# Colorectal cancer stratification in the routine clinical pathway: a District General Hospital experience

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**Abstract**

Colorectal cancer (CRC) has many subtypes with different prognoses and response to treatment. Patients must be characterised to access the most appropriate treatment and improve outcomes. An increasing number of biomarkers are required for characterisation but are not in routine use. We investigated whether CRC can be stratified routinely within a small district general hospital (DGH) to inform clinical decision making at local multidisciplinary team meeting (MDT)/tumor board (TB) level.

We evaluated mismatch repair (MMR) and EGFR signaling pathways using predominantly in-house immunohistochemical (IHC) tests (MSH2, MSH6, MLH1, PMS2, BRAF-V600E, Her2, PTEN, cMET) as well as send away PCR/NGS tests (NRAS, KRAS and BRAF). We demonstrated that many of the tests required for personalised treatment of CRC can be done locally and timely. Send away tests need to be requested shortly after cut-up and this needs to be firmly established in the tissue pathways for the results to be considered at MDT/TB. We have shown that MMR IHC combined with BRAFV600E IHC is practical and easy to perform in a small DGH, has full concordance with DNA-based tests and satisfies the latest NICE requirements for the identification of potential Lynch syndrome (LS) patients.

We provide a framework for the interpretation and presentation of test results. It is a practical classification that clinical pathologists can use to communicate effectively with the clinical team. It is broadly based on molecular subtyping, firmly focused on treatment decisions and dependent on the panel of molecular tests currently available.

## Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide (1.6 million new diagnoses p.a.) and is the fourth most common cause of cancer death (1). The standard treatment for early stage disease has been surgery with or without adjuvant chemotherapy. Whilst the diverse nature of CRC has long been accepted, there has been little clinical reason to recognise this since treatment options have been few and historically determined predominantly by tumour stage and not by disease subtype (2). In the past decade, the therapeutic landscape has become complex with the emergence of numerous new targeted treatments (Figure 1) that are only effective on selected cohorts of patients. Companion diagnostic tests are required to identify potential responders (e.g. *RAS* mutational analysis for drugs targeting the EGFR pathway (3,4). Pathologists are now required to provide companion diagnostics and other prognostic and predictive tests to guide treatment choice and clinicians have to consider the significance of growing numbers of additional tests.

The Multi Disciplinary Team (MDT)/Tumor Board (TB) meeting is the forum where all test results are considered and the management of each cancer patient is agreed. Despite UK guidelines recommending the use of tests such as those for DNA mismatch repair (MMR) on all patients with CRC (5,6), at present only few are managed with the full knowledge of these results (7). The reasons for this non-compliance are unclear. The aim of our study was to understand whether high-quality, full-scale testing for CRC biomarkers can be delivered for all CRC patients in a timely manner, what practical obstacles may prevent implementation, and to provide a framework for laboratories wanting to comply with the guidance. The work was carried out in a small District General Hospital (DGH) to provide proof of principle that these service improvements

are not dependent on facilities and expertise available only in large regional reference centres or teaching hospitals. Our goal was to develop a workflow plan to produce and present molecular pathology data for optimal personalised patient management in time for MDT/TB (within 2 weeks from surgery). Our assessment included IHC for MMR together with *BRAF* V600E, as per NICE guidance (6) and *RAS* mutation status (as a biomarker for EGF-R targeted therapy). In addition, we evaluated PTEN, HER-2 and c-MET. Although these are currently not in guidelines, there is evidence that they influence the effectiveness of EGF-R targeted therapy (8,9) and can be evaluated easily by IHC.

## Materials and Methods

This work was performed prospectively as part of a study to improve the colorectal histopathology service and, as such, required no ethics approval. The study included all patients at Dorset County Hospital who underwent surgical resection for CRC during 2013.

For each resection specimen, a tissue block suitable for the study was identified at cut-up (Day 0). Ideally, this contained tumour plus normal colonic tissue (for control). Tissue was fixed in 10% neutral buffered formalin for 6-72 h at room temperature (16-21$^{o}$C) and paraffin-embedded following routine processing.

*Immunohistochemistry (IHC)*

Eight different IHC tests were performed on each case (Table 1). Three μm sections were cut from the selected block and placed on coated slides. Control tissue was also added to the slide if required. Where available, the patient's initial endoscopic biopsy was identified, sectioned and placed on the same slide. The slides were baked for 45 minutes at 60$^{o}$C. We included endoscopic biopsies to assess whether they could provide robust molecular data for the patients who are treated before surgery. We used the resection data if there was discordance between endoscopic biopsy and resection in this study.

All the IHC was performed on either the Ventana BenchMark XT or Ventana BenchMark ULTRA following the protocols indicated in Table 1. Staining patterns were evaluated according to the criteria in Table 1 on resection specimens and, when available, endoscopic biopsies.

*Controls for IHC*

Colorectal tissue contained sufficient internal positive and negative controls for most of the antibodies. An additional piece of known positive tissue was placed on each slide for the BRAF V600E mutation-specific antibody (VE1) and HER-2/neu antibody (4B5). For the MMR markers, appendix tissue can be used to facilitate validation and control for staining consistency (Figure 2). Quality assurance of our MMR IHC was through the UK NEQAS ICC&ISH assessment scheme.

*Genomic testing: Next Generation Sequencing (NGS) and conventional non-NGS techniques*

After taking all the sections necessary for the in-house IHC work, the tissue blocks were sent to a reference centre (UCL Advanced Diagnostics) for *RAS* and *BRAF* mutation analyses. These requests followed the routine clinical pathways of the hospital and therefore were performed using PCR, pyrosequencing and NGS, depending on the current method used by the reference laboratory. The concordance of some of the mutation data was later assessed using NGS (Ion Ampliseq Cancer Hotspot Panel v2, Thermo Fisher Scientific), as per manufacturer's instructions (10) through a research collaboration with Oxford Molecular Diagnostic Centre.

## Results

A total of 111 patients underwent surgical resection for CRC at Dorset County Hospital during 2013. One patient with neuroendocrine tumour and one patient with no residual tumour after neoadjuvant radiotherapy were later excluded from the analyses, so we assessed 109 cases. Seven cases of rectal cancer and 1 of sigmoid cancer had neoadjuvant radiotherapy. Molecular data for our cohort are summarised in Table 2 and the staining patterns are illustrated in Figure 3.

IHC was performed routinely and was successful in all 109 cases. Routine mutational analyses were requested for *KRAS* (76), *NRAS* (27) and *BRAF* (56) and all carried out successfully. Retrospective NGS analysis was performed on 93 cases. The data obtained from resection specimens was comparable to that obtained from the respective endoscopic biopsy with the exception of PTEN, C-MET & HER-2 (see below).

*MMR status and* BRAF *V600E mutation status*

There was complete concordance between endoscopic biopsy and resection specimen for MMR marker IHC (MSH2, MSH6, MLH1 and PMS2). All 4 MMR proteins were expressed in 93 cases, and classified as proficient for MMR (pMMR). In 16 cases, one or more MMR proteins were abnormal, i.e. absent, and were classified as deficient for MMR (dMMR). Four of these had wild-type BRAF, raising the possibility of Lynch Syndrome (LS). One had loss of MSH6 while the other 3 had loss of both MLH1 and PMS2.

*BRAF* mutation status was evaluated by IHC for all 109 cases. We verified these results in 101 cases using PCR only (n=8), NGS only (n=45) or both (n=48). We were unable to verify the IHC result for the remaining 8 cases due to repeated failure of NGS testing.

IHC-based BRAF mutation status was concordant with NGS & PCR findings except for mutation c.1816G>A (p.Gly606Arg), which was identified with NGS. This is a Tier 3 mutation, of indeterminate significance, distal to codon 600, and was unsurprisingly not picked up by either PCR or IHC. Such cases were therefore deemed non-mutated. There was complete concordance between endoscopic biopsies and resection specimens for BRAF IHC.

KRAS *and* NRAS *mutation status*

*KRAS* mutation status was determined successfully for a total of 105 cases. It was determined by PCR or pyrosequencing only (n=12), NGS only (n=29) or both (n=64). There were 2 cases where a mutation identified by NGS was not picked up by PCR (*KRAS* c.38G>A [p.Gly13Asp] and *KRAS* c.183A>C [p.Gln61His}). Both are Tier 1 mutations and therefore included as *KRAS* mutations within this study.

*NRAS* mutation status was determined successfully in a total of 98 cases by PCR or pyrosequencing only (n=5), by NGS only (n=71) or by both methods (n=22) with full concordance.

*PTEN loss, HER-2 & c-MET over expression*

Where available, these biomarkers were scored on the endoscopic biopsy as well as the resection specimen. There was discordance in PTEN expression between biopsy and resection in 8.3% of cases (7/84). In all discordant cases PTEN was present in the endoscopic biopsy but lost in the resection. Discordant c-MET expression occurred in 25% of cases with biopsy (7/28). These were not the same seven cases as above. In six cases there was a decreased expression in the resection. Discordant HER-2 expression

occurred in just one case (out of 80 with biopsy), where focal 3+ expression was not seen in the biopsy.

## Discussion

We wanted to understand if a DGH IHC laboratory, with support from a reference centre, can carry out up-front/reflex testing to stratify CRC within the timeframe required for informing clinical decision making and we wanted to provide a framework for the production and presentation of this molecular pathology data. We evaluated whether it is possible to perform the selected tests (MMR status, *BRAF* mutation status, *RAS* mutation status, loss of PTEN and overexpression of c-MET and HER-2) within a 2-week turnaround time (TAT) and in time for the local MDT.

We demonstrated that all slide-based tests were easily performed in-house within a 48 hour TAT. The mutation analyses were sent off-site to a reference centre as part of the routine diagnostic pathway and had a TAT of 8-10 working days. It was therefore only possible to collate all the results in time for MDT if a suitable block was selected at the time of cut-up and sent away promptly, without waiting for the diagnostic report. If TAT of referral centres cannot be improved, the prompt dispatch of the tissue block is critical and needs to be inserted into the routine cut-up procedure. A recent study has shown that using a NGS panel approach achieves a median turn-around time of 7 days at a cost which is increasingly competitive compared to single gene testing as more targets are added (10).

*Mismatch repair deficiency and Lynch Syndrome (LS)*

Recent NICE guidance states that all CRC patients, regardless of their age, should have tumour-based testing to assess the risk of LS when first diagnosed (6). LS is the most common cause of hereditary bowel cancer and carries an increased risk of developing other cancers (11). LS is estimated to cause 1,000 cases of bowel cancer each year in UK,

yet fewer than 5% of people with this condition are currently identified (12).

This guidance significantly increases the amount of testing required but our study demonstrates that testing all CRC patients for MMR using in-house IHC in a DGH is feasible. The addition of BRAF IHC allows the identification of *BRAF* V600E mutation-negative patients who require referral to genetic services for further investigations for LS. These IHC tests can be performed on either the endoscopic biopsy or resection specimen, as we demonstrated 100% concordance. NICE guidelines recommend that patients negative for both *BRAF* V600E mutation and MLH1 require an *MLH1* promoter hypermethylation test (6). Only three patients in our cohort (2.8%) fell into this category, demonstrating that in-house IHC would be sufficient for the majority of cases.

MMR testing by IHC can be done quickly and reliably before treatment in order to support clinical decision making as patients with dMMR tumours may have better prognosis (13), may not benefit from adjuvant chemotherapy (14), may benefit from low dose aspirin (15) and respond to immunomodulation through checkpoint inhibitors (16). The FDA has recently granted accelerated approval to Pembrolizumab in certain situations for patients with any type of dMMR solid tumour (17), emphasising the importance of universal MMR testing.

*Implications for targeted/biological therapies*

Prompt identification of dMMR or LS patients is only one aspect of CRC biomarking. It is a rapidly growing and constantly evolving area, but we have demonstrated that a DGH can implement the necessary service improvements to take advantage of new biomarkers and provide high quality testing with adequate turnaround time for patient treatment in a local setting.

The targeting of the EGF-R signaling pathway is a major therapeutic option in CRC (Figure 1) and the regulatory approval for drugs targeting this pathway is dependent on absence of activating mutations in the *RAS* genes. Although *RAS* mutation status was only immediately relevant for four of our patients (those presenting with advanced/metastatic disease), an estimated recurrence rate of 20-30% for stage II and 50-80% for stage III patients (n=18) means that 75% of our cohort would need this data to inform treatment in the near future. This would provide ample justification for immediate reflex testing rather than on-demand at a later date. In fact, immediate reflex testing provides higher quality information in the pathology report that is, most importantly, rapidly accessible upon recurrence. In addition, as we have shown in the prostate setting, reflex testing allows more effective use of service resources thus paradoxically creating capacity (manuscript in preparation).

Evidence suggests that changes in many other molecules along the EGF-R signalling pathways may impact on response to inhibitors of these pathways. For example, the presence of *BRAF* activating mutations affect the response to EGF-R inhibitors such as Cetuximab and Panitumumab (19). Likewise, loss of PTEN, over-expression of c-MET or HER-2 have a negative effect on response to EGF-R inhibitors although there is some controversial literature (8, 20-22). Certainly, the current selection criteria for EGF-R TKI therapy results in a number of treatment failures, suggesting that refinements in the selection are necessary (23).

Since ours was a feasibility study, the MDT did not act upon our additional test results. Nevertheless, we retrospectively evaluated their effect on eligibility for EGF-R targeted therapy. According to current guidelines, 66% of our patients would be eligible for these drugs. The addition of *BRAF* mutation status would bring this down to 45%. The

inclusion of PTEN and HER-2 would reduce this cohort to 31% and, using all our data, we would predict that only 15 patients (14%) would respond optimally. These tests therefore may have huge implications on treatment decisions. While we used published scoring systems for PTEN, c-MET and HER-2 (Table 1), there are consensus issues (22-27), so it is crucial that suitable scoring systems are devised and validated against clinical response for these markers, and an external quality assurance process is established. We found disparities between scores for resection and biopsy tissue, which may be due to fixation/pre-analytical processing or a reflection of disease process.

Other potential targeted therapies may be beneficial to CRC patients. For example, HER-2 overexpression may indicate good response to Trastuzumab and Lapatinib (Heracles Trial, 28), loss of PTEN may indicate good response to mTOR inhibitors, which target the AKT pathway downstream of PTEN (29) and over expression of c-MET may indicate response to MET and MEK inhibitors (MErCuRIC1 trial, 30). The FOCUS4 trial is currently stratifying CRC using biomarkers such as BRAF, PIK3/PTEN and RAS to inform treatment (31). Our study complements this trial by demonstrating the feasibility of using these tests for routine stratification in a small DGH.

*Development of the algorithm*

Many of the authors (CD, TT, IMF, KM and AC) have had long associations with MDT/TB and understand the challenges of presenting ever-increasing molecular pathology data with complex ramifications for treatment decisions. The MDT/TB has only a few minutes allocated to each patient so requires a system of communication that summarises all findings, is easily and quickly interpretable and can guide clinical decisions. We therefore constructed a graphic representation, structured to follow the

then current clinical decision-making. Since then, rapid progress in CRC biomarker research has impacted further on treatment decisions. For instance, immunoscore (32), TILs (tumour infiltrating lymphocytes) and PD-L1 assessment were not widely used at the time of the study and immunomodulation with checkpoint inhibitors was unavailable as a treatment option in 2013.

We have therefore expanded and revised the algorithm to reflect what would be a working classification in 2017 and included test results with prognostic and therapeutic implications as well as traditional anatomical data (Figure 4). This new algorithm is easy to update as new biomarkers emerge and guidelines change and can be customised to reflect local oncological practice.

*Financial implications*

NICE concluded that testing using IHC for MMR plus *BRAF* and *MLH1* promoter methylation is a cost-effective use of NHS resources (6,33,34). Our approach to CRC testing could easily be adopted by all hospitals in response to NICE guidelines. In its most succinct form, this can be done with five IHC tests (MLH1, PMS2, MSH2, MSH6 and BRAF), with a relatively low burden on resources, although the health-economic case is predicated on central funding given the benefits to the NHS lie outside of pathology budgets (33). Additional tests could provide more accurate prediction and prognosis, thus reducing costs and delay caused by ineffective treatment (35).

In the UK there is a large gap between the provision of cancer testing and demand (36). The estimated gap in CRC is the largest and in 2014 affected 10,704 patients (49%) who did not receive testing, potentially missing out on optimal treatment. The UK commissioning system funds tests for systemic anti-cancer treatment centrally whereas

traditional IHC tests are commissioned locally. Funding of some IHC tests has therefore become separated from the funding of the associated targeted treatment. Furthermore, NHS departmental budgets are compartmentalised, so that savings made in Oncology and Surgery through the improvement of outcomes by personalised medicine are unavailable to Pathology. This is serious, as the health-economic case is predicated on central funding, given the benefits to the NHS lie outside of Pathology (33). Such current structures do not therefore fit current requirements and threaten the implementation of improved care pathways with a proven health-economic basis.

The use of IHC for drug selection for breast and lung cancer is well established (37,38). When other modalities of testing have been available, IHC is the most efficient and cost-effective platform (38). Our study demonstrates that IHC has a significant role to play in personalised medicine for CRC. The recent introduction of PD-L1 testing (the biomarker for checkpoint inhibitor therapy that can only be done by IHC in Histopathology services) reinforces its importance.

It is clear that we need a unified strategy to fund all companion diagnostics, irrespective of whether they are test tube-based or slide-based. If we fail to finance appropriately and adequately all tests that allow patients to receive the most appropriate medicine, we fail both patients and all who pay into the health system, as well as making a mockery of health-economic studies.

We hope this work will enable pathologists to take up the challenges of supporting personalised cancer treatment whether they work in large centres or in small hospitals. We acknowledge that the future is moving toward screening large panels of biomarkers and this may even involve liquid biopsies as opposed to tissue biopsies. Once such

systems are established, running in sufficient quantities and can demonstrate concordance and quality, they may well be the most effective way of determining choice of targeted therapy. We are not there yet. We have to fill the gap for the patients of today. This requires education, an understanding of the current limitations as well as the future possibilities and the development of funding streams which are not divisive.

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Figure Legends

**Figure 1**

**EGF-R signalling pathways and points of action of available biological therapy**

EGF-R (or ErbB-1), indicated in pink, is a transmembrane receptor tyrosine kinase; its extracellular domain had a binding site for its soluble ligands. EGF (in mauve) is one of the 7 known ligands for EGF-R. Binding to its cognate ligand results in a conformational change in an EGF-R monomer which exposes the dimerisation site (in blue), allowing two ligand-bound monomers to dimerise. EGF-R can form homodimers or heterodimers with HER-2 (ErbB-2, in green), another member of the EGF-R receptor family. Dimerisation activates phosphorylation of the (intracellular) kinase domain and this leads to activation of a range of downstream signalling pathways. RAS-RAF-MAPK and PI3K-AKT are major kinase cascade pathways that regulate cell proliferation, cell division and apoptosis. There are a number of biological therapies targeting EGF-R interaction with its ligands, EGF-R phosphorylation and the phosphorylation of other downstream molecules. These are indicated in the diagram within the rectangular boxes; therapeutic agents in brackets are currently awaiting regulatory approval. Activating mutations in RAS, RAF and PI3K result in constitutive activation of these kinases and are known oncogenic drivers in CRC. PTEN acts as tumour suppressor gene since it provides an inhibitory loop for the PI3K-AKT pathway and its function is commonly lost in human cancer. Over expression of HER-2 facilitates receptor dimerisation and activation of the intracellular kinase domains even in the absence of EGF-R ligand. All these events can render biological therapy targeting this signalling pathway ineffective.

**Figure 2**

Mismatch Repair – examples of MSH6 staining in control and tumour tissue

**A and B: MSH6 Positive control tissues**

**(A)** Appendix. Strong nuclear staining of the epithelium at the base and lower third-half.

**(B)** Tonsil. Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction whilst the germinal centre B-cells show a strong nuclear staining reaction.

**C, D, E and F: Tumour tissues stained with MSH6 antibody**

**(C)** Tumour positive for MSH6 staining. Both tumour cells (black arrow) and internal controls (red arrow) are stained.

**(D)** Tumour negative for MSH6 staining. Tumour cells are not stained (black arrow). Result is valid as internal controls are stained (red arrow).

**(E)** Invalid results for MSH6 staining. Equivocal staining as internal control cells are not stained (red arrow). Test needs to be repeated.

**(F)** Invalid results for MSH6 staining. Equivocal staining in tumour cells (black arrow). Test needs to be repeated.

**Figure 3**

Examples of immunohistochemistry of formalin fixed paraffin embedded sections from 4 cases, each stained with the following antibodies (BRAF V600E, PTEN, MSH2, MSH6, MLH1, PMS2, HER-2/neu, cMET) on Ventana Benchmark Ultra or XT using the protocols indicated in Table 1.

Case 1 is proficient for mismatch repair proteins (pMMR), negative for BRAF V600E mutation, shows loss of PTEN and no over-expression of HER-2 or c-MET. Loss of PTEN indicates that this patient may not respond to therapy targeting the EGF-R signalling pathway, but may respond to mTOR inhibitors.

Case 2 is MMR deficient for mismatch repair proteins MLH1 and PMS2 (dMMR) and positive for BRAF v600E mutation (therefore not a suspected Lynch Syndrome (LS) patient). There is preservation of PTEN and only weak expression of HER-2 and c-MET. This patient is unlikely to respond to therapy targeting the EGF-R signalling pathway but may respond to immunomodulation through checkpoint inhibitors and benefit from low dose aspirin .

Case 3 is pMMR, negative for BRAF v600E mutation, shows loss of PTEN, over-expression of HER-2 and weak expression of c-MET. This patient is unlikely to respond to therapy targeting the EGF-R signalling pathway, but may respond to Trastuzumab, Lapatinib or mTOR inhibitors.

Case 4 is deficient for mismatch repair proteins MSH2 and MSH6 (dMMR), negative for BRAF v600E mutation (therefore a suspected LS patient). There is no loss of PTEN, weak expression of HER-2 and over expression of c-MET. This patient is likely to respond to therapy targeting the EGF-R signalling pathway, as well as MET and MEK inhibitors. The patient may also respond to immunomodulation through checkpoint inhibitors and benefit from low dose aspirin.

**Figure 4**

Example of CRC algorithm report for presentation to MDT/Tumor Board

The top section reports all biomarkers. Dashed lines separate lines of therapy. Results are entered by turning the relevant boxes green. Square boxes relate to therapy. In this example the CRC is dMMR with mutant *BRAF* and therefore unlikely to be LS; it is not suitable for 5FU but may benefit from low dose aspirin and immune modulation with checkpoint inhibitors. Treatment targeting EGF-R pathways is unlikely to be effective in view of *BRAF* mutation and HER-2 amplification unless in combination, however anti-HER-2 therapy may be effective. The arrows indicating treatment options can be edited according to local practice.

The bottom section gives an indication of prognosis based on conventional pathological findings (e.g. T N M), biomarkers (e.g. *BRAF*) and other indicators such as Immunoscore. Assessment of prognosis (risk) is important in deciding how aggressive should be the treatment.

**Acknowledgements**

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## Table 1: Details of IHC performed and scoring system

| Antibody | Vendor | Clone | Conc. or Ready to Use (RTU) | Retrieval Solution & time | Antibody Incubation time in mins (at 37°C) | Platform, Detection System & Amplification | Scoring System |
|---|---|---|---|---|---|---|---|
| BRAF V600E | Spring Bioscience / Roche | VE1 | RTU | CCI, 64 mins | 32 mins | XT, OptiView, OV Amp 4_4 | Present or absent in tumour tissue. BRAF V600E mutation present if positive staining. |
| PTEN | Spring Bioscience | SP218 | 1:100 | CCI, 64 mins | 32 mins | XT OptiView, OV Amp 4_4 | PTEN scored as present or absent in tumour tissue. Scoring system based on Sangale et al. (39) |
| MSH2 | Roche | G219-1129 | RTU | CCI, 32 mins | 32 mins | XT, OptiView, No Amp | Mismatch Repair (MMR) proteins (MSH2, MSH6, MLH1, PMS2) scored as present or absent in tumour tissue MMR Deficient if 1 or more proteins show loss of expression. MMR Proficient if all 4 proteins present. |
| MSH6 | Roche | 44 | RTU | CCI, 64 mins | 16 mins | XT, OptiView, OV Amp 4_4 | |
| MLH1 | Roche | M1 | RTU | CCI, 32 mins | 16 mins | XT, OptiView, OV Amp 4_4 | |
| PMS2 | Roche | EPR 3947 | RTU | CCI, 72 mins | 32 mins | XT, OptiView, OV Amp 8_8 | |
| anti HER-2/neu | Roche | 4B5 | RTU | CCI, 36 mins | 16 mins | ULTRA, UltraView, No Amp | HER-2/neu scoring system based on that used by Seo et al. (40). HER-2 overexpression indicated by a score of 2+/3+ or 3+. |
| anti-Total c-MET | Roche | SP44 | RTU | CCI, 64 mins | 16 mins | ULTRA, UltraView, No Amp | Scoring system based on Bardelli *et al.* (9) Low = Score of 0 or 1. High = Score of 2 or 3. |

**Table 2: Molecular data for 2013 cohort (n= 109)**

| | MMR PROFICIENT (MMR+) (93/109 cases = 85.3%) | | | MMR DEFICIENT (MMR-) (16/109 cases = 14.7%) | | | ALL CASES combined |
|---|---|---|---|---|---|---|---|
| Molecular test | No. of cases | MMR+ data set | % of MMR+ data set | No. of cases | MMR- data set | % of MMR- data set | % of total data set |
| *BRAF* MUTATION | 10 | 93 | 10.8% | 12 | 16 | 75.0% | 20.2% |
| *RAS* MUTATION | 34 | 89 | 38.2% | 2 | 16 | 12.5% | 34.3% |
| PTEN LOSS | 23 | 93 | 24.7% | 4 | 16 | 25.0% | 24.8% |
| HER-2 3+ | 5 | 93 | 5.4% | 0 | 16 | 0.0% | 4.6% |
| c-MET HIGH | 48 | 93 | 51.6% | 4 | 16 | 25.0% | 47.7% |

Figure 1

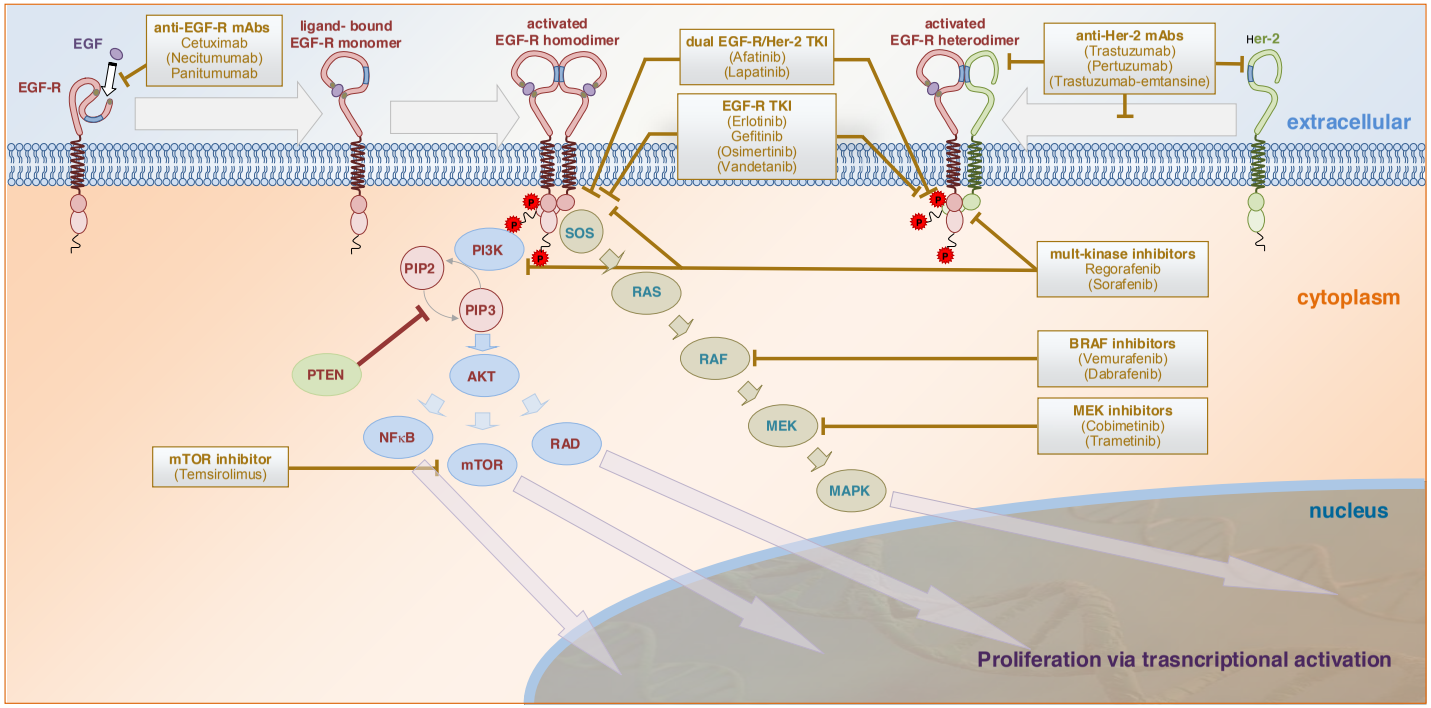

EGF
EGF-R
anti-EGF-R mAbs
Cetuximab
(Necitumumab)
Panitumumab
ligand- bound
EGF-R monomer
activated
EGF-R homodimer
dual EGF-R/Her-2 TKI
(Afatinib)
(Lapatinib)
EGF-R TKI
(Erlotinib)
Gefitinib
(Osimertinib)
(Vandetanib)
activated
EGF-R heterodimer
anti-Her-2 mAbs
(Trastuzumab)
(Pertuzumab)
(Trastuzumab-emtansine)
Her-2
extracellular
cytoplasm
SOS
PI3K
PIP2
PIP3
PTEN
AKT
RAS
RAF
MEK
MAPK
NFκB
mTOR
RAD
mTOR inhibitor
(Temsirolimus)
mult-kinase inhibitors
Regorafenib
(Sorafenib)
BRAF inhibitors
(Vemurafenib)
(Dabrafenib)
MEK inhibitors
(Cobimetinib)
(Trametinib)
nucleus
Proliferation via trasncriptional activation

Figure 2

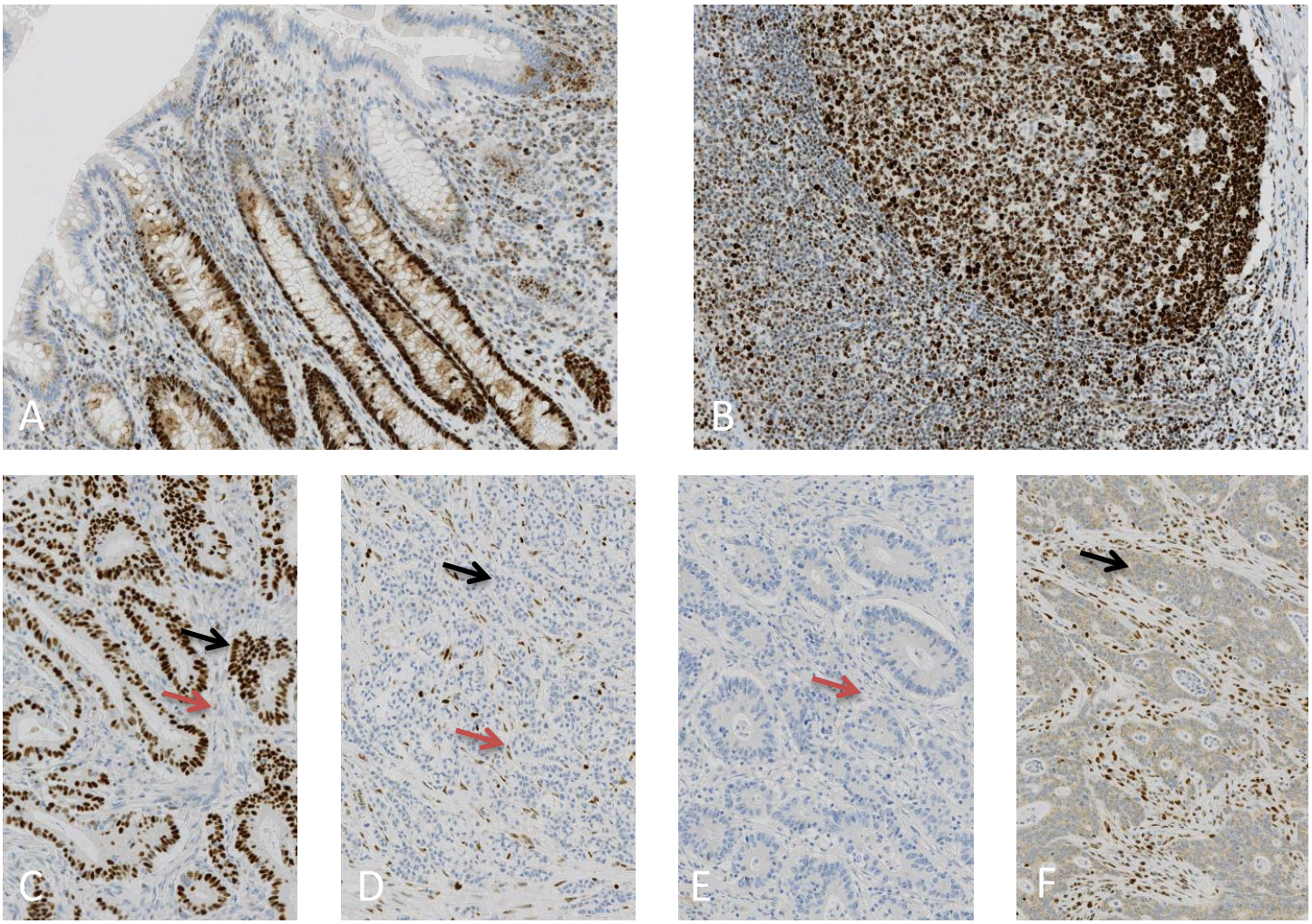


Figure 3

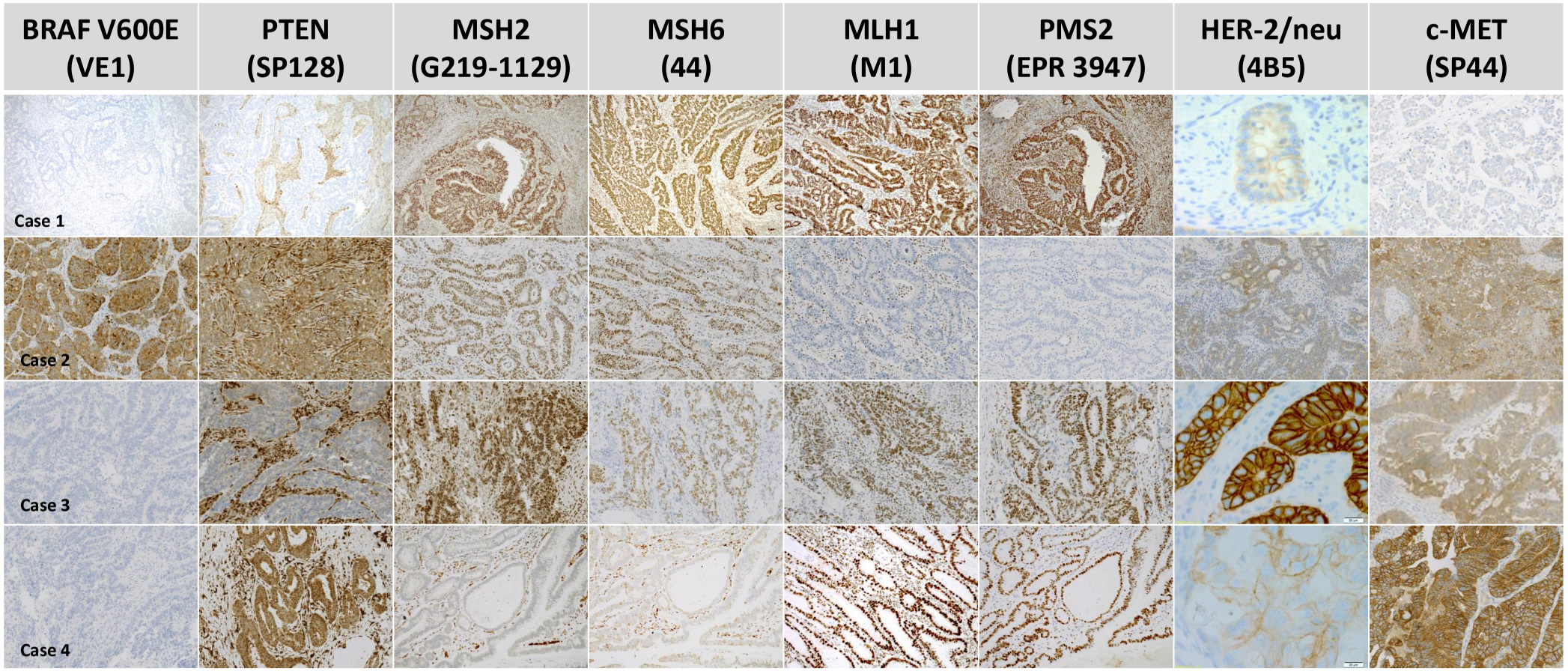
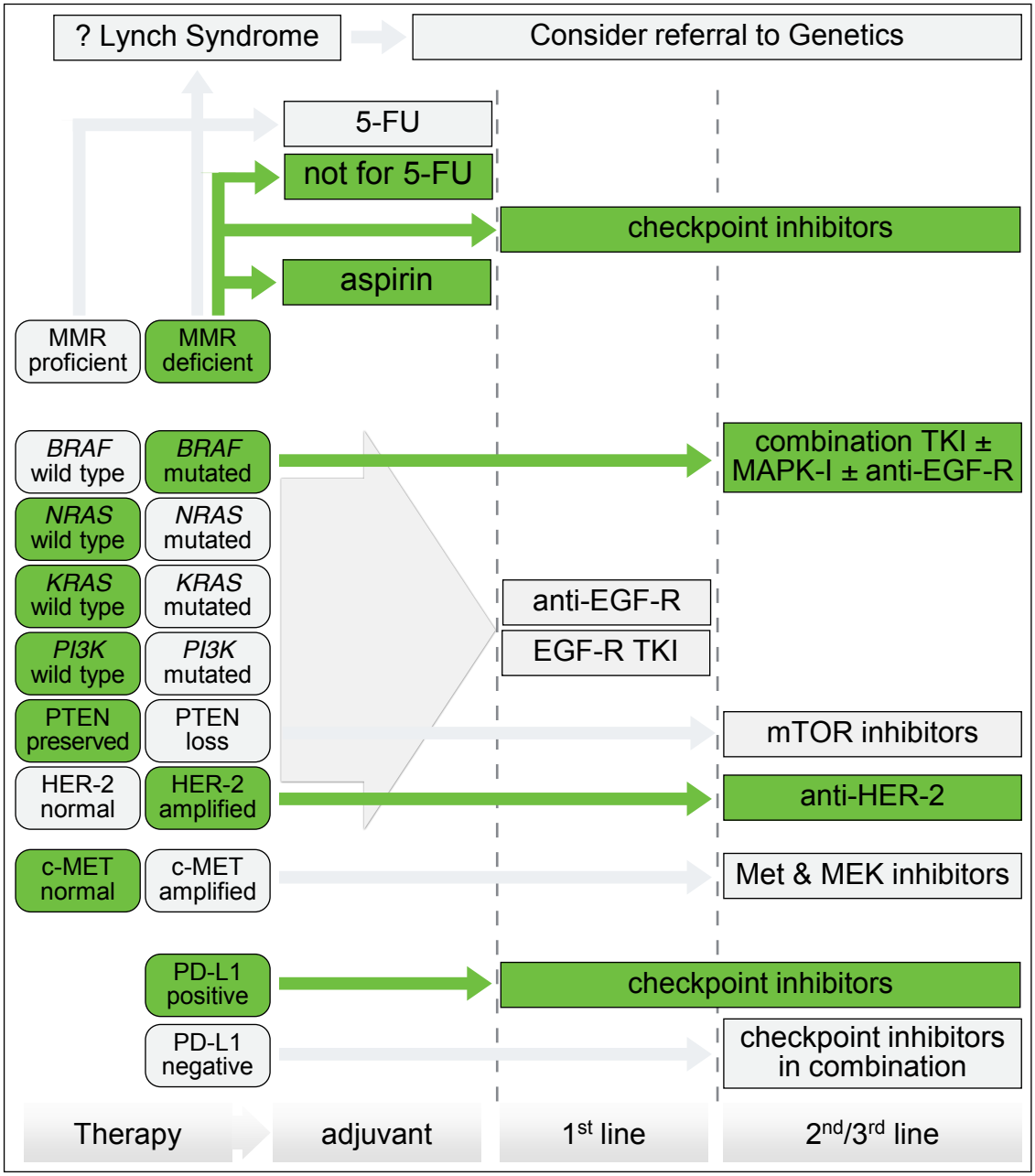
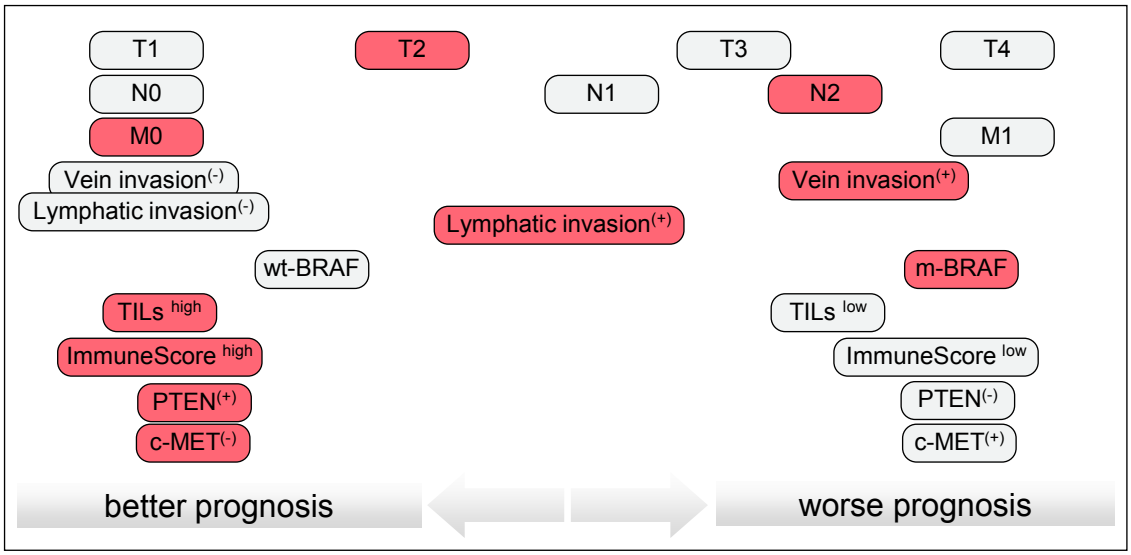

BRAF V600E (VE1)
PTEN (SP128)
MSH2 (G219-1129)
MSH6 (44)
MLH1 (M1)
PMS2 (EPR 3947)
HER-2/neu (4B5)
c-MET (SP44)
Case 1
Case 2
Case 3
Case 4

Figure 4


? Lynch Syndrome
Consider referral to Genetics
5-FU
not for 5-FU
checkpoint inhibitors
aspirin
MMR proficient
MMR deficient
BRAF wild type
BRAF mutated
combination TKI ± MAPK-I ± anti-EGF-R
NRAS wild type
NRAS mutated
KRAS wild type
KRAS mutated
anti-EGF-R
EGF-R TKI
PI3K wild type
PI3K mutated
PTEN preserved
PTEN loss
mTOR inhibitors
HER-2 normal
HER-2 amplified
anti-HER-2
c-MET normal
c-MET amplified
Met & MEK inhibitors
PD-L1 positive
checkpoint inhibitors
PD-L1 negative
checkpoint inhibitors in combination
Therapy
adjuvant
1st line
2nd/3rd line


T1
N0
M0
Vein invasion(-)
Lymphatic invasion(-)
T2
wt-BRAF
TILs high
ImmuneScore high
PTEN(+)
c-MET(-)
N1
Lymphatic invasion(+)
T3
N2
Vein invasion(+)
m-BRAF
TILs low
ImmuneScore low
PTEN(-)
c-MET(+)
T4
M1
better prognosis
worse prognosis