Molecular Diagnostic Service for Cancer

Operational Policy

Index

1. Introduction

This document defines the responsibilities and operational procedures governing the Oxford NHS / BRC Molecular Diagnostic Service for Cancer. The aim of the service is to provide accurate and timely information about genetic abnormalities detected in solid tumours in order to inform treatment decisions and prognosis.

1.1 Objectives

1.1.1 Objectives of the Molecular Diagnostic Service for Cancer process

- To ensure compliance with NICE guidelines
- To ensure appropriate, cost-effective use of genetic tests, avoiding unnecessary test duplication
- To ensure maximal information is obtained from limited diagnostic material with analyses performed in the most clinically relevant order
- To ensure accurate, clinically informative reports are issued and disseminated to appropriate personnel within defined specimen turn-around times
- To provide high quality genetic information from samples obtained during ethically approved research trials

1.1.2 Objectives of this operational policy

- To define the responsibilities of local laboratories and the Molecular Diagnostic Service for Cancer in delivering the service
- To outline the processes involved in generating genetic information for solid tumours including sample reception, processing and reporting

2. General Operational Principles

The laboratory is designated by the commissioning groups and by the Thames Valley Cancer Network and is managed by the Oxford University Hospitals (OUH) NHS Trust.

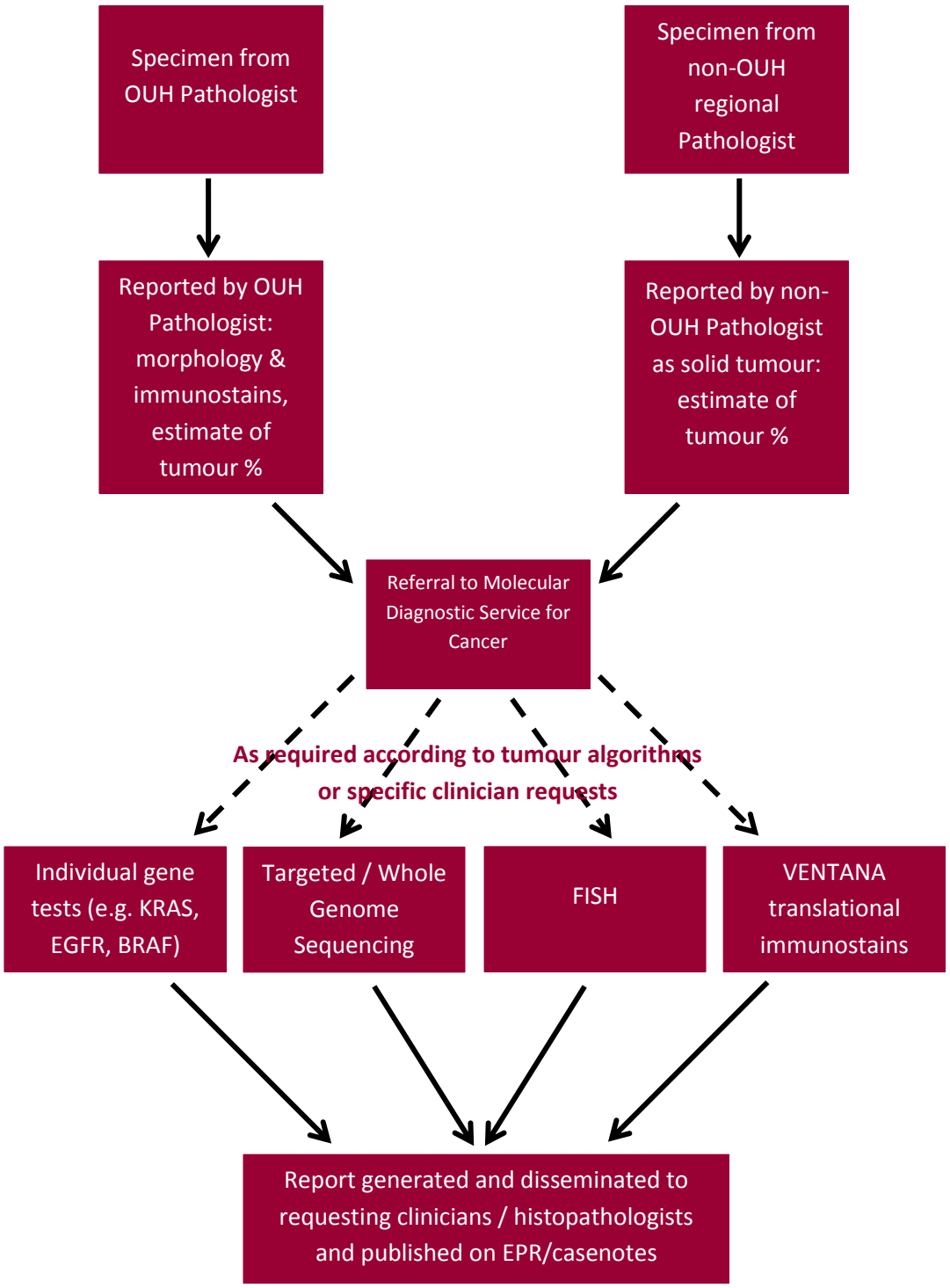
It is directed by a single head of service who reports to the Chief Executive of OUH via the Trust's Cancer Lead. Responsibilities include the design of investigational algorithms, oversight of the use of resources for diagnostics and research and standards of reporting.

The laboratory has a single pathology reception where all samples are received for initial processing and from which they are redirected for requested / algorithmically determined tests.

A combined molecular report is issued to the requesting histopathologist and clinician within the designated test turnaround time. It is the responsibility of the requesting histopathologist to integrate the genetic information into the histopathology report.

3. Detailed description of work-flows and procedures governing the Molecular Diagnostic Service for Cancer

3.1 Overview of sample pathway



3.2 Central Specimen Reception

3.2.1 Contact details and shipment address

Email: molpath@nhs.net

Telephone: 01865 572769

Address for slides: Oxford Molecular Diagnostics Centre
Molecular Haematology
Level 4, John Radcliffe Hospital
Headington
Oxford OX3 9DU

Address for blocks: Molecular Pathology Requests
Department of Cellular Pathology
Level 1 Academic Block, John Radcliffe Hospital
Headington
Oxford OX3 9DU

3.2.2 Request forms

See appendix 1

Request forms are also available from the Oxford Molecular Diagnostics Centre website

http://www.oxford-translational-molecular-diagnostics.org.uk/sites/default/files/Molecular%20Diagnostic%20Request%20form%20100513_0.pdf

This form should be emailed to molpath@nhs.net and also sent accompanying the tumour sample.

In order to ensure that the most appropriate investigations are performed on the tumour sample particularly if there is limited material available it is helpful to supply as much of the clinical information indicated on the request form as possible although it is appreciated that not all this information may be available at the time the specimen is sent.

3.2.3 Timing of requests

Samples are batched and performed on a weekly basis. For samples to be processed in a particular week they must be in the laboratory by 12 noon on Monday, or 12 noon on Tuesday in the case of a Bank Holiday.

3.3 Specimens

3.3.1 FFPE for genetic tests

10 serial sections of 5 microns (or 5 serial sections of 5 microns if the area of the tumour is greater than 2 cm^2) should be cut and placed on glass slides (multiple sections can be placed on a single glass slide). These should be sent along with an immediately preceding H&E or immunostained section with areas of malignant cell density >70% (i.e. the malignant cells themselves constitute >70% of the nucleated cells in that area) marked by the referring pathologist. The higher the density of malignant cells in the marked section the greater the probability of detecting a low frequency mutation, however samples with malignant cell density <70% can be analysed and in such situations the area with the highest density of malignant cells should be marked. When preparing pathological material for DNA analysis it is essential that equipment is completely clean (particularly the microtome blade and water bath) to prevent cross-contamination; this can be achieved by wiping with ethanol until all debris is removed.

OR

Alternatively a punch biopsy from the tissue block encompassing the area with highest density of malignant cells can be sent (along with an estimation of tumour percentage in that region); this method is currently under development and this operational policy will be updated when an SOP for this procedure is available.

3.3.2 FFPE for FISH / VENTANA immunohistochemistry

Sections should be prepared as for genetic tests and placed on positively charged glass slides. An immediately preceding H&E or immunostained section should also be sent with the areas with the highest density of tumour cells marked. The specific number of slides required is specified in individual tumour algorithms.

3.3.3 Cytological material for genetic tests

Cytological material should be made into a cell block and processed as for FFPE tissue blocks. Alternatively as much material as possible can be put on slides (e.g. smears, touch preps) and sent.

4. Algorithms guiding the testing of tumour samples

The individual tumour site algorithms are designed to provide genetic information which will

- Allow access to targeted therapies (e.g. gefitinib, cetuximab)
- Prevent the inappropriate use of high cost therapies likely to be detrimental to the patient
- Allow compliance with NICE guidelines

- Allow access to Cancer Drug Fund (but not NICE approved) therapies
- Allow access to clinical trials

The order of tests is designed to prevent unnecessary testing (e.g. looking for a second mutually exclusive mutation) and ensure that clinical questions are answered in order of importance particularly when there is limited pathological specimen available for analysis.

Each algorithm includes the rationale for inclusion of the specific tests (e.g. available targeted therapy, NICE guideline). Details of the individual tests are given in appendices 2 & 3.

4.1 Metastatic Colorectal Carcinoma

Samples are evaluated as follows:

1. KRAS mutation testing (cobas or Cancer Gene Panel; see below for test choice)
2. For OUH specimens BRAF mutation testing performed (cobas or Cancer Gene Panel; see below for test choice)*
3. NRAS mutation testing (if requested, performed on Cancer Gene Panel)*
4. Cancer Gene Panel (if requested)*
5. DNA storage (if sufficient)

*All of these tests are available for non-OUH samples if requested, but will not automatically be performed.

Rationale

1. **KRAS mutation testing**
   The CRYSTAL[1] and OPUS[2] studies demonstrated that some patients with wild type KRAS derived clinical benefit from the EGFR-targeting monoclonal antibody cetuximab. [3] These data has led to NICE recommending the use of cetuximab[4] in combination with FOLFOX or FOLFIRI chemotherapy as first-line treatment for patients with metastatic colorectal carcinoma where the primary tumour has been resected or is resectable and metastases are limited to the liver and the patient is fit enough to undergo surgery should the metastases become resectable after treatment with cetuximab. Subsequent analysis of the OPUS study suggested patients with an activating KRAS mutation did worse when treated with a combination of cetuximab and FOLFOX than those treated with FOLFOX alone.[5]

   The current National Cancer Drug Fund List permits the use of cetuximab in patients with metastatic colorectal carcinoma with wild type KRAS in the following circumstances (in addition to that recommended by NICE TA176): First line in combination with irinotecan-based chemotherapy, second or third line in combination with irinotecan-based chemotherapy, third or fourth line as a single agent.[6]

   If fewer than three genetic mutation tests are requested on a specimen, KRAS mutation testing is performed using the cobas system (see appendix 2 for further details) which looks for all common activating mutations. If all three individual genetic mutation tests are requested on a specimen, KRAS mutation testing is performed using the Cancer Gene Panel

(see appendix 3 for further details) which looks for all common activating mutations. Of note the technologies used and mutations looked for when performing KRAS testing is currently undergoing NICE evaluation.[7]

2. BRAF mutation testing

Given BRAF is found downstream of KRAS in the EGFR pathway there is some suggestion that mutations in this gene may affect the efficacy of anti-EGFR therapies e.g. cetuximab. Presently there is insufficient evidence to base treatment decisions upon the presence or absence of these mutations,[8] however research in this area continues and in a recent systematic review, patients with mutations in BRAF had a statistically significant worse prognosis than patients with wild type BRAF.[9]

Specific BRAF inhibitors exist which have been adopted into clinical practice for certain malignancies (e.g. vemurafenib in malignant melanoma)[10] and worldwide there are clinical trials of such therapies in colorectal cancer.

If fewer than three genetic mutation tests are requested on a specimen, BRAF mutation testing is performed using the cobas system (see appendix 2 for further details). If all three individual genetic mutation tests are requested on a specimen, BRAF mutation testing is performed using the Cancer Gene Panel (see appendix 3 for further details).

3. NRAS mutation testing

Given that even amongst KRAS wild-type patients the response rate to anti-EGFR monoclonal antibodies (cetuximab or panitumumab) is relatively low, retrospective analyses have searched for other mutations which might predict lack of response to this therapy. One such analysis demonstrated that activating mutations of NRAS were associated with a poor response to cetuximab.[11] A more recent prospective-retrospective analysis of the PRIME trial demonstrated that those patients with mutations in non-exon 2 KRAS, NRAS and BRAF had an inferior response (PFS and OS) to the combination of panitumumab and FOLFOX chemotherapy compared to those with wild-type RAS and BRAF. Furthermore the addition of panitumumab to FOLFOX in this mutated population did not improve PFS or OS.[12]

NRAS testing is performed using the Cancer Gene Panel (see appendix 3 for further details).

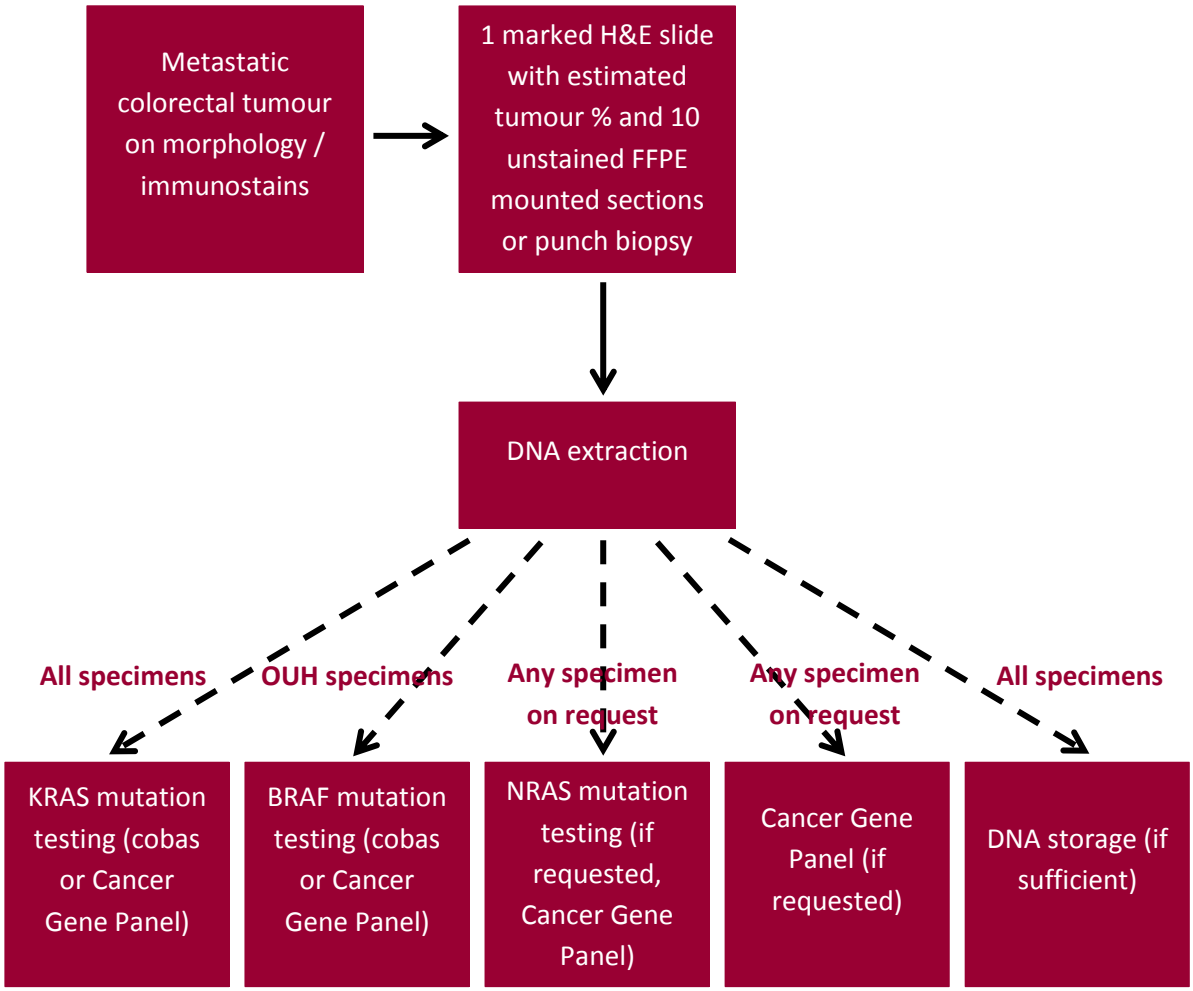
4. Cancer gene panel

For details of this test please see appendix 3.

5. DNA storage

To allow further genetic tests to be done if / when these are available and of clinical utility for the individual patient concerned.

Summary Algorithm (Please see appendix 4 for all test turnaround times)



4.2 Metastatic Malignant Melanoma

Samples are evaluated as follows:

1. BRAF mutation testing
2. Cancer Gene Panel (OUH specimens only)
3. DNA storage (if sufficient)

Rationale:

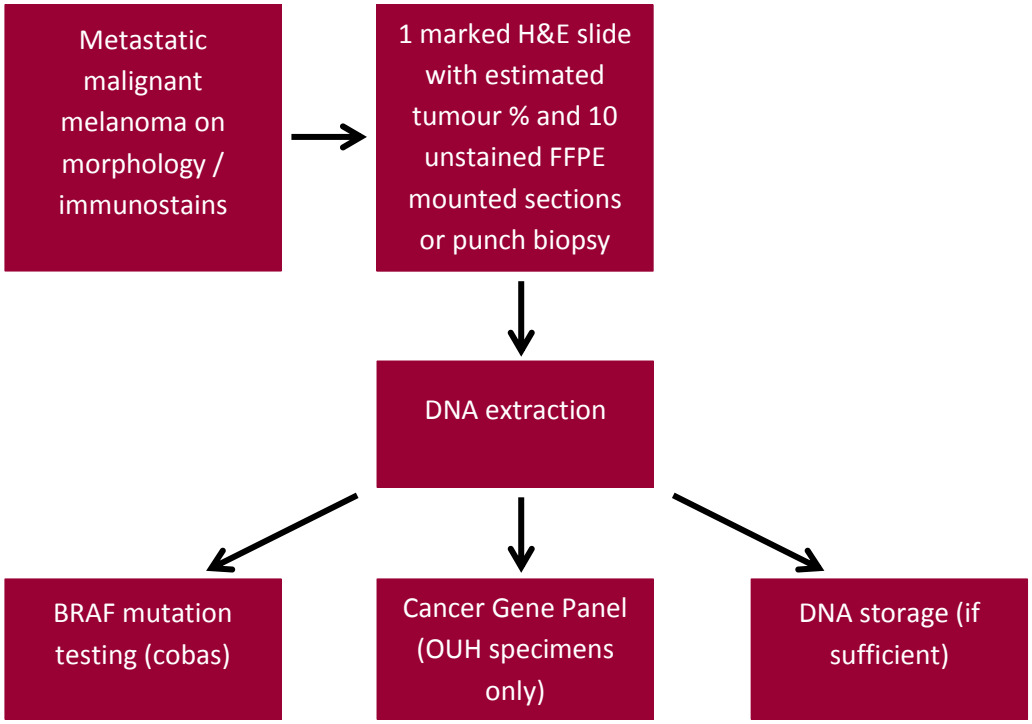
1. **BRAF mutation testing**
   The BRIM-3 trial demonstrated that some patients with the V600E BRAF mutation derived clinical benefit from the BRAF inhibitor vemurafenib,[10] while subsequent studies have shown the drug to effective in patients harbouring the V600K BRAF mutation.[13] These observations are reflected in the NICE guidance which recommends the use of vemurafenib to treat BRAF V600 mutation-positive unresectable or metastatic melanoma.[14]

BRAF mutation testing is currently performed using the cobas system (see appendix 2 for further details).

2. **Cancer gene panel**
   For details of this test please see appendix 3.

3. **DNA storage**
   To allow further genetic tests to be done if / when these are available and of clinical utility for the individual patient concerned.

Summary Algorithm (Please see appendix 4 for all test turnaround times)



4.3 Non-resectable or Metastatic Non Small-Cell Lung Carcinoma

Samples are evaluated as follows:

1. EGFR mutation testing
2. ALK rearrangement testing performed if negative for EGFR mutation
3. Cancer Gene Panel (OUH specimens only)
4. DNA storage (if sufficient)

Rationale

1. **EGFR mutation testing**
   The EURTAC[15] and OPTIMAL[16] trials demonstrated that some patients with activating mutations of EGFR derived clinical benefit from the EGFR inhibitor erlotinib whilst the IPASS trial[17] demonstrated similar benefit for gefitinib in the same patient population. These

observations are reflected in the NICE guidance which recommends erlotinib[18] or gefitinib[19] as first line treatment for locally advanced or metastatic non-small cell lung carcinoma shown to have an activating EGFR mutation.

EGFR mutation testing is currently performed using the cobas system (see appendix 2 for further details) which is one of the technologies recommended by NICE to investigate for activating EGFR mutations.[20]

2. ALK rearrangement testing

The PROFILE 1007 trial[21] demonstrated that some patients with rearrangements of the ALK gene (usually but not exclusively with the ELM4 gene) derive clinical benefit from the ALK-targeting tyrosine kinase inhibitor crizotinib. On the basis of this data crizotinib has received a conditional marketing authorisation from the EMA for the treatment of adults with previously treated ALK-positive advanced non-small cell lung cancer. Although it received an unfavourable NICE appraisal[22] (currently being appealed) on the grounds of cost-effectiveness, it is currently available via the cancer drugs fund[23] for the licenced application and there are clinical trials evaluating its use in the first-line setting.[24]

The gold-standard method for detecting ALK rearrangements is via FISH. This is performed using the Vysis ALK Break Apart FISH Probe Kit. Given an ALK rearrangements is considered to be a driver mutation in non-small cell lung cancer, and driver mutations are usually mutually exclusive, only non-OUH specimens which are negative for an activating EGFR mutation (as determined by the cobas assay) and OUH specimens negative for an activating EGFR mutation (as determined by the cobas assay) and BRAF and KRAS mutations (as determined by the cancer gene panel) undergo ALK testing. OUH specimens subjected to ALK-rearrangement testing are also currently undergoing immunohistochemistry using a fully automated technique to validate this as a screening method for ALK rearrangements. (VENTANA ALK immunohistochemistry).

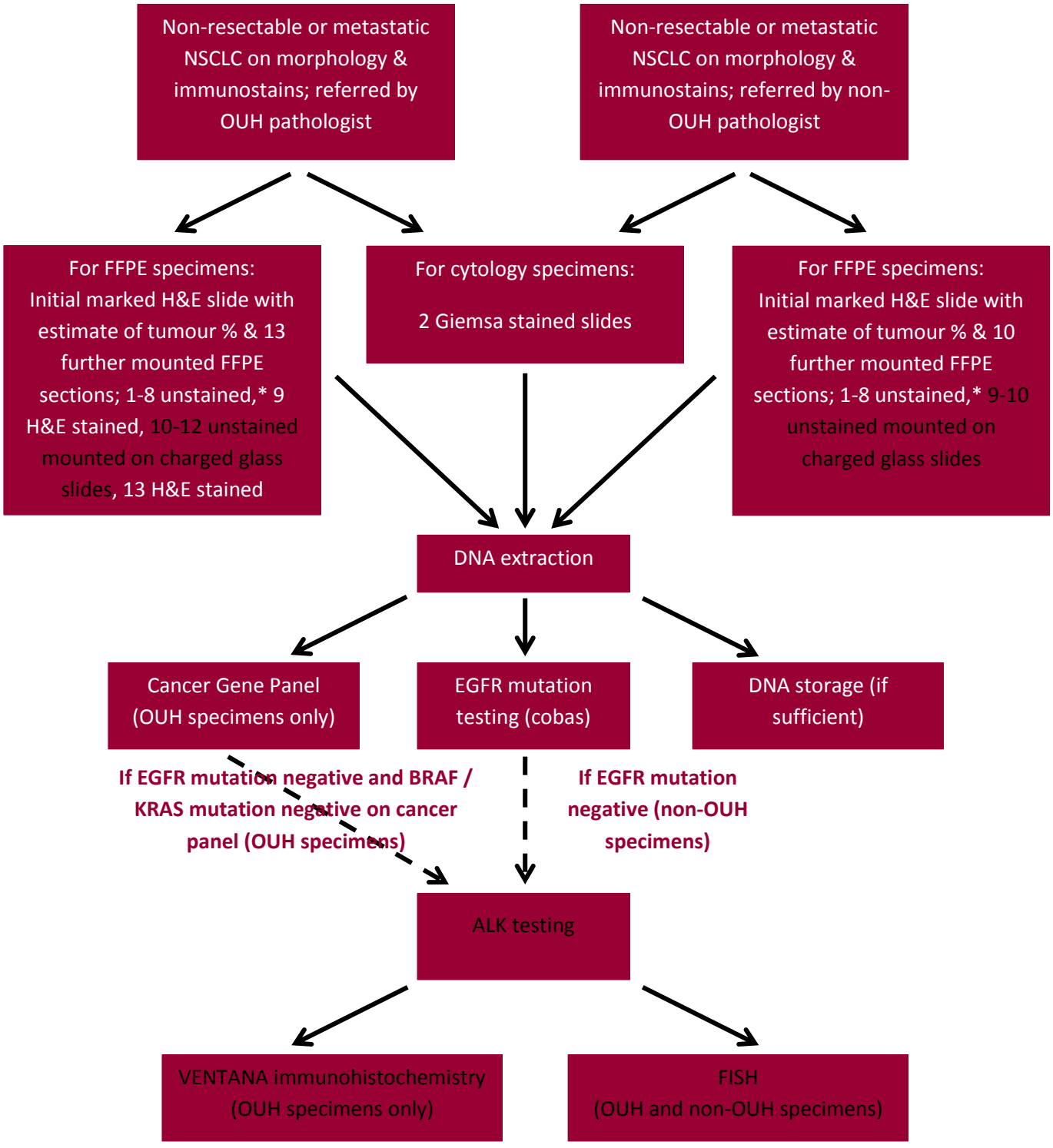
3. Cancer gene panel

For details of this test please see appendix 3.

4. DNA storage

To allow further genetic tests to be done if / when these are available and of clinical utility for the individual patient concerned.

Summary Algorithm (Please see appendix 4 for all test turnaround times)



***Slides 1-8 can be replaced with a punch biopsy of the tumour block, but charged slides with guide H&E slides are required for ALK testing.**

5. Appendices

5. 1 Appendix 1: Request Form

OXFORD Molecular Diagnostics Centre

MOLECULAR DIAGNOSTICS REQUEST FORM FOR CLINICIANS:
EGFR/ K-RAS/ B-RAF/46 Gene Cancer Panel, other tests by prior arrangement

Patient Details *(to be completed by referring clinician)*		
Surname:	Forename:	Sex: F/M
Address:	Date of Birth:	OUH Hospital Number (if known):
	NHS Number:	
NHS/ Private Patient/ Research *(please delete as applicable)*	Referring Hospital:	
Date of biopsy:	Address for Invoice:	
Referral hospital pathology case number & block number/ letter:	Date of request:	

Tumour Details	
Sample type: unstained sections on coated slides/ cytological specimen/ other *(please delete as applicable)*	
Tumour histology:	Site of tumour in this biopsy:
Primary/Metastasis/Not known *(please delete as applicable)*	Primary site of tumour (e.g., lung, colon):
Area of tumour inside ring: cm^2	Estimated Percentage Tumour in sample: %

Test Details

Test (delete as applicable): EGFR/ K-RAS/ B-RAF/46 Gene Cancer Panel
other (please state):
Does patient fulfil NICE guidelines for:
Gefitinib/ erlotinib (175/192/162-lung cancer) Yes / No/ Unknown *(please delete as applicable)*
Cetuximab (TA176 - colorectal cancer) Yes / No/ Unknown *(please delete as applicable)*
Vemurafenib/ ipilimumab (melanoma) Yes / No/ Unknown *(please delete as applicable)*
Imatinib for c-kit positive GIST Yes / No/ Unknown *(please delete as applicable)*
46 Gene Cancer Panel Yes / No/ Unknown *(please delete as applicable)*

Laboratory Procedure: Ten 5 micron sections are required (or five if marked tumour area >2cm^2), mounted on microscope slides. Multiple sections can be placed on a single slide. Please clean microtome blade and water-bath thoroughly before cutting sections, to avoid cross-contamination and false positive results. Please also include an H&E stained section from the same block with the tumour boundary marked. Tissue in this ring must be >70% tumour. For cytological material, please cut cell blocks as for tissue blocks or send maximum available material (smears, touch preps etc) on slides.

Referring clinician and/ or pathologist:.....

Referring clinician/ pathologist telephone/ bleep/ pager .....

E-mail address(es) for report.....

Requests, Molecular Diagnostics Centre, Molecular Haematology, Level 4 John Radcliffe Hospital, Oxford OX3 9DU. Tel 01865 572769 E-mail oxford.molecularhaem@nhs.net
http://www.oxford-translational-molecular-diagnostics.org.uk/

5.2 Appendix 2: Cobas testing

Individual gene mutation testing for KRAS, BRAF & EGFR is performed using the cobas 4800 system by Roche.

KRAS

The cobas KRAS mutation test kit is a TaqMelt real-time PCR assay designed to detect the presence of common mutations in codons 12, 13 and 61 of the KRAS gene which account for approximately 95% of all activating mutations in the KRAS gene. Mutations at these locations all cause constitutive activation of the protein and have all been associated with resistance to anti-EGFR therapy. [25,26] It should be noted that mutations outside these codons **will not** be detected by this assay. The sensitivity (i.e. % mutant detectable DNA in a background of wild-type DNA) of this assay is 5%.

BRAF

The cobas BRAF V600 mutation test is a TaqMelt real-time PCR assay primarily designed to detect the presence of the 1799T>A mutation which results in a V600E substitution (valine to glutamic acid) in the BRAF protein and constitutive activation of the RAF pathway. Approximately 10% of mutations at this site result in an alternative amino acid substitution[27] such as V600K, V600R, V600D, all of which increase the catalytic activity of BRAF.[28] In preliminary testing by Roche, some V600D and V600K mutations were detected with this assay, but the absence of detecting a V600 mutation does not rule out one of these rarer mutations being present. Mutations outside codon 600 **will not** be detected by this assay. The sensitivity (i.e. % mutant detectable DNA in a background of wild-type DNA) of this assay is 5%.

EGFR

The cobas EGFR mutation test is a real-time PCR test for specific common mutations in exons 18, 19, 20 & 21 (together account for >90% activating mutations) of the EGFR gene which cause constitutive activation of the protein. The mutations detectable by this assay are indicated in the table below:

Mutation
G719X (G719S / G719A / G719C)* in exon 18
29 deletions and complex mutations* in exon 19
T790M in exon 20
S768I in exon 20
5 insertions in exon 20*
L858R in exon 21

*The test detects the presence of these mutations but cannot distinguish between them.

This test can detect both mutations which are likely to indicate a favourable clinical response with a specific tyrosine kinase inhibitor (e.g. erlotinib or gefitinib)[29] and those which are likely to suggest acquired resistance to these drugs. [30] Mutations in exons 18-21 outside the tested region and in

other exons **will not** be detected by this test. The sensitivity (i.e. % mutant detectable DNA in a background of wild-type DNA) of this assay is 5%.

5.3 Appendix 3: Cancer Gene Panel

Background

The Cancer Gene Panel is a targeted next generation sequencing assay able to look for mutations in defined regions across 50 genes* frequently mutated in a variety of tumours. It uses IonTorrent technology which employs sequencing by synthesis: When a complementary nucleotide is incorporated into the DNA strand during synthesis a hydrogen ion is released which is detected by a semiconductor. Sequential exposure to the four different nucleotides allows determination of the gene sequence. The 207 target regions span parts of the exons listed in the following table.

Gene	Exons (partially covered)	Gene	Exons (partially covered)
ABL1	4, 5, 6, 7	**IDH2**	4
AKT1	3, 6	**JAK2**	14
ALK	23, 25	**JAK3**	4, 13, 16
APC	14	**KDR**	6, 7, 11, 19, 21, 26, 27, 30
ATM	8, 9, 12, 17, 26, 34, 35, 36, 39, 50, 54, 55, 56, 59, 61, 63	**KIT**	2, 9, 10, 11, 13, 14, 15, 17, 18
BRAF	11, 15	**KRAS**	2, 3, 4
CDH	3, 8, 9	**MET**	2, 11, 14, 16, 19
CDKN2A	2	**MLH1**	12
CSF1R	7, 22	**MPL**	10
CTNNB1	3	**NOTCH1**	26, 27, 34
EGFR	3, 7, 15, 18, 19, 20, 21	**NPM1**	11
ERBB2	19, 20, 21	**NRAS**	2, 3, 4
ERBB4	3, 4, 5, 6, 7, 8	**PDGFRA**	12, 14, 15, 18
EZH2	16	**PIK3CA**	2, 5, 7, 8, 10, 14, 19, 21
FBXW7	5, 8, 9, 10, 11	**PTEN**	1, 3, 5, 6, 7, 8
FGFR1	4, 7	**PTPN11**	3, 13
FGFR2	7, 9, 12	**RB1**	4, 6, 10, 11, 14, 17, 18, 20, 21, 22
FGFR3	7, 9, 14, 16, 18	**RET**	10, 11, 13, 14, 16
FLT3	11, 14, 16, 20	**SMAD4**	3, 4, 5, 6, 8, 9, 10, 11, 12
GNA11	5	**SMARCB1**	2, 4, 5, 9
GNAQ	5	**SMO**	3, 5, 6, 9, 11
GNAS	8, 9	**SRC**	14
HNF1A	3, 4	**STK11**	1, 4, 5, 6, 8
HRAS	2, 3	**TP53**	2, 4, 5, 6, 7, 8, 10
IDH1	4	**VHL**	1, 2, 3

* Prior to xxxxx a 46 gene Cancer Gene Panel was used for this assay which has slightly different gene and exon coverage to that listed in the above table. If you require specific information with regards to gene / exon coverage please contact the laboratory.

It should be noted that usually only small sections of the above exons are targeted by the assay (those known to contain frequent mutations across a range of tumours) and that mutations outside these regions **will not** be detected.

Samples

The assay requires genomic DNA which can be extracted from FFPE specimens, cell blocks (all of which are macro-dissected to ensure the highest possible tumour percentage) and cytology specimens. The minimum amount of DNA required for a likely successful test result is 10 ng. The recommended minimum percentage of tumour in the supplied specimen is 30%. Again, the assay can, on occasion, be attempted with a lower percentage of tumour although the likelihood of missing a low level mutation is higher.

This test is currently automatically performed on

- OUH melanoma specimens
- OUH non-small cell lung carcinoma specimens

It is regularly performed on colorectal carcinoma specimens (on request) and is available for any malignant specimen (providing sufficient DNA is available) at the request of the treating clinician.

Results

In order to be reported as a mutation the nucleotide(s) in question must have coverage of at least 500 reads (i.e. >= 500 separate DNA strands including this region of DNA) and the mutation must represent at least 4% (hotspot region) or 8% (non-hotspot region) of the nucleotides measured at this position.

The assay is validated for results from nine genes present on the panel (**BRAF**, **EGFR**, **KIT**, **KRAS**, **NRAS**, **PDGFRA**, **PIK3CA**, **PTEN**, **TP53**), the remainder are reported on a research only basis.

If detected, mutations are reported using a Tier structure;

- **Tier 1 mutation**: This is a mutation for which there is an approved course of clinical action (e.g. a BRAF mutation in melanoma, EGFR mutation in non-small cell lung carcinoma or KRAS mutation in colorectal carcinoma)**.**
- **Tier 2 mutation**: This is a mutation which although not having a defined approved course of clinical action as determined by randomised controlled trials, nevertheless is likely to promote informed clinical decision making, for example through entry into a clinical trial. Examples of such mutations include NRAS mutations in melanoma where MEK inhibitors may be of benefit,[31] and KIT and PIK3CA mutations for which clinical trials of targeted therapies may be available. [32]
- **Tier 3 mutation**: This is a mutation which has been previously described in a malignancy (as listed in the COSMIC database) but whose significance is as yet undetermined.
- **Tier 4 mutation**: This is a mutation which has, as yet, not been previously described in a malignancy (as listed in the COSMIC database) and therefore the significance of which is unknown.

The cancer gene panel is reported with reference to known local treatment pathways and available clinical trials, My Cancer Genome (website providing information on clinical trials by mutation and tumour location) and information available from the EGAPP (Evaluation of Genomic Applications in Practice and Prevention) Working Group (organisation concerned with developing a 'systematic approach for assessing the validity and utility of emerging genetic tests' and providing 'guidance on the appropriate use of such tests in specific clinical scenarios'). [33]

In view of the fact this is a novel test providing results whose clinical significance is, as yet, often unknown, we are keen to have feedback as to how any results have altered clinical management in order to generate some clinical utility data for the assay.

5.4 Appendix 4: Turnaround times

The turnaround times for the various tests performed by the molecular diagnostic service for cancer within the laboratory are listed in the following table:

Test	Turnaround time	Notes
KRAS mutation (cobas)	5 working days	If received in laboratory by 12 noon on Monday
KRAS mutation (cancer gene panel)	1 week	If received in laboratory by 12 noon on Monday
BRAF mutation (cobas)	5 working days	If received in laboratory by 12 noon on Monday
BRAF mutation (cancer gene panel)	1 week	If received in laboratory by 12 noon on Monday
EGFR mutation (cobas)	5 working days	If received in laboratory by 12 noon on Monday
NRAS mutation (cancer gene panel)	1 week	If received in laboratory by 12 noon on Monday
Cancer Gene Panel	2 weeks	If algorithmically determined (i.e. OUH melanoma or NSCLC specimen) or formally requested and received in laboratory by 12 noon n on Monday
ALK rearrangement testing by FISH	2 weeks	From decision to perform ALK rearrangement testing (i.e. EGFR mutation negative for external samples or EGFR, BRAF and KRAS mutation negative for OUH samples)
ALK rearrangement testing by VENTANA immunohistochemistry (if sufficient slides received)	2 weeks (once validated)	From decision to perform ALK rearrangement testing (i.e. EGFR mutation negative for external samples or EGFR, BRAF and KRAS mutation negative for OUH samples)

6. Abbreviations

ALK Anaplastic lymphoma kinase

BRAF v-raf murine sarcoma viral oncogene homolog B

COSMIC Catalogue Of Somatic Mutations In Cancer

DNA Deoxyribonucleic acid

EGAAP Evaluation of Genomic Applications in Practice and Prevention

EGFR Epidermal Growth Factor Receptor

ELM4 echinoderm microtubule-associated protein-like 4

EMA European Medicines Agency

FFPE Formalin fixed paraffin embedded

FISH Fluorescence in situ hybridization

FOLFIRI Folinic acid, fluorouracil & irinotecan

FOLFOX Folinic acid, fluorouracil & oxaliplatin

H&E Hematoxylin & Eosin

KIT v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog

KRAS Kirsten rat sarcoma viral oncogene homolog

MEK mitogen-activated protein kinase kinase

NICE National Institute for Health and Care Excellence

NRAS Neuroblastoma RAS viral (v-ras) oncogene homolog

NSCLC Non-small cell lung carcinoma

OS Overall survival

OUH Oxford University Hospitals NHS Trust

PCR Polymerase chain reaction

PDGFRA platelet-derived growth factor receptor, alpha polypeptide

PFS Progression free survival

PIK3CA phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha

PTEN phosphatase and tensin homologue

SOP standard operating procedure

TP53 tumour protein p53

7. References

[1] Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009; 360:1408–17.

[2] Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 2009; 27:663–71.

[3] Bokemeyer C, Van Cutsem E, Rougier P, et al. Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. Eur J Cancer 2012; 48:1466-75.

[4] National Institute for Health and Care Excellence. Cetuximab for the first-line treatment of metastatic colorectal cancer. [TAG176]. London: National Institute for Health and Care Excellence. 2009;

[5] Jimeno A, Messersmith W, Hirsch F et al. KRAS mutations and sensitivity to epidermal growth factor receptor inhibitors in colorectal cancer: practical application of patient selection. J Clin Oncol. 2009; 27:1130-6.

[6] National Cancer Drugs Fund List (Updated 30 July 2013).

[7] National Institute for Health and Care Excellence. Diagnostic Assessment Report commissioned by the NIHR HTA Programme on behalf of the National Institute for Health and Clinical Excellence – Protocol. London: National Institute for Health and Care Excellence. 2013;

[8] Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: can testing of tumour tissue for mutations in EGFR pathway downstream effector genes in patients with metastatic colorectal cancer improve health outcomes by guiding decisions regarding anti-EGFR therapy? Genet Med 2013; 15:517-27.

[9] Safaee Ardekani G, Jafarnejad S, Tan L et al. The prognostic value of BRAF mutation in colorectal cancer and melanoma: a systematic review and meta-analysis. PLoS One. 2012; 7:e47054.

[10] Chapman P, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011; 364:2507-16.

[11] De Roock W, Claes B, Bernasconi D et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010;11:753-62.

[12] Douillard J, Oliner K, Siena S et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med. 2013; 369:1023-34.

[13] Sosman J, Kim K, Schuchter L, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. N Engl J Med. 2012; 366:707-14.

[14] National Institute for Health and Care Excellence. Vemurafenib for treating locally advanced or metastatic BRAF V600 mutation-positive malignant melanoma. [TAG269]. London: National Institute for Health and Care Excellence. 2012;

[15]Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2012; 13:239-46.

[16] Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2011;12:735-42.

[17] Mok, T. Phase iii, randomized, open-label, first line study of gefitinib vs carboplatin/ paclitaxel in clinically selected patients with advanced non-small-cell lung cancer (NsCLC) (iPAss) [Abstract LBA2]. Ann. Oncol. 2008; 19 (Suppl. 8), viii1.

[18] National Institute for Health and Care Excellence. Erlotinib for the first-line treatment of locally advanced or metastatic EGFR-TK mutation-positive non-small-cell lung cancer. [TAG258]. London: National Institute for Health and Care Excellence. 2012;

[19] National Institute for Health and Care Excellence. Gefitinib for the first-line treatment of locally advanced or metastatic non-small-cell lung cancer. [TAG192]. London: National Institute for Health and Care Excellence. 2010;

[20] National Institute for Health and Care Excellence. Epidermal growth factor receptor tyrosine kinase (EGFR-TK) mutation testing in adults with locally advanced or metastatic non-small-cell lung cancer [DG9]. London: National Institute for Health and Care Excellence. 2013;

[21] Shaw A, Kim D, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 2013; 368:2385-94.

[22] National Institute for Health and Care Excellence. Lung cancer (non-small-cell, anaplastic lymphoma kinase fusion gene, previously treated) – crizotinib [ID499]. London: National Institute for Health and Care Excellence. 2013;
[23] National Cancer Drugs Fund List (Updated 30 July 2013)
[24] ClinicalTrials.gov
[25] Normanno N, Tejpar S, Morgillo F et al. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. Nat Rev Clin Oncol. 2009; 6:519-27.
[26] Loupakis F, Ruzzo A, Cremolini C et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer 2009; 101:715-21.
[27] http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/
[28] Wan P, Garnett M, Roe S et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004; 116:855-867.
[29] Rosell R, Moran T, Queralt C et al. Screening for epidermal growth factor receptor mutations in lung cancer. New England Journal of Medicine 2009; 361:958-969.
[30] Balak MN, Gong Y, Riely GJ, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. Clin Cancer Res. 2006;12:6494-6501.
[31] Ascierto P, Schadendorf D, Berking C et al. MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study. Lancet Oncol 2013; 14:249-56.
[32] http://www.mycancergenome.org/
[33] http://www.egappreviews.org/