COLORECTAL CANCER CHEMOTHERAPY
A short summary
Based on the Clinical practice guidelines for the prevention, early detection and management of colorectal cancer. (Cancer Council Australia)

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<th>Meaning</th>
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<tr>
<td>APC</td>
<td>Adenomatous polyposis coli</td>
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<tr>
<td>CAP</td>
<td>Consumer Advisory Panel (of the AGITG)</td>
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<tr>
<td>CRC</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>EGF(R)</td>
<td>Epidermal growth factor (receptor)</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular signal–regulated kinases (= MAP kinase)</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>-ib</td>
<td>Selective inhibitor</td>
</tr>
<tr>
<td>-mab</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase (= ERK)</td>
</tr>
<tr>
<td>MEK</td>
<td>MAP kinase kinase (MAPK-ERK kinase)</td>
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<tr>
<td>MMR</td>
<td>Mismatch Repair gene</td>
</tr>
<tr>
<td>MOOC</td>
<td>Massive Open On-line Course</td>
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<tr>
<td>MSI/MSI-H</td>
<td>Microsatellite instability (MSI-high)</td>
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<tr>
<td>PD(-L)</td>
<td>Programed Death receptor(Ligand)</td>
</tr>
<tr>
<td>RAS, RAF</td>
<td>Refer to both genes and proteins found in CRC tumours</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>TGA</td>
<td>Therapeutic Goods Administration</td>
</tr>
<tr>
<td>THF</td>
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</tr>
<tr>
<td>VEGF(R)</td>
<td>Vascular endothelial growth factor (receptor)</td>
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Preface

This document has been primarily written to assist the members of the Consumer Advisory Panel of the Australasian Gastro-Intestinal Trials Group (AGITG)/GI Cancer Institute to understand terms used in treatment of colorectal cancer. The author is a member of the Panel.

As the drugs for the treatment of colorectal cancer work on processes associated with cell replication, it may be helpful to first understand a little of the processes that go on inside the nucleus of a cell. Some resources that explain some of these processes are outlined in the Bibliography.

Colorectal cancer (CRC) treatment is necessarily complicated and evolving. This attempt to explain the process simply may fail. Please advise of any errors or omissions – there will be some, in part because sources differ and in part because of the author’s misunderstanding or lack of research.

Finally but very importantly, note that this paper follows closely and refers to the Clinical practice guidelines for the prevention, early detection and management of colorectal cancer. (see https://wiki.cancer.org.au/australia/Guidelines:Colorectal_cancer). Links to specific sections of that document are included in this document (as footnotes) for ease of reference. These Guidelines will set key directions in CRC treatment in Australia for the foreseeable future and represent a very up to date and detailed exposition of CRC treatment. So it is necessary that this paper to closely follow them.

Nothing in this paper is, or should be taken to be, advice of any kind.

Brian Wall

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Introduction

Chemotherapy for Colorectal Cancer – Some history


Prior to World War II there was no effective cancer chemotherapy. The early drugs were sometimes found incidentally – e.g. mustine is very close to mustard gas used against troops in WW I. It was noticed that many poisoned soldiers’ records showed low number of white blood cells – those that if mutated, can develop into leukaemia and lymphoma, and it was later found that mustine (very closely related to mustard gas) was effective in blood and lymph cancers¹.

A good example of how other early anti-cancer drugs were developed, and of particular interest in colorectal cancer (CRC) is the discovery in 1957 of fluorouracil (or 5-fluoro-uracil, or 5FU, the number indicating where on the uracil molecule the introduced fluorine atom is).

After 60 years, fluorouracil is still a mainstay of CRC treatment because it has been to be shown to be very effective.

¹ For more on this interesting story see http://scienceblog.cancerresearchuk.org/2014/08/27/mustard-gas-from-the-great-war-to-frontline-chemotherapy/
Fluorouracil was synthesised following observations of more rapid uptake of uracil into tumour cells than normal cells in rats. A scientist, Charles Heidelberger, looking at the toxicity of fluorine compounds at the time decided to add a fluorine atom to the uracil base, making fluorouracil, which was then found to have potent anti-tumour activity\(^2\).

In part because fluorouracil by mouth does not reliably reach the circulation (due to poor absorption and metabolism by the liver) and in the search for other effective drugs, similar drugs have been developed such as capecitabine, which can be given by mouth (orally) and is metabolised to fluorouracil by the tumour cells. Other related compounds have been found to be useful for other tumour types (e.g. gemcitabine), or as anti-infective agents (e.g. acyclovir, vidarabine). This group of closely related anti-cancer agents are sometimes referred to as fluoropyrimidines.

While fluorouracil is effective it is not highly selective, and so there is a range of untoward effects that cannot be completely avoided but can be managed. This is the case with most of the traditional anti-cancer drugs. Rapid advances in genetics and molecular biology have occurred in the last 20 to 30 years, giving us a range of (relatively) selective adjuncts to traditional treatment. For example there are the \(-\text{mabs}\) (ending in \(-\text{mab}\) because they are derived from monoclonal antibodies) and the \(-\text{ibs}\) (which are selective \textit{inhibitors} of a particular biochemical process in the tumour but not found in other cells) as well as other selective inhibitors or stimulators of cell processes to prevent cancer growth.

While the adverse effects of the targeted therapies are different they can still be very serious, so they are certainly not without risk. In tumours like melanoma they have been a great advance, but for CRC they have not delivered a game-changing breakthrough, although they do provide alternatives in metastatic disease that is unresponsive to traditional first line treatments. The place of targeted therapies in CRC is not currently as central as in some other cancer types, and they still have serious adverse effects.

The rapid emergence of targeted therapies means finding the relative place of them in treatment, alongside or instead of the older proven treatments, is a further incentive for more CRC treatment research.

**Sources of the material covered in this paper.**

The key sources in the compilation of this paper are the Draft and final versions of the \textit{Clinical practice guidelines for the prevention, early detection and management of colorectal cancer} (Cancer Australia) (hereinafter referred to as the Guidelines); the Prescribing Information published by a drug’s sponsors and approved by the TGA; the US National Cancer Institute’s Dictionary website\(^3\) and other sources acknowledged in the text. The references and a bibliography consisting mainly of links to material on the internet are provided at the end of the paper.

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\(^2\)Uracil is an essential base in RNA and is the one base that is not the same as the one in the DNA from which it is copied. See Appendix 1 for an outline of this.

\(^3\)https://www.cancer.gov/publications/dictionaries/cancer-terms
The drugs traditionally used in colorectal cancer – still the mainstay
The principal drugs used to treat CRC continue to be non-targeted but tried and tested, well understood therapies used in combination. This paper will briefly outline critical properties of particular drugs in use, then go on to outline the key combinations of the drugs together. Later in the paper it will summarise some of the specifically targeted agents that affect for example a single enzyme system.

**Fluorouracil**
Fluorouracil is administered by intravenous injection or infusion in combination with other drugs, usually for Stage III\(^4\) or later CRC.

Fluorouracil closely resembles an essential building block for DNA (thymine) and for RNA (uracil). See Appendix 1 for an outline of this aspect. Fluorouracil itself is inactive and is converted in cells into two principal active metabolites (FdUMP and FUMP) that:

- interfere with the synthesis of DNA by blocking an enzyme (thymidylate synthetase) so the essential base thymine is then not available for making DNA;
- interfere with RNA processing, being taken into the RNA in place of something else (UMP) and thus inhibiting cell growth.

Fluorouracil is not absorbed orally. After intravenous administration, fluorouracil is distributed throughout body tissues and fluids. The plasma half-life\(^5\) is 8 to 22 minutes. Fluorouracil disappears from the blood within four hours. If prolonged blood levels are required it needs to be given by infusion.

Common side effects stem from uptake by tissues that are rapidly turning over, such as those in the mouth and lining of the gut, and so include inflammation of the mouth, loss of appetite, nausea and diarrhoea, hair loss, and inflammation of the skin. It affects blood cell counts, and also has some cardiovascular effects. Fluorouracil is usually given at the same time as folinic acid\(^6\) (leucovorin) and this makes the GI and other side effects worse (but the treatment more effective).

Fluorouracil is used in combination with other drugs to treat CRC. These combinations are touched on later.

**Capecitabine**
Capecitabine was designed as an orally administered (i.e. by mouth) precursor (pro-drug) of fluorouracil. It is changed into fluorouracil (ultimately)\(^7\) within tumours by an enzyme that is found in tumours in much higher concentration than it is in non-tumour tissues, providing some selectivity.

After oral administration, capecitabine is rapidly and almost completely absorbed, followed by conversion to the metabolites. Its effects and side effects are similar to those of fluorouracil. It is usually given in twice daily doses during the treatment period.

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\(^4\) Stages are: Stage I: Confined to bowel wall; Stage II: Invading through bowel wall without nodal metastases; Stage III: Lymph node metastases; Stage IV: Distant metastases (From Townsville Hospital Medical Oncology Handbook, 4\(^{th}\) ed, 2016).

\(^5\) The time it takes for the level in the plasma/blood to fall by 50%. In this case, it is relatively short.

\(^6\) Folinic acid is used as the preferred name for this as it is the Australian Approved Name but it is often referred to as leucovorin and the terms are essentially equivalent. Calcium folinate is the form in the medicinal preparations but the strength/dose is measured as folinic acid not calcium folinate.

\(^7\) It is first changed to an intermediate compound by the liver on absorption and that is then converted to fluorouracil.
Trifluridine/Tipiracil
The drug combination of Trifluridine/Tipiracil (Lonsurf, Orcantas) has recently been approved for use in Australia. Trifluridine is another fluoropyrimidine, i.e. of the same general class as (but not the same as) fluorouracil.8

Trifluridine is rapidly metabolised by the body’s enzymes so tipiracil, which inhibits these enzymes, is given with trifluridine, so that trifluridine is not broken down before it can take effect.

The combination is approved in Australia:

“for the treatment of adult patients with metastatic colorectal cancer (mCRC) who have been previously treated with, or are not considered candidates for fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapies, anti-VEGF agents, and anti-EGFR agents.”

Dosage is by mouth, and is given twice a day for five days in week 1, five days in week 2, then two weeks rest before any further treatment cycles. The side effects of the combination are most commonly on the blood and on the GI tract, of a similar type to those of other fluoropyrimidines.

Folinic acid (calcium folinate, leucovorin, tetrahydrofolate (THF))
Folinic acid is the active form of folic acid, and folic acid has to be changed to folinic acid in the body before it is still further metabolised. Giving folinic acid bypasses this first step. As we are attempting to ensure greater than normal physiological levels, this first step must be jumped.

Folinic acid is used in CRC with fluorouracil and similar drugs. It enhances the effects of the active metabolite of fluorouracil (FdUMP) by stabilizing the binding of FdUMP to its target enzyme, and so prolonging the anti-cancer activity.

Folinic acid must be given by injection. Following administration, it rapidly enters the general body pool. Distribution occurs throughout the body. The active metabolite 5-methyl THF then appears, which becomes the major circulating form of the drug. Peak levels are observed at 1.5 hours following intravenous administration. The half-life (for total folinic acid active metabolites) is reported as 6.2 hours.

By prolonging the effect of the fluorouracil metabolites, it also increases the side effects. Folinic acid by itself is well tolerated (but has no anti-cancer activity). The combination with fluorouracil may cause severe inflammation of the gastrointestinal tract from the mouth down, resulting in diarrhoea and dehydration, and severe discomfort. The most common dose-limiting adverse reactions are severe oral inflammation and diarrhoea. The gut toxicity and myelosuppression (decreased bone marrow activity resulting in fewer red and white blood cells and platelets) can be life threatening.

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8 Trifluridine is derived from thymine which has fluorine atoms instead of hydrogen atoms on the –CH3 group. (Appendix 1 shows the thymine molecule. In that diagram the –CH3 is at 10 o’clock.) Trifluridine uses fluorine instead of hydrogen in the same place on thymine as fluorouracil has fluorine on uracil. So there are similarities between trifluridine and fluorouracil but they are by no means the same.
**Oxaliplatin**

Oxaliplatin belongs to a class of platinum-based compounds in which the platinum atom is complexed with other structures. It displays activity in a variety of tumours, including CRC.

The absorption, distribution, metabolism and excretion is complex. After a 2-hour infusion, 15% of the administered platinum is present in the circulation, the remaining 85% being rapidly distributed into tissues or eliminated in the urine. Irreversible binding to red blood cells and plasma occurs.

The mechanism of action of oxaliplatin is not completely understood. Oxaliplatin is metabolised by the body into other forms that interact with DNA, cross-linking the DNA strands\(^9\), inhibiting DNA replication and RNA transcription\(^10\), as well as other non-specific cell toxicity.

The sponsor’s approved Product Information states oxaliplatin “demonstrates synergistic action” (an effect greater than the sum of the two alone) when given in combination with fluorouracil. It also says oxaliplatin shows effectiveness in various cisplatin-resistant models (cisplatin being the longest used platinum-based cancer drug, and thus the benchmark for this type of drug.)

Neurological adverse effects are the dose-limiting toxicity. Symptoms like numbness in the hands or feet occur in 85-95% of patients. These symptoms usually develop at the end of the 2-hour oxaliplatin infusion or within a few hours, stop within the next hours or days, and often recur with further cycles. These effects may worsen on exposure to cold. Sensitivity to cold is common, especially soon after infusion but continues for weeks or months for some, and more rarely permanently.

It is used in combination with fluorouracil and folinic acid in some regimens (e.g. FOLFOX).

**Irinotecan**

Irinotecan is a derivative of camptothecin, a cytotoxic substance extracted from an Asian tree *Camptotheca acuminata*.

Camptothecins interact with the enzyme Topoisomerase I\(^11\), which relieves torsional (twisting) strain in DNA by causing reversible single strand breaks. Failure to repair these breaks leads to double-strand DNA damage, which disrupts cell growth and leads to cell death.

Irinotecan is a precursor (pro-drug) to the metabolite SN-38\(^12\) which is 1,000 times as potent in preventing re-linkage of these broken single strands by topoisomerase I.

Continuing DNA synthesis is necessary for irinotecan to exert its cytotoxic effects, as it works in the period of the cell reproductive cycle when new DNA is being formed (called the S-phase).

After intravenous infusion irinotecan has a mean (average) terminal elimination half-life of about 6 to 12 hours. The mean terminal elimination half-life of the active metabolite SN-38 is about 10 to 20 hours.

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\(^9\) I think of it as tangling them
\(^10\) Production of RNA leading to formation of proteins.
\(^11\) Enzymes that cut one of the two strands of double-stranded DNA, relax the strand, then re-join the strand.
\(^12\) 7-ethyl-10-hydroxy-camptothecin
Colorectal cancer treatment in practice

Introduction
Colorectal cancer, like most other solid tumours, is treated by a mix of surgery, radiotherapy and chemotherapy. The focus here is solely on the drug treatment, and almost solely on the anti-tumour drugs, not those used in conjunction with the chemotherapy for other reasons, such as corticosteroids or drugs for nausea. This is in an effort to keep the outline simple, not because they are not important.

Stage 1 and Stage 2 CRC (no spread to the outside the colon or rectum wall) are usually treated by surgery alone. Late Stage 2 may occasionally be treated with drugs, but stages 3 and 4 will be. The Guidelines state “Adjuvant chemotherapy for stage II cancers can be considered on a case-by-case basis but cannot be considered a standard of care.”

The Guidelines divide treatment into the sections, some of which are focussed on surgical alternatives alone but it will be used as a framework for the ensuing discussion of treatments.

- Adjuvant therapy for colon cancer.
- Neoadjuvant and adjuvant therapy for rectal cancer.
- Management of resectable locally recurrent disease and metastatic disease.
- Management non-resectable locally recurrence disease and metastatic disease.
- The role of systemic therapies in non-resectable metastatic disease.

The various combination drug regimens used (FOLFOX, FOLFIRI etc) are not outlined in full in this paper as they are too numerous and too likely to change. Reference should be made to the EviQ website of the Cancer Institute NSW for details of these regimens (https://www.eviq.org.au/). Nevertheless, below are a few common acronyms and indication of their derivation:

FOLFOX – includes FOLinic acid, Fluorouracil and OXaliplatin.

FOLFIRI – includes FOLinic acid, Fluorouracil and IRInotecan.

CAPOX (XELOX) – CAPecitabine (Xeloda™) and OXaliplatin.

The Guidelines use FULV and FLOX but these do not appear to be standard regimens. They are just abbreviations, using the name leucovorin (LV or just L) instead of folinic acid.

A detailed outline of the common CRC chemotherapy regimens is given in the Guidelines in the section entitled Systemic chemotherapy treatment options for first-line treatment and the following sections deal with later treatment options.

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14 You must register to access the EviQ website but registration is simple and free. Once registered follow the links to Medical Oncology > Colorectal > Chemotherapy Protocols.
Definition of adjuvant and neo-adjuvant therapy

Adjuvant therapy is any treatment that is given in addition to a standard curative cancer treatment such as surgery. By convention, the term ‘adjuvant’ is reserved for postoperative treatment, while ‘neoadjuvant’ refers to treatment given prior to the definitive surgery.¹⁶

Adjuvant therapy for colon cancer

The Guidelines recommend postoperative fluorouracil or capecitabine plus folinic acid regimens in stage III disease, with the addition of oxaliplatin for persons under 70 (e.g. one of the FOLFOX or XELOX regimens). For persons over 70, no benefit from oxaliplatin added to adjuvant treatment has been found. However, there is a case for the addition of oxaliplatin in the elderly where there are metastases. The difference remains unexplained.¹⁷

The Guidelines state that three well designed trials failed to demonstrate a benefit from the addition of irinotecan to fluorouracil in patients with stage II or III colon cancer; and that the addition of targeted biologic agents to conventional adjuvant therapy has not led to any patient benefit in the adjuvant setting.¹⁸

Neoadjuvant and adjuvant therapy for rectal cancer

The decisions about neoadjuvant chemotherapy are not easily summarised as it is intimately linked with radiotherapy, for which chemotherapy is used as a sensitizer, at least in part. Outcomes can be very grave, and precise location and staging are required to determine treatment. For detailed discussion, refer to the Guidelines.¹⁹ Fluorouracil by infusion or capecitabine by mouth are the drugs most commonly used.

The Guidelines state that there is not strong evidence of success of adjuvant therapy with fluorouracil or capecitabine for rectal cancer, and the use of other agents is not warranted on present evidence. Patients with tumours higher in the rectum may do better.²⁰

Management of non-resectable locally recurrent and metastatic disease

Clearly the presentation of recurrences present challenges that are unique to each patient, and treatments are therefore tailored. Chemotherapy is but one small part, and regimens when administered systemically are similar (fluorouracil and folinic acid) as well as other drugs via innovative treatment routes. These include:

- Selective Internal Radiation Therapy (SIRT) using radioactive micro-spheres that after arterial infusion are trapped in the finest arteries in the liver tumour site;²¹ plus e.g. modified FOLFOX6 ± bevacizumab;
- Trans-Arterial Chemo-Embolisation (TACE) with irinotecan drug-eluting beads;
- Hepatic Arterial Infusion (HAI) with fluorouracil and folinic acid.

The Guidelines recommend use of these treatments only in trials and in other special situations.²² They are mainly included here so that the treatments and acronyms have some familiarity to CAP members if they come across them.

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²¹ For a fuller explanation see https://www.insideradiology.com.au/sirt/
**The role of systemic therapies in non-resectable metastatic disease**

Improvements in treatment of metastatic CRC have been made using traditional treatments supplemented by tailored and other therapies. The main drugs mentioned in this section of the Guidelines\(^\text{23}\) have already been discussed in this paper. Clearly, decisions as to which to use when will mostly be on a case-by-case basis as the adverse effects and likely benefit will need to be balanced for each individual.

This section of the Guidelines does provide a detailed exposition of treatment regimens with the first-line and subsequent therapies, as well as a good outline of the clinical aspects of molecular typing (RAS, BRAF, MSI etc). There is also a range of other emerging biomarkers whose significance is still being researched.

Key points include:

- RAS testing should be carried out on all patients at the time of diagnosis of metastatic colorectal cancer. If not wild type (i.e. mutant type) the anti-EGFR treatments (cetuximab and panitumumab) are not effective.
- BRAF mutations are a negative prognostic indicator. Most current therapies are less effective in the group with BRAF mutations.
- MSI testing may be a predictive marker for the use of immune checkpoint inhibitors in the treatment of patients with metastatic colorectal cancer (but it is early days).

**Treatment with targeted therapies.**

Comprehending this part of this summary, and the sections following, may be helped if one has some understanding of what the MAPK pathway is, as well as some other biochemical concepts. To that end a brief description of these is given in Appendix 2. A little of what is there will be repeated here to aid understanding, but generally it will be assumed the reader has read Appendix 2.

As already indicated targeted therapies are often either –mabs (ending in –mab because they are derived from monoclonal antibodies) or –ibs (which are selective inhibitors of a particular biochemical process in the tumour) although in the case of CRC, the useful –ibs are scarce: only one is included in the Guidelines.

**Monoclonal antibodies in use (sometimes on a trial basis) for CRC**

Monoclonal antibodies are antibodies produced by identical cells (clones of each other) grown in laboratory culture. Each type of cell culture is engineered to produce an antibody of a certain type/structure that has a specific effect on the tumour cells, which is not achievable by the body’s immune response alone.

The guidelines set out in some detail the use of some of these agents to treat metastatic CRC.\(^\text{24}\) A simple outline of how they work and what they are for is given below.

**Cetuximab (Erbitux)**

Cetuximab is a monoclonal antibody that binds to epidermal growth factor receptors (EGFR) more strongly than the natural proteins (ligands) thus preventing triggering of the pathways that lead to tumour cell activation. It is mostly used in patients who do not have RAS or RAF mutations, which are ‘downstream’ of


the point on the cell surface at which cetuximab works (see Appendix 2). It is administered by IV infusion. It is approved in Australia for

“the treatment of patients with epidermal growth factor receptor (EGFR)-expressing, RAS wild-type metastatic colorectal cancer

  in combination with infusional 5-fluorouracil/folinic acid plus irinotecan
  in combination with irinotecan in patients who are refractory to first-line chemotherapy
  in first-line in combination with FOLFOX
  as a single agent in patients who have failed or are intolerant to oxaliplatin-based therapy and irinotecan-based therapy. ” (Sponsor PI).

Panitumumab (Vectibix)
The pathway of action of Panitumumab is very similar to that of cetuximab and its use is also similar, as are its approved indications.

Bevacizumab (Avastatin)
Bevacizumab is a monoclonal antibody that binds to and neutralises the biologic activity of human vascular endothelial growth factor (VEGF). This reduces the growth of blood vessels to tumours, thus inhibiting tumour growth. It has been shown to produce a small but significant increase in progression-free survival or overall survival in CRC when given as part of a regimen including other proven therapies. In Australia it is approved for use in a number of cancers, including CRC. For CRC it is approved:

  in combination with fluoropyrimidine-based chemotherapy ... for the treatment of patients with metastatic colorectal cancer.

Ramucirumab (Cyramza)
Ramucirumab like Bevacizumab binds VEGF (Receptor 2) or VEGFR-2, which is key to the stimulation of VEGF induced angiogenesis (blood vessel formation). So it interferes with a tumour getting the necessary blood supply.

It has a host of serious adverse effects. It is not yet approved for use in CRC in Australia but is in the US “in combination with FOLFIRI, for the treatment of metastatic colorectal cancer with disease progression on or after prior therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine.”

Aflibercept
This is a –mab-like structure consisting of two parts: one a fragment of VEGFR-1 and the other of VEGFR-2, attached to a ‘tail’ of human immunoglobulin. The sponsor’s Approved Production Information states that “Aflibercept blocks the activation of VEGF receptors and the proliferation of endothelial cells, thereby inhibiting the growth of new vessels that supply tumours with oxygen and nutrients.”

It has a range of serious side effects often associated with bleeding. It is approved for use in CRC in Australia:

  in adults with metastatic colorectal cancer previously treated with an oxaliplatin-containing regimen.
Kinase inhibitors
Protein kinases are enzymes that add a phosphate group to a protein and so modify its function – typically rendering it active or inactive (switching it on or off).

A protein kinase inhibitor blocks the action of one or more protein kinases. The kinase inhibitors are mostly small molecules, not large like proteins, but can be large e.g. –mabs.

Regorafenib
Regorafenib (Stivarga) is a tyrosine kinase inhibitor interferes with a number of these enzymes involved in cancer. Tyrosine kinases transfer phosphate to other proteins in the cell to switch them on or off.

It can (rarely) cause severe liver toxicity which may be fatal. It is essentially used when everything else has failed.

It is given orally, and is approved for

treatment of patients with metastatic colorectal cancer (CRC) who have been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF therapy, and, if RAS wild type, an anti-EGFR therapy.

(Regorafenib is also approved for use in GIST.)

No other kinase inhibitor is used in the treatment of CRC at present.

Other drugs

Tegafur/Gimeracil/Oteracil
The combination therapy Tegafur/Gimeracil/Oteracil (aka S-1) is mentioned in the Guidelines but is not discussed here. It is not yet approved for use in Australia. If it becomes more frequently used a section on them can be prepared but the biochemistry/pharmacology is complex and so is omitted to keep the focus on more mainstream treatments at this stage.

Pembrolizumab
Similarly Pembrolizumab is mentioned in the Guidelines, in the context of MMR-deficient (MSI-high) CRC, but is not yet available in Australia for CRC, other than in the possible context of a clinical trial.
References and links

References
(Note: References used in Appendices are listed in the relevant Appendix.)


Cancer Institute NSW eviQ website https://www.eviq.org.au/


General materials referred to or listed to aid readers’ understanding of CRC treatment. What is Chemotherapy and how does it work? https://theconversation.com/explainer-what-is-chemotherapy-and-how-does-it-work-76403

Scitable: Essentials of Genetics https://www.nature.com/scitable/ebooks/essentials-of-genetics-8/contents

Hallmarks of Cancer: http://teachercenter.insidecancer.org/browse/Hallmarks%20of%20Cancer/

Historical outlines

The Enemy Within: 50 years of fighting cancer. https://vimeo.com/54898062

Epigenetics


Griffins C. TEDx talk Epigenetics and the influence of our genes https://www.youtube.com/watch?v=JTBg6hqeUTg


The MAPKinase pathway https://www.youtube.com/watch?v=r7GoZ9vFCY8

https://www.youtube.com/watch?v=oDjDUUhGVsI

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Appendix 1..The structure of the bases of DNA and RNA and the relationship with fluorouracil.

The genetic code of DNA is made up of only four basic building blocks (bases) that link to each other in a special way – each pairing with a particular one of the others in a chain, which is tightly spiralled. It must be ‘unzipped’ for cell replication.

RNA is made up of three of the four same bases, but in RNA uracil takes the part of the fourth one, played by thymine in DNA. Thymine and uracil are very similar but critically different molecules.

The chemical structure of the four bases of DNA are shown in a ‘simple’ way below: (from http://www.atdbio.com/content/5/Nucleic-acid-structure accessed 20 June 2017. A brief outline of the structure of DNA and RNA is given at that site).

These building blocks fall into two types. Adenine and guanine are purines and cytosine and thymine are pyrimidines, as is uracil. Hence the reference to the class of fluoropyrimidine drugs, all of which have the pyrimidine structure in there somewhere, with fluorine and other entities hooked on to that basic structure.

The similarity of thymine, uracil and fluorouracil is readily seen when they are side by side.

The structures of Thymine (from DNA) Uracil (from RNA) and Fluorouracil

Note that the structure of uracil is the same as thymine minus the methyl group (the -CH₃ at 10 o’clock on the thymine molecule in the above figure). Fluorouracil has a fluorine atom added to the uracil molecule in the position where thymine had a methyl group.
Appendix 2  A simplified explanation of some of the biomarkers of significance to cancer therapies using ‘targeted treatments’.

A greater understanding of the biology of cancer at the cellular and subcellular level has identified cellular markers that indicate when certain drugs may or may not work – resulting in so called tailored therapy. This has been particularly successful in e.g. some blood cancers and melanoma, but seems to be still in its infancy for CRC.

The clinical significance of the key biomarkers in CRC, as well as the significance of left-sided and right-sidedness in tumour behaviour, is outlined in the Guidelines.\(^{25}\)

The key biological pathways for CRC are briefly outlined below, to aid understanding how and why some of the targeted treatments work.

The MAPK pathway and its place in colorectal cancer occurrence and management.

*This is a complex topic. This brief summary may be better understood after watching the videos on MAPK pathways - see links included in the list at the end of the paper.*

Colorectal cancer is frequently associated with mutations affecting proteins on a complex pathway that controls cell growth and survival. This MAPK pathway (aka the ERK pathway or the RAS-RAF-MEK-ERK pathway) has a number of steps that can go wrong resulting in CRC and detection of these ‘errors’ can indicate likely success of the targeted therapies. An attempt to outline the key features of the pathway simply is given below, as a precursor to trying to understand the targeted therapies better.

The first step in activation is the binding of a ‘ligand’ – a protein that binds to a receptor on the surface of a cell. In CRC one ligand of great importance is Epidermal Growth Factor (EGF) which binds to an EGF Receptor (EGFR) on the cell surface. Once this happens two EGFRs get together (dimerise) and they have phosphate radicals attached to them within the cell (by other proteins – phosphatases). This switches on a cascade or chain reaction of proteins that bind to others, in particular the RAS proteins, of which KRAS and to a lesser extent NRAS are important in CRC. Following some further intermediate steps the RAS (now bound to something called GTP) is able to attract another protein group to the cell surface – the RAF proteins (A-RAF, B-RAF and C-RAF). Following still further steps these RAF proteins can activate MEK (which also other names) and that in turn activates MAPK (aka ERK). MAPK/ERK enters the nucleus of the cell and activates a range of factors that lead to transcription – the production of genes involved in cell proliferation. Normally this process is kept under control in the cell by still more protein kinases that switch RAS off.

The take-home message of all of the above is that certain triggers on a cell surface cause a chain reaction involving numerous intermediate proteins that ultimately give rise to a messenger (MAPK) that enters the cell nucleus and cause cell multiplication. These processes are all in a very delicate balance all the time.

Things occasionally go wrong with the proteins or the genes involved in these processes. In CRC these mistakes are often found and they give rise to e.g. RAF or RAS proteins that are abnormal. These abnormal states signify the sub-type of tumour it is and what might work against it. That is what we are about to consider.

Mutations of significance in colorectal cancer

The MAPK pathway can become overactive through mutation in several ways. Following is a brief description of the key ones.

One of the common ones is mutation of the RAS protein gene leading to mutated RAS that is resistant to the enzymes (protein kinases) that switch RAS off. Thus the cells may become tumour cells. Mutated KRAS or NRAS proteins are found in nearly half of cases of CRC.

A second mutation affects BRAF and this is found in up to 10% of CRC cases. Two mechanisms are thought to play a role in BRAF mutation. One is as a result of a repair gene called APC being faulty and this leads to inherited CRC. The other mechanism is epigenetic (non-heritable change – not a change to the DNA itself but how the nucleus of the cell handles the DNA within it). It should be noted that KRAS and BRAF mutations never occur together – if you have one you do not have the other.

BRAF mutation means that targeted anti-EGF therapy using –mabs like cetuximab will not work as the cause of the CRC is ‘downstream’ of that step.

KRAS mutations usually mean cetuximab will not work for the same reason, but there is said to be a subgroup of people with a particular type of KRAS mutation for whom this is not the case, and they may respond to –mab therapy (Al-Hajeili et al 2017).

The hunt is on for drugs that will inhibit RAS and RAF. BRAF inhibitors have been found to be effective in melanoma (which also has BRAF mutations) but not for CRC so far.

The anti- EGFR (epidermal growth factor receptor) monoclonal antibodies cetuximab and panitumumab are only effective in patients whose do not have the RAS mutations (i.e. their RAS is said to be of the wild-type). This is because these –mabs work to block cancer growth prior to the point of the RAS abnormality and so are ineffective.

Similarly, patients with the BRAF mutation are not likely to respond to cetuximab and panitumumab.

Vascular Endothelial Growth Factors (VEGF)

At the beginning of this section it was said EGF was an important ligand, but it is not the only one. Another important class is VEGF (VEGF-1, VEGF-2 and others), which when stimulated dimerise to set off a similar pathway to EGF, but this time it results in the development of blood vessels (angiogenesis). Tumours clearly require oxygen (thus blood) and they can stimulate VEGF activation to ensure development of the necessary blood vessels to keep them going. Drugs that block VEGF activation will prevent an adequate blood supply form developing and the tumour cells stop growing or die.
Mismatch Repair Gene deficiency – or Microsatellite Instability

Another important biological indicator is mutation of a mismatch repair gene (MMR).

If the MMR gene does not do its job (MMR deficient) one gets errors in the DNA. If these errors are not repaired you get short bits of DNA called microsatellites accumulating. When these short bits of genetic material build up or become abnormally short you are said to have microsatellite instability (or MSI high (MSI-H)). This is an indicator of tumours that do not respond well to fluorouracil when it is given as adjuvant therapy. However, metastases may still respond (Al-Hajeili et al 2017).

Checkpoint Inhibitors

Checkpoint inhibitors block the destruction of activated T cells (blood cells that are trying to fight a cancer). The T cell destruction process can be initiated by both the cancer cells and by the body’s own natural processes.

Checkpoint inhibitors prevent triggering of the (charmingly named) Programmed Death receptors (PD-1 and sometimes PD-2) on the T cell by another protein (a Ligand), of which there are two types: PDL-1 and PDL-2. These ligands may come from a white cell (tissue macrophage) or from the tumour. Using a checkpoint inhibitor to restrain activation of the Programmed Death receptors by ligands PDL-1 and-2, allows the T cells to live longer and thus work harder against the cancer, i.e. the drug enhances the body’s own immune response to the tumour. The drug does not attack the tumour directly. Unfortunately the enhanced immune response can lead to side effects, as the immune response becomes too strong in other non-target cells.

MMR-deficiency (MSI-H) may signal a person for whom the use of a checkpoint inhibitor is appropriate. There is evidence of effectiveness of pembrolizumab (a checkpoint inhibitor) in persons who are MSI-H, which if corroborated in larger trials would be fortunate, as this sub-group of CRCs are relatively resistant to most other treatments.

Note that pembrolizumab’s mode of action is quite unlike the other –mabs discussed in this paper. It remains experimental (as at January 2018) and is not approved for use in Australia for CRC. It may be available in future via clinical trial.

References:


26 Macrophages engulf bacteria and other unwanted matter in the body, but Tumour Associated Macrophages (TAMs) have a different, complex role. They are a positive prognostic indicator in some cancers but negative for others.