MOLECULAR GENETICS REPORT TEMPLATE FOR SOMATIC MUTATION TESTING IN SOLID TUMOURS

September 2016

Objective 6.2: Consolidating access to molecular testing

This document is made available to health care professionals to provide them with guidance in the design of their molecular genetics reports. It is an update of the document published in 2012 by INCa to account for the progressive implementation of targeted next-generation sequencing (NGS) technology. This technology notably enables the analysis of multiple genes in parallel, resulting in the identification of variations, the clinical value of which is not always known.

It has been drafted on the basis of the proposals of a working group made up of members of the hospital cancer molecular genetics platforms with a view to harmonising the delivery of results of screening tests for somatic mutations of theranostic interest in solid tumours. It has been submitted to the 28 cancer molecular genetics platforms for review.

The document is organised in three sections:

I. A molecular genetics report template for somatic mutation testing

II. An accompanying note explaining the references used to draft this document

III. An example of a somatic mutation screening test report for a lung cancer patient.

I. MOLECULAR GENETICS REPORT TEMPLATE FOR SOMATIC MUTATION TESTING

NAME OF LABORATORY

Title of test

TEST NO.: [unique sample code or ID assigned by somatic genetics laboratory]

- Patient: Surname First name Birth name
  Date of birth Sex: F/M
- Performed using the specimen referenced [piece ID No. in original pathology laboratory]
  dated [sampling date]
- Received on [date of arrival of specimen to platform]
- Prescribed by: [prescriber's surname, first name and contact details] on [date of prescription]
- Reason for requested test: (specify the clinical context of the request here)

ANATOMO-CYTO-PATHOLOGICAL DETAILS:

- Specimen type [surgical specimen, biopsy, cytology, etc.] and organ:
- Histological type and tumour status (primary, metastases and origin):
- Pathologist responsible for diagnosis: [surname, first name and contact details of pathologist responsible for initial diagnosis]
- Tumour material type: [FFPE, frozen material] and specimen type [fragment, puncture, ctDNA, etc.]
- Test conducted on a region selected by Dr [platform pathologist]
  containing X% tumour cells.

RESULTS

- **Variants of known clinical impact** class 5, with MA in the disease, to be described as per HGVS nomenclature

Gene	Reference sequence	Variant	Protein modification
E.g. EGFR	NM_ 005228.3	c.2573T>G	p.Leu858Arg

- **Variants to be discussed at Molecular RCP review** classes 4 and 5, with no MA in the disease, to be described as per HGVS nomenclature

Gene	Reference sequence	Variant	Protein modification
E.g. MET	NM_ 001127500.1	c.2942-2A>G	splicing variant

- **Other variants for which the predictive value is unknown** class 3, to be described as per HGVS nomenclature

Gene	Reference sequence	Variant	Protein modification

- Genes for which no anomalies were detected provide list, as per HGVS nomenclature
- Uninterpretable result for provide list genes due to insufficient sequencing depth
- Uninterpretable result [specify reason]
- Test not feasible [specify reason]

Issued in on [date of report]
Signature

CONCLUSION / INTERPRETATION OF RESULTS BASED ON THE CURRENT STATE OF THE ART:

- Within the limits of the procedures used, no mutations were detected in exon(s) X of the *X* gene. *Optional comment for colorectal cancer patient with no KRAS mutation: No anti-EGFR antibody therapy resistance mutations detected.*
- Presence of [standard name, e.g. L858R] mutation of the *X* gene imparting sensitivity to [name of targeted therapy class].
- Presence of [standard name] mutation of the *X* gene imparting resistance to [name of targeted therapy class].
- Presence of *X* gene mutation of indeterminate predictive value of response to [name of targeted therapy class.].
- Within the limits of the procedures used, no mutations were detected in exon(s) X of the *X* gene. Given the low % (< X %) of tumour cells present in the sample tested, this result is non-contributive: it is therefore recommended to perform a test on a specimen with a higher tumour cell content.
- Given the quality and/or [select applicable term] quantity of the DNA extracted from the specimen provided, the test is not feasible and/or [select applicable term] interpretable. A further specimen would be required.
- Test not feasible due to [necrotic tissue, depleted piece].
- Presence of [class 4 or 5 modification, standard name] gene mutation to be discussed at molecular RCP review. [option to add details on the existence of clinical trials on this gene, particularly for the AcSé programme]
- Detection of increase/decrease in copies of the [class 4 or 5 modification, standard name] gene suggesting the existence of gene amplification/deletion. This result must obligatorily be verified using a validated complementary procedure. The clinical interest of this modification should be discussed at the molecular RCP review.

METHOD

- Test conducted using a specimen (piece / number of unstained slides / chips / frozen tissue) fixed with [if information is available], after macrodissection [or using other method] of the region of interest.
- Tumour genome DNA extraction method [if a commercial kit is used: name and version]
- Analytical method [if a commercial kit is used: name and version], sequencer used, sensitivity, minimum depth, list of genes and exons analysed, reference genome version used, coverage level of exons and reference sequence [accession number]
- Analytical pipeline version

II. ACCOMPANYING NOTE

This molecular genetics report template has been drafted with a view to harmonising the delivery of results of screening tests for somatic mutations of theranostic interest in solid tumours.

When multiple genes are studied, it would seem important to issue **a single report** for all these tests so as to provide an **overall interpretation** of the result. If the biomarkers are not all studied in parallel, the results should be sent sequentially, and a definitive report should summarise the results.

WORKING DOCUMENTS

To draft this report template, the working group based its work on the following documents:

- International Classification of Diseases for Oncology (ICD-O-3). Percy C, Fritz A, Jack A, Shanmugarathan S, Sobin L, Parkin DM, Whelan S. World Health Organization, 2008.
- Best practices for theranostic somatic mutation testing in solid tumours (INCa document, August 2010).
- Final report of the 2011 pilot EQA scheme for *EGFR* mutation testing in non-small cell lung cancer.
- General report – ESP KRAS External Quality Assessment Scheme 2011. The reports were analysed according to a list of criteria based on four reference documents: (1) ISO15189: 2007 and ISO DIS 15189:2011 Medical laboratories - Particular requirements for quality and competence (International Organization for Standardization), (2) ISO17025: 2005 General requirements for the competence of testing and calibration laboratories (International Organization for Standardization), (3) van Krieken JH et al. KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program. Virchows Arch. 453(5):417-31 (Nov. 2008) et (4) Gulley M.L. et al. Clinical laboratory reports in molecular pathology. Arch. Pathol. Lab Med. 131, 852-863 (2007).
- Molecular genetics reports of platforms involved in the working group.
- The next steps in next-gen sequencing of cancer genomes. D. Neil Hayes and William Y. Kim; J Clin Invest, 2015;125(2):462-8.

ITEMS FEATURED IN THE REPORT TEMPLATE

The items featured in the report template were determined on the basis of the best practice guidance document for theranostic testing (INCa document) and European quality control guidelines defined for *EGFR* and *KRAS* mutation testing.

- The items defined using "Best practices for theranostic somatic mutation testing in solid tumours" have been categorised according to the type of information provided for optimal clarity.

  Test-related information: unique sample code or ID assigned by laboratory /Patient's surname, first name and date of birth / Piece or specimen ID No. in original laboratory /Sampling date /Date of arrival of specimen to platform / Prescriber's surname, first name and contact details (if available) / Date of prescription / Type of test requested.

  Anatomopathological information: specimen type (surgical, biopsy, cytology, etc.) /Histological type / Organ and tumour status (primary, metastasis, etc.) /Surname, first name and contact details of pathologist responsible for initial diagnosis / Percentage of tumour cells in sample tested / Name and simplified address of pathologist who classified the specimen. It is recommended to use existing frames of reference when structuring these data (ICD-O-3 code, ADICAP code).

  Method-related information: exhaustive list of sequenced and interpreted mutations / Method used with analytical sensitivity / Reference sequence.

  Result-related information: mutation identified and annotated as per HGVS nomenclature / Comments on results.

- The final report of the 2011 pilot EQA scheme for EGFR mutation testing in non-small cell lung cancer recommends the following points:

  The patient should be identified using 3 items: surname/first name, birth name, sex - date of birth – laboratory patient/sample ID.

  The following items should feature in the report:

  - clinical context of the request;
  - date of request, date request received, report printing date;
  - reference of original laboratory;
  - method: procedure used (if a commercial kit is used, its name and version should be mentioned) and analytical sensitivity;
  - result: characterise the mutation by a nucleotide modification as per HGVS nomenclature (except for exon 19 deletions) and provide the reference sequence (accession number).

- The final report of the ESP KRAS External Quality Assessment Scheme 2011 recommends:

  - mentioning the contact details of the prescriber and of the pathology laboratory that provided the specimen tested.

TARGETED NGS TEST RESULTS ON GENE PANEL

For all variants for which a result is delivered, it is recommended to specify: the name of the gene, the reference genome sequence (code NM_), the description of the mutation and the corresponding protein modification. The results should be given as per HGVS nomenclature.

NGS techniques provide a large amount of information that needs to be classified according to the level of clinical validation. Actionable mutations and those specified in the prescription should be

highlighted. It is proposed to classify results according to the UNCseq classification (Hayes and Kim, 2015) which is summarised as follows:

- **Class 5:** actionable variant. This class may in turn be detailed according to the level of clinical development of targeted therapies or their prognostic value:
    - variant with MA in this disease;
    - variant with MA in another disease;
    - variant for which targeted therapies are available in clinical trials;
    - variant for which preclinical data are available;
    - variant with prognostic significance.
- **Class 4:** variant with no known theranostic effect, but with a potential activating (oncogene) or harmful (suppressor gene) effect
- **Class 3:** variant with no known effect in the literature. Modelling data may provide guidance on a mutation effect.
- **Class 2:** potentially neutral variant
- **Class 1:** variant known as constitutional polymorphism.

Variant grading system adopted:

- **Variants of known theranostic impact (Class 5, variants with MA in the disease)**
- **Variants to be discussed at Molecular RCP review (Class 5 with no MA in the disease and class 4):** This category includes all non-validated variants with a known potential functional impact. It is recommended to discuss these mutations at the molecular RCP review with a view to envisaging directing patients towards relevant clinical trials in particular. For these mutations, it is recommended to specify, in the results, the clinical knowledge invoked for the classification of the variant ("clinical impact" column). However, it is not recommended to detail all of the data leading to this classification in the report (SIFT data, Polyphen, literature, etc.).
- **Other variants for which the predictive value is unknown (class 3):** This category includes all somatic variants of unknown clinical impact.
- **Variants that it is not recommended to indicate in the report:**
    - Constitutional polymorphisms (class 1)
    - Intronic or silent mutations with no effect on splicing

Note: Due to the quantity of information generated by NGS techniques, it is essential to classify the information and highlight the most clinically relevant results as well as the mutations specified in the prescription. This classification may be performed in different ways according to the options offered by the laboratories' software (order of presentation of information, different fonts, tables, etc.).

Allelic frequency: It is not recommended to specify the allelic frequency in all the reports. However, in some cases, this information may be used if it is a source of additional information. This

particularly applies in the case of discrepancies between the allelic frequency and the cellularity which may suggest the presence of tumour subclones. The allelic frequency may also be specified to assist the interpretation of mutations detected in tumour suppressor genes.

Copy-number variation (CNV): bioinformatic algorithms for detecting copy-number variation are available. This information is not reliable enough to establish a diagnosis, but may be used to advise in-depth testing with another procedure if the gene shows clinical interest.

Coverage: It is not necessary to specify the sequencing depth for all variants, but the report should specify the list of exons for which a coverage deficiency exists.

Method: Library kit reference / sequencer used / diagnostic sensitivity / analytical pipeline version. In the case of locally developed panels, it is necessary to specify the list of genes and exons of the panel (+/- NM_) as well as the reference genome version used. It may also be useful to specify whether the exons are partially or fully covered, particularly for suppressor genes.

ITEMS THAT MAY OPTIONALLY FEATURE IN THE MOLECULAR GENETICS REPORT TEMPLATE

The EQA scheme for EGFR mutation testing in non-small cell lung cancer suggests using the double-signature procedure for the report **so as to avoid input errors**. This does not seem to be feasible in all cases, but is proposed as an option.

CONCLUSION/INTERPRETATION OF RESULTS

- The final report of the 2011 pilot EQA scheme for EGFR mutation testing in non-small cell lung cancer states that:

  Interpretation is essential and must: (1) refer to "EGFR tyrosine kinase inhibitors" and not to a specific molecule and (2) establish the association between mutation and EGFR-TKI sensitivity without prescribing treatment. There is no benefit in reporting variants of no clinical impact

- The final report of the ESP KRAS External Quality Assessment Scheme 2011 specifies the following point:

  In cases of *KRAS* mutation in colorectal cancer, it is necessary to establish the association between mutation and anti-EGFR antibody resistance and **in the event of absence of mutation,** it is necessary to specify that the patient exhibits no anti-EGFR antibody resistance

- Moreover, the working group has made the following proposals:

  Bibliographic references: it does not seem necessary to cite a reference publication for drugs subject to an MA restricting administration to the presence or absence of molecular alterations

  ***EGFR* mutations in non-squamous lung cancers:**

  - mutations interpreted as imparting EGFR tyrosine kinase inhibitor sensitivity: exon 19 deletions and G719X, L858R and L861Q point mutations. No comment modulating the sensitivity according to the identified mutation is required. No comment on the activating effect of the mutation is required
  - mutations interpreted as imparting 1st and 2nd generation EGFR tyrosine kinase inhibitor resistance: T790M mutation. The T790M mutation is described as imparting 3rd generation tyrosine kinase inhibitor sensitivity.

- mutations interpreted as being of indeterminate predictive value of response to EGFR tyrosine kinase inhibitors: mutations other than the mutations cited above

RAS mutations in colorectal cancers:

- In the absence of *RAS* mutation, specify that the patient exhibits no anti-EGFR antibody resistance

Mutations identified within the scope of the AcSé programme:

- Presence of MET/ALK/ROS1/BRAF gene mutation for which an AcSé type clinical trial is available. AcSé trials are aimed at patients with no other therapeutic options and who cannot be included in other clinical trials (academic or industrial)
- Add the link to the relevant internal site:
  AcSé crizotinib:
  http://www.unicancer.fr/rd-unicancer/le-programme-acse/essai-acse-crizotinib
  AcSé vemurafenib:
  http://www.unicancer.fr/rd-unicancer/le-programme-acse/essai-acse-vemurafenib

Other identified variants (NGS):

- Presence of X gene mutation to be discussed at molecular RCP review. Details on the clinical impact of the mutation may be added in conclusion.
- Detection of increase/decrease in copies of the X gene suggesting the existence of gene amplification/deletion. Further testing with another procedure would help verify the presence of this modification.

Detailed information on the therapeutic indications and monitoring of drugs can be viewed on the ANSM and EMA websites

REPORT FORMATTING

The final report of the 2011 pilot EQA scheme for EGFR mutation testing in non-small cell lung cancer makes the following recommendation:

One-page report formatting:

If the report takes up more than one page, the patient ID, result, interpretation and signature should feature on the first page. The pages should also be numbered.

III. SAMPLE REPORT FOR A LUNG TUMOUR

XX HOSPITAL SOMATIC GENETICS LABORATORIES

Screening test for somatic mutations of *EGFR, KRAS, BRAF, PI3KCA,HER2*

TEST NO.: 1324

- **Patient** Name: **SMITH John** Birth name
- Date of birth: **30/10/1952** Sex: **M**
- Performed using the specimen referenced **12.0004** dated **02/01/2016**
- Received on **16/01/2016**
- Prescribed by: **Dr DURAND Cécile (avenue du Général de Gaulle, Paris)** on **10/01/2016**
- Reason for requested test: **somatic mutation testing as part of the therapeutic care plan of a patient suffering from adenocarcinoma of the lung**

ANATOMO-CYTO-PATHOLOGICAL DETAILS

- Specimen type: **bronchial biopsy**
- Histological type and tumour status: **adenocarcinoma of the lung**
- Pathologist responsible for diagnosis: **Dr MARTIN Michel (Avenue Jean Jaurès, Paris)**
- Test conducted on a region selected by **Dr LAMBERT Céline**
- Specimen: biopsy, piece fixed and included in paraffin, 50% tumour cells

RESULTS

- **Variants of known clinical impact**

Gene	Reference sequence	Variant	Protein modification
EGFR	**NM_ 005228.3**	**c.2573T>G**	**p.Leu858Arg**

- **Variants to be discussed at Molecular RCP review**

Gene	Reference sequence	Variant	Protein modification
MET	**NM_001127500.1**	**c.2942-2A>G**	**Splicing variant**

- **Other variants for which the predictive value is unknown**

Gene	Reference sequence	Variant	Protein modification

- **Genes for which no anomalies were detected:**
  AKT1, ALK, BRAF, ERBB2, ERBB4, FGFR2, FGFR3, HRAS, KIT, KRAS, MAP2K1, NRAS, PDGFRA, PI3KCA

CONCLUSION / INTERPRETATION OF RESULTS BASED ON THE CURRENT STATE OF THE ART

- **Presence of EGFR gene p.L858R mutation imparting EGFR tyrosine kinase inhibitor sensitivity.**
- **P**resence of MET gene c.2942-2A>G mutation inducing exon 14 skipping in the gene. MET mutations are covered by the AcSé crizotinib clinical trial (http://www.unicancer.fr/rd-unicancer/le-programme-acse/essai-acse-crizotinib). AcSé trials are aimed at patients with no other therapeutic options and who cannot be included in other clinical trials (academic or industrial)
- No anomaly in the other genes tested

Issued in **Paris** on **23/01/2016**
Signature

METHOD

- Test conducted using a specimen fixed with buffered formol and included in a paraffin block, after macrodissection of the region of interest.
- Tumour genome DNA extraction method. **if a commercial kit is used: name and version**
- Mutation testing on the following genes using the NGS method (**kit name and version, sequencer used, sensitivity, minimum depth, analytical pipeline version**), list of genes and exons tested and reference sequence
- *EGFR* gene mutation testing using **test method if a commercial kit is used: name and version,** analytical sensitivity **as a % of mutated cells in wild-type cell background noise,**
  list of mutations under test, reference sequence (NM_005228.3) and reference genome version

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The report template has been submitted to the 28 cancer molecular genetics platforms for review.