ARIEL Trial – Guidance on RAS (KRAS/NRAS) testing results interpretation

Scope: All ARIEL Sites – document explains RAS (KRAS/NRAS) results interpretation and align these to eligibility criteria as per the ARIEL protocol

We understand there are several possible reporting methods for RAS (KRAS/NRAS) testing across NHS trusts. However, the below guidance is designed to explain how to interpret the most common format of reports.

1. ARIEL RAS (KRAS/NRAS) result interpretation

The following information is a guide to reading and interpreting the KRAS and NRAS results within a pathology report. Guidance has been provided by the Pathology Lead Prof Nick West. The key information we aim to derive from this report is:

- Are any mutations present?
- If mutations are present, which codons are they displayed in?

The key information we are looking for will either be in the main body or the technical information of the report, however, this may need some further understanding to decipher this. As a minimum it is recommended that the assay should cover:

- KRAS codons 12/13/59/61/117/146
- NRAS codons 12/13/59/61

In many cases, BRAF will also be tested along with KRAS and NRAS, although the BRAF status is not relevant to the ARIEL trial.

The example below is an MSI plus report from the Newcastle GLH laboratory and is the standard testing used by centres across the North East and Yorkshire Region.

Example report 1 displays RAS testing results with no mutation detected.

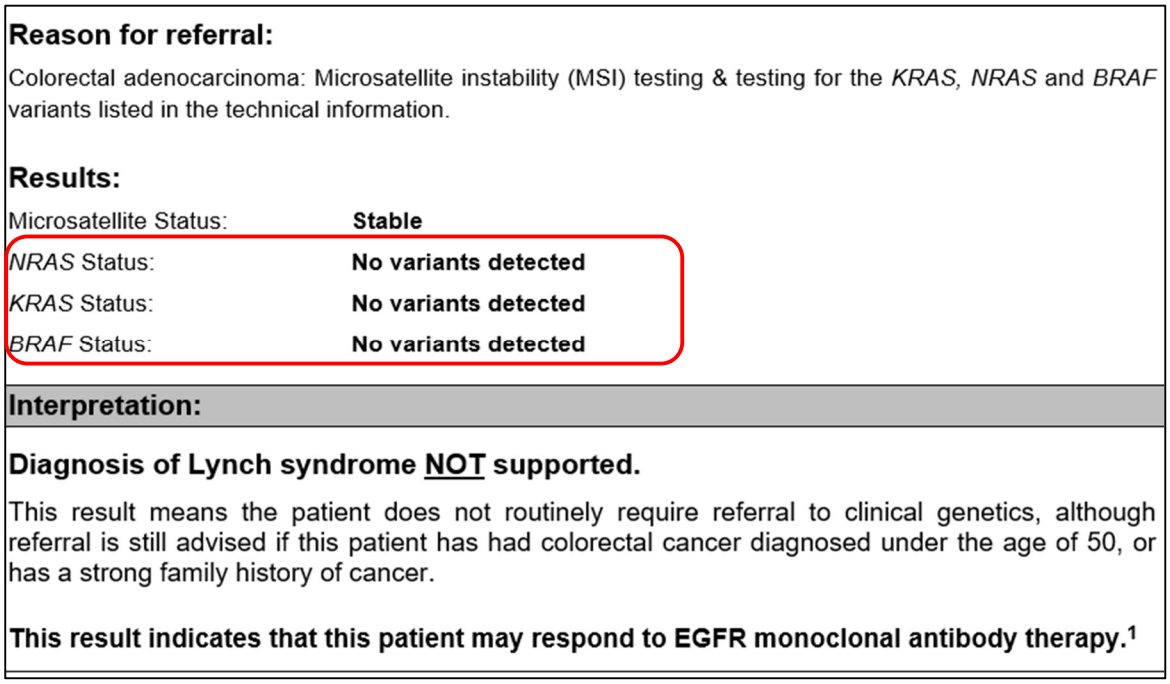
Example report 2 displays RAS testing results with a mutation present.

It should be easy to determine if any mutations are present/detected by looking at the first section of the report [1]. This section will highlight whether any mutations are present within the tested

sample and the location. In the first example, it is clearly shown that there are no mutations.

Mutations may be referred to as 'variants' by some laboratories.

Figure 1. Example report 1 displays RAS testing results with no mutation detected.



Reason for referral:

Colorectal adenocarcinoma: Microsatellite instability (MSI) testing & testing for the *KRAS*, *NRAS* and *BRAF* variants listed in the technical information.

Results:

Microsatellite Status:	**Stable**
NRAS Status:	**No variants detected**
KRAS Status:	**No variants detected**
BRAF Status:	**No variants detected**

Interpretation:

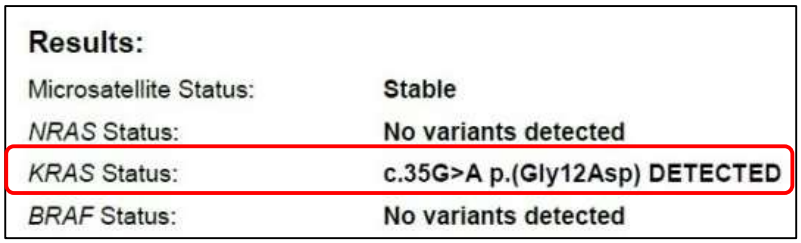
Diagnosis of Lynch syndrome NOT supported.

This result means the patient does not routinely require referral to clinical genetics, although referral is still advised if this patient has had colorectal cancer diagnosed under the age of 50, or has a strong family history of cancer.

This result indicates that this patient may respond to EGFR monoclonal antibody therapy.[1]

In cases where a mutation is present, it will be displayed as per the example below [2]. Any result other than 'No variants detected' means there is a mutation within the patient sample unless it is a fail or equivocal result.

Figure 2. Example report 2 displays RAS testing results with a mutation present.



Results:

Microsatellite Status:	**Stable**
NRAS Status:	**No variants detected**
KRAS Status:	**c.35G>A p.(Gly12Asp) DETECTED**
BRAF Status:	**No variants detected**

1.1. How to identify individual codon results:

The RAS result eCRF on MACRO requires you to identify the individual results for each codon tested. These individual results can be found later in the report under the 'Technical Information' section.

If there is no mutation present (e.g. figure 1), you need to check the report to determine which codons have been tested by the assay in order to complete the result input on MACRO. This information is usually stated in the technical information section of the report (see figure 3).

The areas covered by the assay might be summarised by base number, codon number or both. Each codon in the gene is composed of three bases. The composition and order of the bases determines the amino acid produced. For example, the first codon in KRAS that is usually assessed is codon 12, which is composed of bases 34,35 and 36. If the codon is normal, the amino acid produced should be glycine (abbreviated as Gly).

The technical information below shows the areas of both genes covered by the MSI-plus assay. The first area of the KRAS gene assessed is "c.34 & c.35 (p.Gly12)", which means that codon 12 is included in the assay by assessing for mutations at bases 34 and 35 potentially affecting the production of the glycine ammino acid. You can see that this assay covers KRAS codons 12, 13, 59, 61, 146, NRAS codons 12, 13, 18, 59, 61, 146, and BRAF codon 600.

Figure 3. Example report technical information section.

TECHNICAL INFORMATION

Microsatellite instability (MSI) or Microsatellite stable (MSS) status & variant testing has been performed using a bespoke Next Generation Sequencing (NGS) assay targeting 14 mononucleotide repeats and the target regions defined below. MSI classification is taken from the NGS data where the MSI marker median read depth is ≥100x. Targeted *BRAF*, *KRAS*, and *NRAS* variant genotype is taken from the NGS data with a minimum read depth of 250x. The minimum tumour content required for microsatellite instability classification is 10%. The sensitivity for targeted variant detection is approximately 5% allele frequency. Data analysis performed using a locally developed MSI Pipeline (v2.0.0). Nomenclature is based on sequence accession numbers NM_033360.3 (*KRAS*), NM_004333.4 (*BRAF*) and NM_002524.4 (*NRAS*) where base 1 is the A of the initiation ATG codon.
[1]Douillard *et al.* (2013) *N Engl J Med* 369:1023-34

Targeted Variants:

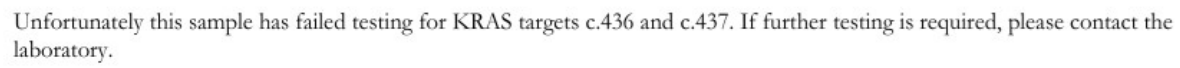
Pathogenic Single Nucleotide Variants & insertion/deletions at the following positions:

Gene	Variant positions	Gene	Variant positions
KRAS	c.34 & c.35 (p.Gly12)	*NRAS*	c.34 & 35 (p.Gly12)
KRAS	c.37 & c.38 (p.Gly13)	*NRAS*	c.37 & 38 (p.Gly13)
KRAS	c.175G>A p.(Ala59Thr)	*NRAS*	c.52G>A p.(Ala18Thr)
KRAS	c.181, 182 & 183 (p.Gln61)	*NRAS*	c.175G>A p.(Ala59Thr)
KRAS	c.436 & 437 (p.Ala146)	*NRAS*	c.181, 182 & 183 (p.Gln61)
BRAF	c.1799T>A p.(Val600Glu)	*NRAS*	c.436 & 437 (p.Ala146)

We cannot exclude the presence of rare variants mirroring the targeted nucleotide change in this test.

If any of the core codons are not assessed by the assay then you should enter "not tested" into MACRO. Occasionally one or more codons may fail or provide equivocal results, in which case the relevant macro entry should be made (see figure 4).

Figure 4. Example report text where some codons have failed.


Unfortunately this sample has failed testing for KRAS targets c.436 and c.437. If further testing is required, please contact the laboratory.

In the above example, only the bases are provided but these can be divided by 3 to get the codon number i.e. this relates to failed testing at KRAS codon 146.

When it comes to identifying which codon a mutation is present in, if a mutation is detected, the same principles apply. In figure 2, the KRAS mutation detected is summarised as "c.35G>A p.(Gly12Asp)". This means that a point mutation has been detected in the KRAS gene at base 35 in codon 12 with a substation of the G base for an A base leading to a change in the amino acid from glycine to aspartic acid. The actual type of mutation is not important as we are just looking to see if a mutation has occurred. This should be recorded on MACRO as a KRAS codon 12 mutation. All other codons tested on the assay would be wild type in this case. Occasionally double mutations exist in the same codon, however, for the purposes of data entry, we will just need this to be recorded as 'Mutant' for the relevant codon on MACRO. If double mutations are present in more than one codon, both codons should be marked as 'mutant'.

IMPORTANT

PLEASE ENSURE THE RAS (KRAS/NRAS) RESULTS HAVE BEEN REVIEWED AND APPROVED BY THE PI BEFORE THE PATIENT PROCEEDS ANY FURTHER ON THE TRIAL.

If there are any questions regarding results interpretation, please email us at ARIEL@leeds.ac.uk, we are happy to help.