-PSA and phi score calculation, total PSA and free PSA (prostate).

Before taking any samples, view the key information for each test (pre-analytics, turnaround time, required documents*, etc.) on **www.eurofins-biomnis.com** > Test guide section > **Test code**

Analysis codes

- Melanoma NGS Panel: **MELA**
- Oncological FISH **MOHC4**
- MGMT methylation: **MGMT**
- Non-invasive molecular test for bladder cancer monitoring : **URMOL**
- MSI test: **MICSA**
- Somatic BRCA1/2: **BRCAS**
- Pan-organ NGS panel: **PAN**
- TMB test: **TMB**

*Required documents

- Test request form - Oncology - Solid tumours (ref. B9-INTGB)
- Histopathology report
- Lymphoma study: test request form - "Hematologic malignancies" (Ref. B8-INTGB)

Turnaround (FISH and NGS): 10 days (one extra week if verification by Sanger sequencing is required)

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Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Michielin O et al. Ann Oncol. 2019 Dec 1;30(12):1884-1901. PMID: 31566661

WHO Classification of Soft Tissue and Bone Tumours (5th ed) – IARC Lyon 2019

WHO Classification of Central Nervous System Tumours - IARC Lyon 2016

WHO Classification of Tumours of the Urinary System and Male Genital Organ – IARC Lyon 2016

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues – IARC Lyon 2017

Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. F Mosele et al. Ann Oncol 2020 Aug 24;S0923-7534(20)39971-3. PMID: 32853681

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Websites https://www.mycancergenome.org/
https://www.e-cancer.fr/
https://www.cancer.gov/

Abbreviations

TKI	Tyrosine-kinase inhibitor
MDCM	Multidisciplinary consultation meeting
TMB	Tumour Mutational Burden

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