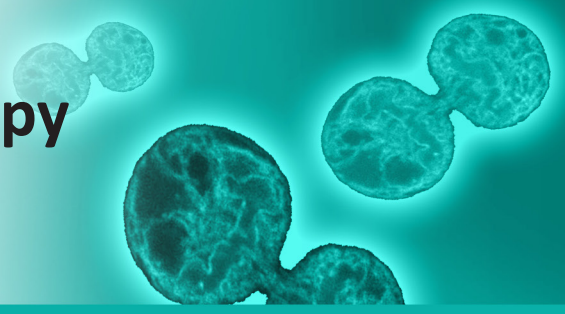# *BRAF*: from gene to cancer therapy
Teachers' notes

Overview of the activity

Malignant melanoma is a type of skin cancer that originates in melanocytes, specialised pigment cells found in the skin. The main cause of malignant melanoma is overexposure to UV light, e.g. from the sun or sunbeds. Mutations in the *BRAF* gene on chromosome 7 are associated with 50% of malignant melanoma cases.

In this activity the students take on the role of genome researchers. They interpret real Cancer Genome Project datasets to identify sites of mutation in the *BRAF* gene. This data is produced on next generation DNA sequencing machines from melanoma biopsies and healthy tissue from actual patients.

Using the data presented to them, students identify the significance of the *BRAF*^{V600E} mutation in malignant melanoma and use the online database COSMIC to compare their findings to that of cancer researchers. Using Information cards they discuss in groups the significance of the *BRAF*^{V600E} mutation and its implications in the treatment of metastatic melanoma.

This activity demonstrates the journey from cancer gene discovery to drug development. It also provides a complementary activity to the existing *KRAS* cancer activity which uses gel capillary sequence data (http://www.yourgenome.org/teachers/kras.shtml).

Aim of the activity

The aim of the activity is to encourage students to interpret and discuss real data from a DNA sequencing project. Through group discussions students can consider the impact of cancer gene discovery, the development of targeted cancer therapies and the challenges of treating malignant melanoma.

Estimated time: 60–90 minutes depending on time taken for introduction and discussion. Activity could be spread over two lessons.

Group size: Students can work in groups of up to 5 people.

Activity preparation

The following tasks need to be completed before starting the activity:
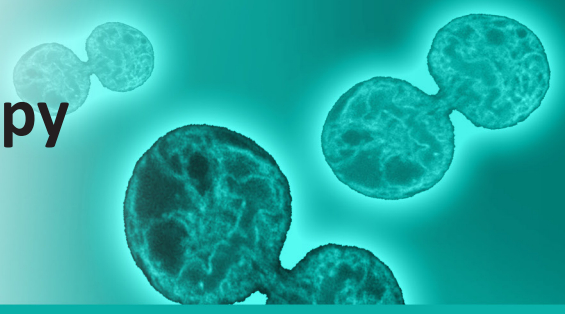
BRAF banner (1 banner per group)

Print all of the sheets that make up the *BRAF* banner. These can be printed on either A4 or A3 paper. We recommend laminating the sheets to prevent damage, however this is not essential. Cut along the dashed lines; these indicate where to trim and overlay the sheets to prevent large gaps forming in the gene sequence. Stick all of the sheets together with adhesive tape.

Note: An A3 gene sheet is provided as a smaller alternative to the banner.

BRAF: from gene to cancer therapy
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Mutation markers (1 set per group)

Print a set of mutation markers and cut these out. We recommend that these are laminated so they can be reused. Apply reusable adhesive, e.g. Blu-Tack®, on the back of each marker.

Note: If using the A3 gene sheet instead of the banner, marker pens or small stickers can be used as mutation markers.

Information cards (1 set per group)

Print one set of information cards per group and cut these out. We recommend that these are laminated so they can be reused.

Set up computers

Set up computers ready for the lesson and ensure they are connected to the internet. You may want to set up shortcuts to the COSMIC database (www.sanger.ac.uk/genetics/CGP/cosmic) prior to starting the lesson.

Running the activity

To run the activity you will require:

- Teachers' notes
- Introductory PowerPoint
- *BRAF* sequence data sheets
- Student worksheets
- Codon wheel
- Information cards
- Computers with internet access*
- *BRAF* banner or A3 *BRAF* gene sheet (1 per group)
- Mutation markers e.g. stickers (1 set per group)

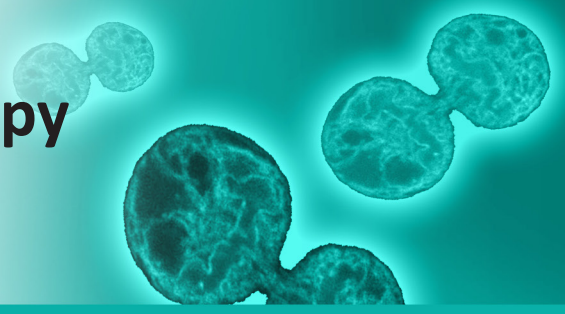*If computers are not available for the students, a demonstration by the teacher is possible as an alternative.

Introducing the topic and the activity (10 minutes)

The "*BRAF* and malignant melanoma" presentation provides students with an overview of cancer. It introduces the concept that cancer is not a single disease but over 200 different diseases that arise due to changes in DNA.

Slides 4 and 5 allow the class to discuss the causes of different cancers in the UK and worldwide including malignant melanoma, cervical cancer and lung cancer.

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Slides 6 to 11 discuss the role of mutations and cancer genes in the development of cancer.

Slides 12 to 14 provide information on malignant melanoma.

Slides 15 to 21 introduce the activity and the various components the students will use for the activity.

If you would like to use fewer slides you can hide slides in the presentation. To do this open the presentation in PowerPoint and select the slide(s) you wish to hide in the side bar. Click the right mouse button and select "hide slide".

Part 1: Interpreting the data (5–10 minutes)

Each group will receive 10 data sheets. These are divided into two sections. The first data set on the sheet shows the DNA sequence from a tumour sample from a patient with malignant melanoma. The second data set is the DNA sequence from a blood sample from the same patient, which provides a reference to compare against the tumour DNA sequence.

The worksheets have three sections to complete: the consensus DNA sequence, the complimentary strand DNA sequence and the amino acid sequence.

To complete the consensus DNA sequence, students must write down the commonly occurring bases in the sequence read, completing each box as a codon of three bases.

To complete the complementary DNA sequence row of the table, students must work out the complementary

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bases from the consensus sequence.

To complete the amino acid sequence students must translate the codons of the complementary DNA sequence into amino acids. It is important that students take note of the direction in which the sequence is read. The *BRAF* gene is read from right to left. This is indicated by the 5' and 3' markers on the complementary DNA strand row of the table, the direction of the arrowhead in the gene region box and the order of the codon number boxes at the bottom of the table.

Understanding the sequence data

The image shows an example of sequence data as a cancer researcher would see it. The key features of the display are highlighted and explained.
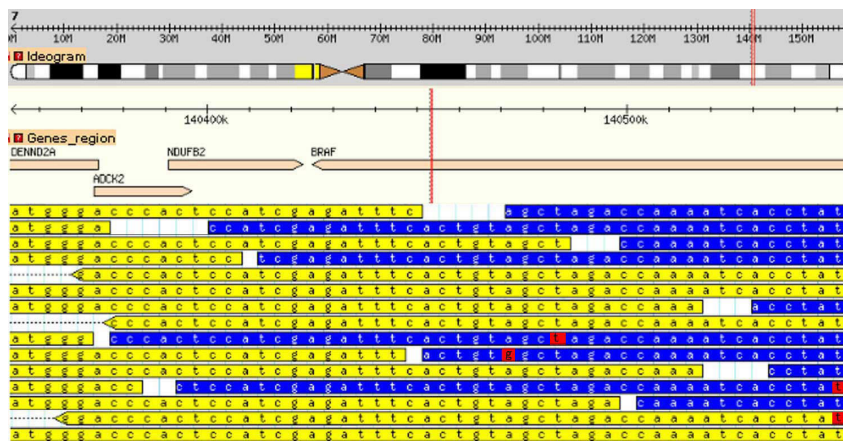
Ideogram: This represents chromosome 7 on which the *BRAF* gene is located. The red line shows the region where *BRAF* is found.

Gene regions: This represents the genes found in this region.

Sequence reads: This is the data produced by the DNA sequencing machine.

Red boxes indicate a change from the reference *BRAF* sequence.

The **ideogram** represents the entire chromosome showing the location of the *BRAF* gene.

The **gene regions** represent the genes found in this region of the chromosome. The arrowhead shows the direction in which the gene is transcribed. This will give you an indication of which direction you should read the data, i.e. left to right or right to left.

The sequence reads are the data produced by the DNA sequencing machine.

The **yellow** sections represent DNA sequence read along the strand from right to left (5' to 3').

The **blue** sections represent DNA sequence read along the strand from left to right (3' to 5').

Red boxes indicate a base change in the DNA sequence compared to the reference human genome sequence. A single red box on its own can indicate an error by the sequencing machine. If there is a line of red boxes this can indicate a mutation. A true result will be displayed as a base change occurring multiple times in the same location on both blue and yellow reads. If the mutation is found on just the yellow or blue reads it could be an error.

Mark up the mutations

Once students have completed all 10 sheets they must complete the table in their worksheet. They should then
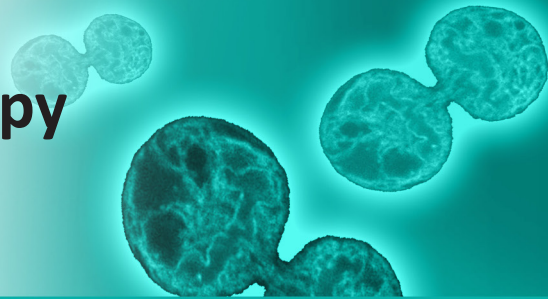
mark all the mutations they have found on either the gene banner or gene sheet provided using a marker pen or mutation markers. An example banner is shown below:

BRAF: from gene to cancer therapy
BRAF gene (exon 15)

DNA sequence	**AAT**ATA**TTT**CTT**CAT**GAA**GAC**CTC**ACA**GTA**AAA**ATA**GGT**GAT

Amino acid	N	I	F	L	H	E	D	L	T	V	K	I	G	D
Codon acid no.	581	582	583	584	585	586	587	588	589	590	591	592	593	594

Part 2: Consult COSMIC (5–10 minutes)

The next stage of the activity is to confirm how the students' findings compare with those of other scientists by consulting the COSMIC database. If time is limited, or there are no computers available for the students to use, this section can be delivered as a demonstration by the teacher.

COSMIC (Catalogue Of Somatic Mutations In Cancer), is a project run by the Wellcome Trust Sanger Institute that catalogues gene sequence changes associated with cancer. COSMIC is a live database that is continually updated, so numbers will change as data is added. It is free to use from the website http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/ and provides a resource for cancer researchers across the world.

To navigate the database, follow the instructions below:
1. On the homepage in the search box click on *By Gene*.

2. Type in BRAF and press the red *Go* button.

3. Select BRAF, the first entry in the list. (please note that sometimes you may be taken straight to step 4 by the website).

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4. In the *Mutation Analysis* box on the right of the screen, click on the *Histogram* button to see a graphical representation of the known mutations in the *BRAF* gene.

5. Enter the amino acid range on the *BRAF* banner (581-621) into the boxes to the right of the histogram and click on the red *Apply* button. This will display the region of the gene that you have been studying in more detail.

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6. You should see a bar on the histogram that corresponds to the position where you have found mutations. You will see that the bar at this position is divided into sections depending on which mutation has occurred and the number of samples with the mutation. Hover over the bar to see the type of mutations and number of samples (mutation count) at position 600. The largest section of the bar represents samples with the same substitution (T>A) as seen in your samples.
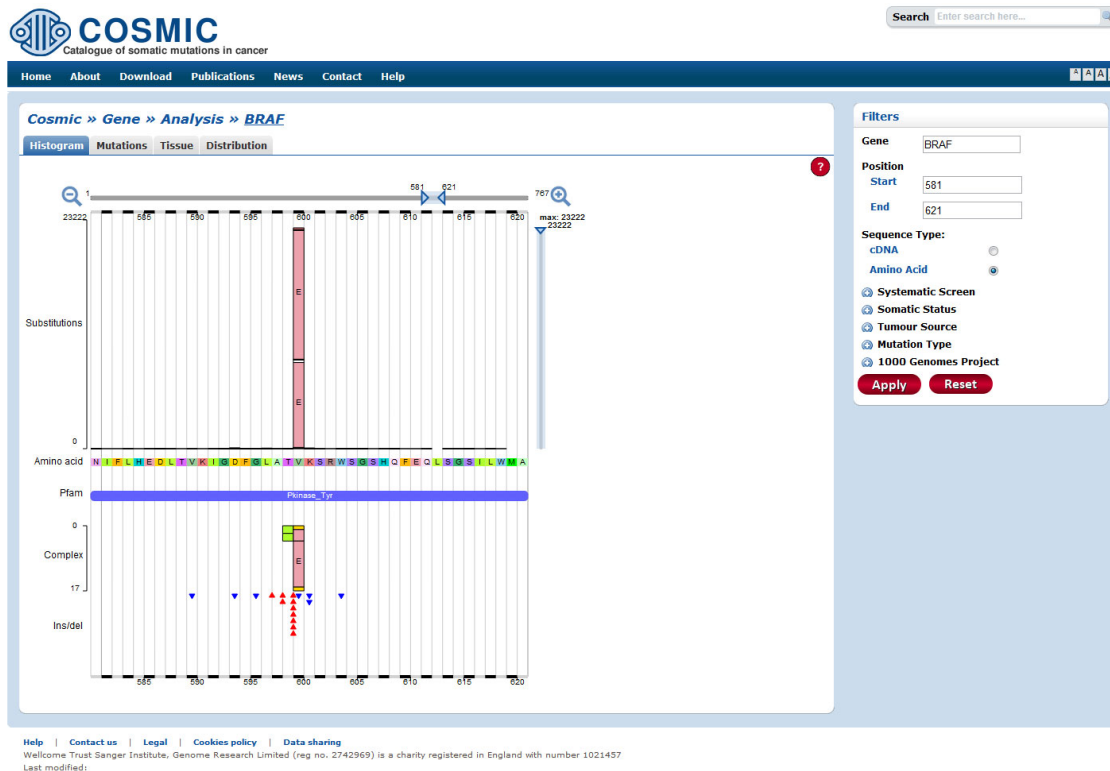
7. Click on the largest section of the bar to display more information about the T>A mutation.

8. To find out more about the tissue type, click on the *Tissue Distribution* tab.
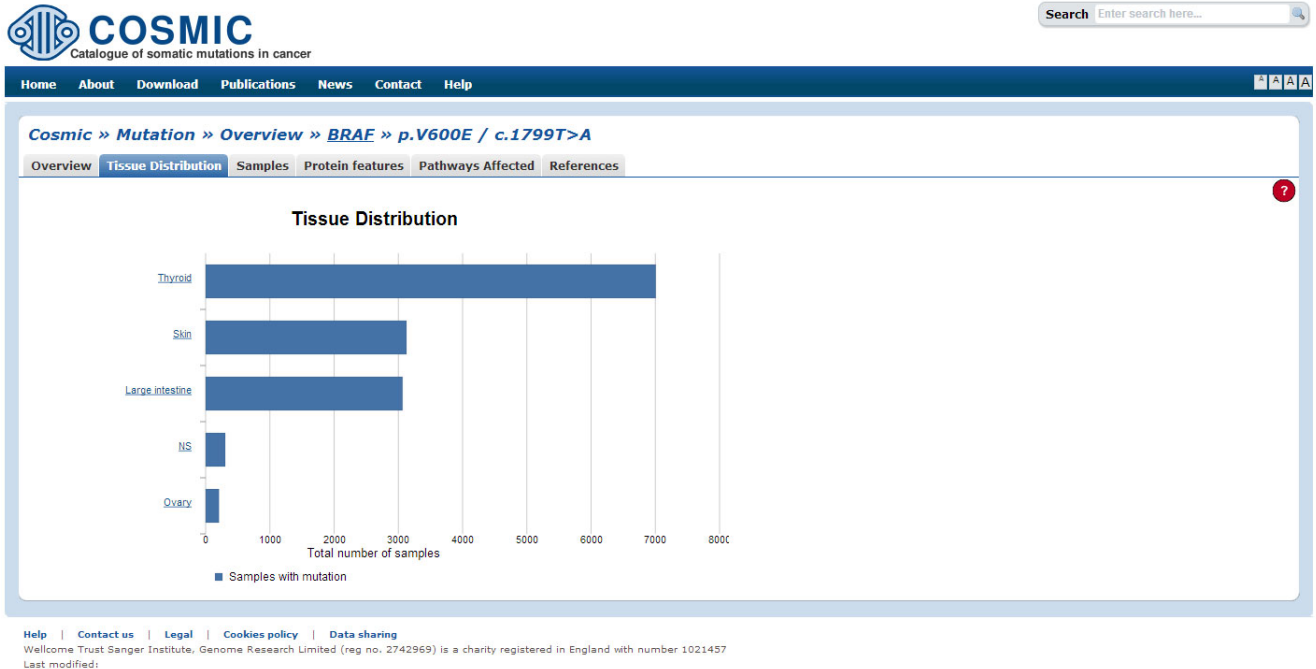
9. Hover the mouse over the tissue type to get figures for the number of samples with mutations.

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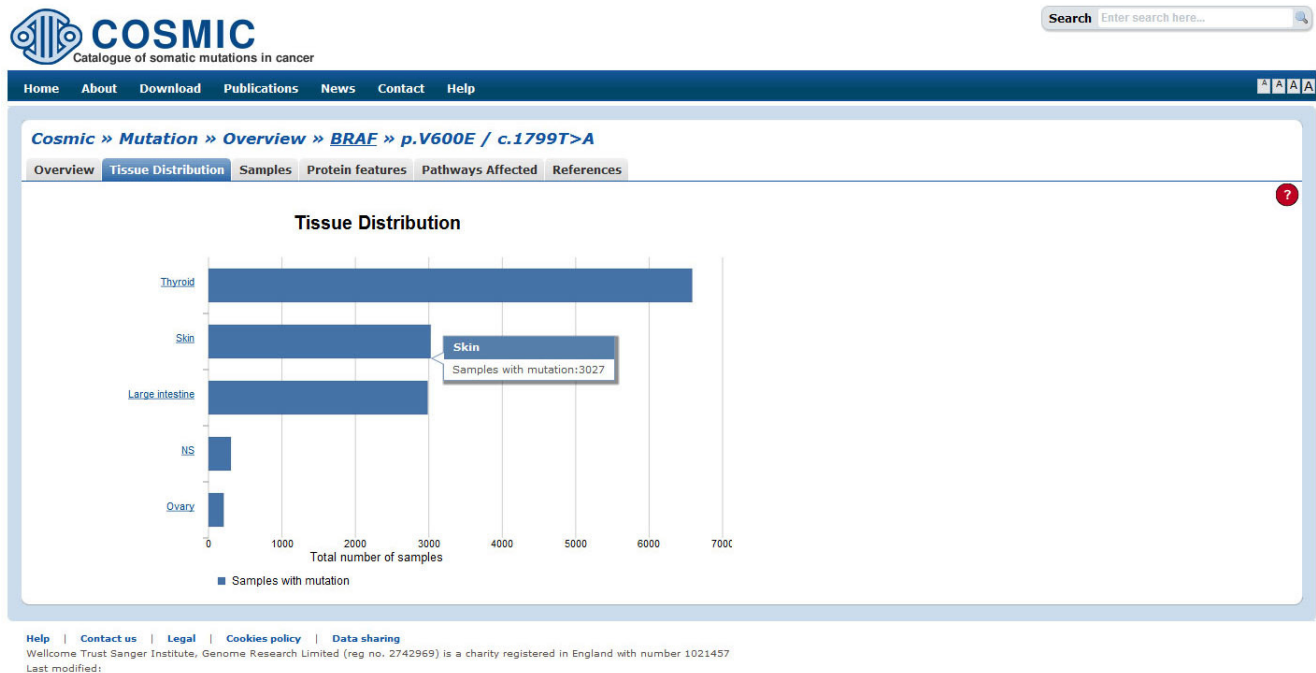
10. If you want to find out more about other genes associated with skin cancer, click on the *Skin* label of the graph.

11. To identify the most commonly mutated genes in skin cancer samples click on the *Genes with Mutations* tab.

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12. Sort the data in the *Mutated samples* column from highest to lowest by clicking on the arrow at the top of the column twice.

Students will have to calculate the percentage of mutated samples using the data in the table.
By navigating the database the students will be able to answer the questions in Part 2 of their worksheet.

The importance of sample size

You may want to explain to the students the importance of sample size in scientific studies. To be able to determine if a mutation in a gene is associated with a particular type of cancer it is important to test a large number of samples. This ensures that any conclusions you make from your results that relate to the general population are reliable and robust.

The "Top 20 genes" on the skin tissue profile (see step 10 above) feature some genes that show a100% mutation rate. This is where all samples have mutations in the gene of interest. However, many of these results are based on very small sample sizes of just 5 or 6 tissue samples. More reliable conclusions on the frequency of these mutations in the general population can be made with larger sample sizes of thousands of tumour specimens.
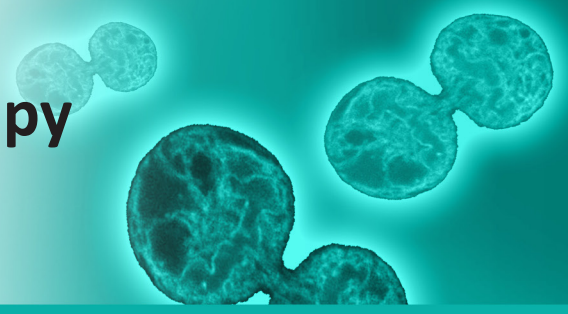
Part 3: Data discussion (20–30 minutes)

The final stage of the activity is for the students to look at their findings alongside details about malignant melanoma from Information cards. They use this information to answer three questions on the role of *BRAF* in malignant melanoma and possible treatment strategies for the disease.

Once students have completed their worksheets, they can feed back their findings and the outcomes from their discussions to the rest of the class.

Wrap up discussion

To wrap up the activity, run through the answers (Slides 22–35) encouraging the students to feed back their answers and summarise the outcomes of their group discussions.

Activity answers and discussion points

Slides 22–35 in the presentation show the answers to the different sections of the activity so that you can discuss them with the group.

Part 1: Interpreting the data

The results of the data analysis are shown below:

Sample	Mutation Yes/No	Codon Number	Healthy codon sequence	Tumour codon sequence	Healthy amino acid	Tumour amino acid
1	Yes	600	GTG	GAG	V	E
2	No	-	-	-	-	-
3	Yes	600	GTG	GAG	V	E
4	No	-	-	-	-	-
5	Yes	600	GTG	GAG	V	E
6	No	-	-	-	-	-
7	Yes	600	GTG	GAG	V	E
8	No	-	-	-	-	-
9	Yes	600	GTG	GAG	V	E
10	No	-	-	-	-	-

Question 1: Is there a pattern in the distribution of the *BRAF* mutations?

50% of the samples have the same V600E mutation. By consulting COSMIC we are able to see whether this mutation is common in other samples.

When marked up on the banner or gene sheet the distribution of mutations should look like this:

BRAF: from gene to cancer therapy
Teachers' notes
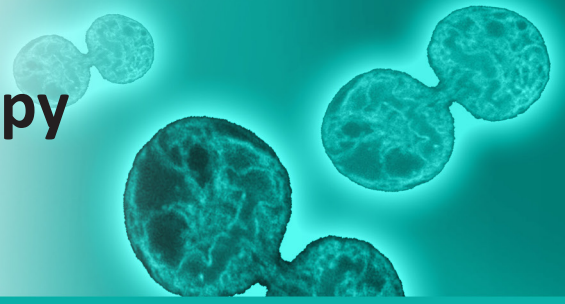
BRAF: from gene to cancer therapy
BRAF gene (exon 15)

DNA sequence	**AAT**ATA**TTT**CTT**CAT**GAA**GAC**CTC**ACA**GTA**AAA**ATA**GGT**GAT**TTT**GGT**CTA**GCT**ACA**GTG**AAA**TCT**CGA**TGG**AGT**
Amino acid	N I F L H E D L T V K I G D F G L A T V K S R W S
Codon acid no.	581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605

Why is 50% significant?

Current figures suggest that 50% of malignant melanoma patients carry the V600E mutation. This suggests that it could be a possible target for drug therapies.

Part 2: Consult COSMIC

Question 2: Are there any mutations that match your findings?

When the students compare their results with those found on the COSMIC database they will see that the most commonly occurring mutation in the *BRAF* gene is found at amino acid 600. The most common mutation at this point is V600E which is the mutation found in the samples on their worksheets. Over 19,000 samples on the COSMIC database have the V600E mutation in the *BRAF* gene as found in the students' worksheet samples.

You can see there are other regions along the *BRAF* gene with mutations but V600E is the most commonly occurring mutation in the gene.

Question 3: In which tissues are *BRAF* mutations commonly found?

The COSMIC database shows that *BRAF* mutations are most commonly found in the following tissues:
- thyroid
- large intestine
- skin
- ovary.

Question 4: What is the most relevant tissue type for malignant melanoma? What are the most commonly mutated genes?

BRAF is one of the most commonly mutated genes linked to skin cancer as it is seen in the top 20 genes associated with skin cancers on the COSMIC database. You may want to display the live page (click on the live page hyperlink on the presentation slide) to show the students the latest results in COSMIC.

Other genes of note in the top 20 for skin cancer include *NRAS*, another oncogene in the same signalling pathway as *BRAF*, and *CDKN2A*, a tumour suppressor gene that plays an important role in controlling the cell cycle and preventing tumour formation. These are featured on one of the Information cards.

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Part 3: Data discussion

Question 5: How does the mutation have an impact on cell growth and division? What are the consequences of this?

Slide 27 provides an animation of the role of the BRAF protein in a normal, functioning cell and the effect of the mutated BRAF^{V600E} protein in a cancer cell.

Students should have identified from the information cards that BRAF is part of the MAPK-ERK signalling pathway and plays a role in cell growth, proliferation and survival.

BRAF is a protein kinase, an enzyme that can transfer a phosphate group from a molecule of ATP to a protein in the cell. This process is known as phosphorylation and functions as an "on" or "off" switch in many cellular processes.

How is the MAPK-ERK pathway involved in cell division?

The MAPK-ERK pathway is involved in the initiation of cell division in response to growth factors. When a growth factor binds to a protein receptor on a cell's surface the following events occur which lead to cell division:

1. A growth factor binds with a protein receptor at the cell surface. This leads to the receptor becoming phosphorylated and switched on.
2. The receptor binds to the RAS protein via a group of adapter proteins. This activates the RAS protein.
3. The activated RAS protein phosphorylates and activates the BRAF protein.
4. BRAF phosphorylates and activates the next protein in the signalling cascade, MEK
5. MEK phosphorylates and activates the ERK protein.
6. ERK phosphorylates and activates transcription factors that control the expression of genes that are involved in initiating cell division and proliferation.

This process is outlined in the diagram on the right.
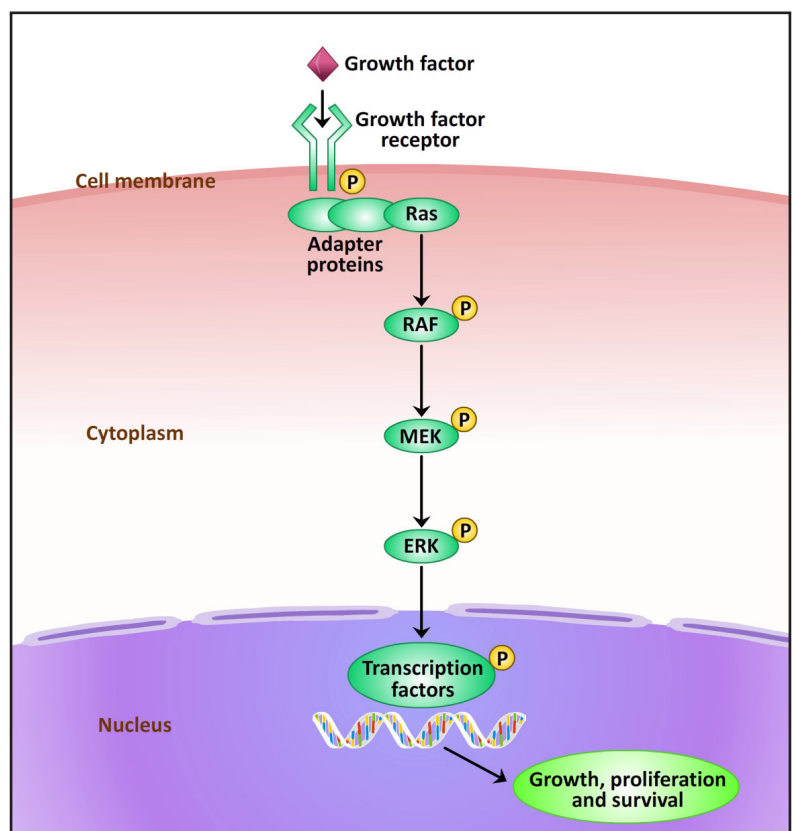
When mutated, the *BRAF* gene encodes a protein called BRAF^{V600E}. This protein is permanently activated, regardless of whether it has been phosphorylated by the proteins upstream of it in the signalling pathway. It

therefore continually sends signals to proteins downstream of it in the pathway resulting in cell proliferation. This can lead to tumour formation if other regulatory genes, such as tumour suppressor genes, are also mutated and cell division becomes completely unregulated.

It is important to emphasise that this is just one pathway in a much larger network of signalling pathways involved in controlling cell growth and proliferation. Slide 28 shows this.

Question 6: How can DNA sequence data be used by the research community to improve cancer treatment?

The V600E mutation in BRAF and its link to malignant melanoma was discovered by scientists at the Wellcome Trust Sanger Institute in Cambridge and the Institute of Cancer Research in London. The work was published in the journal Nature in 2002.

Sequencing cancer genomes enables researchers to identify commonly occurring mutations in different cancers. This can provide scientists with a list of potential targets for drugs. New treatments can be developed that specifically target particular mutated proteins in the cancer. These drugs can stop the mutated protein from functioning and kill the cancer cells.

In 2011, nearly ten years after the discovery of *BRAF* and its role in human cancer, a targeted BRAF V600E inhibitor, vemurafenib (Zelboraf), was approved for use in the USA and Europe. Other BRAF inhibitors are also being developed.

This case study can be used to demonstrate the time it takes to go from discovering a gene mutation involved in cancer to producing a licensed drug that specifically targets the misfunctioning gene product.

Question 7: Treating melanoma patients

Question 7 encourages the students to consider the advantages and disadvantages of different treatments for malignant melanoma and demonstrates the challenges of treating the disease.

Malignant melanoma is the most aggressive form of skin cancer. However, if it is diagnosed in the early stages, i.e. before it has the chance to spread to other parts of the body, it can be cured by surgically removing the lesion. This treatment is used in 80% of melanoma cases.

The graph on the Information card titled "Melanoma statistics" shows that the death rate from malignant melanoma is higher in men compared to women. Students may ask why this is the case. One possible reason for the higher death rate in males may be that men are less likely to cover up in the sun or use sun protection cream to reduce exposure to UV light. Men are also more likely to delay going to the doctor if they see symptoms of melanoma such as an irregular or inflamed mole. Better awareness of the signs of melanoma is essential to ensure the disease is diagnosed early and treated before it progresses. If the disease progresses to advanced or metastatic melanoma the prognosis is very poor. The survival rate for patients with metastatic malignant melanoma is low with only 10-20% of patients surviving for two years or more. Most patients live for 6–15 months after diagnosis.

Short model answers for the two questions are outlined below. More information is provided in the discussion

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points on the following pages.

Question 7a: How would you identify if an advanced melanoma patient carried the *BRAF* mutation? What treatment would you prescribe and why?

If you were presented with a melanoma patient you could carry out a diagnostic test to determine if the patient's tumour carried a *BRAF*^{V600E} mutation. A **cobas**® 4800 BRAF V600 Mutation Test is one example of such a diagnostic test. Alternatively you could have a sample of DNA from the tumour sequenced to determine if it contained the *BRAF*^{V600E} mutation. If the patient carried the *BRAF*^{V600E} mutation they could be treated with the targeted BRAF inhibitor vemurafenib. This drug specifically targets cells with this mutation and clinical trials have demonstrated that vemurafenib can significantly reduce the risk of the disease progressing and improves overall short term survival for patients with the *BRAF*^{V600E} mutation.

Question 7b: How would you treat an advanced melanoma patient without the *BRAF* mutation? Explain your reasons.

If a melanoma patient does not carry the *BRAF*^{V600E} mutation they cannot be treated with vemurafenib, as their tumours do not contain the mutated protein targeted by the drug.

Possible treatment options for this patient include surgical removal of the tumour and surrounding lymph nodes followed by radiotherapy or chemotherapy treatments to try to ensure that all cancer cells have been destroyed. If the tumour cannot be removed by surgery, radiotherapy or chemotherapy treatment alone could be prescribed.

If the cancer was more advanced and metastatic the patient could be treated with an immunotherapy such as ipilimamub. This would stimulate the patient's immune system to attack the cancer cells. This therapy can also be used in combination with chemotherapy.

Key discussion points for treatments for advanced melanoma

What treatments are available for advanced melanoma?
Below is a breakdown of the advantages and disadvantages of the treatments featured on the Information cards.

Chemotherapy– dacarbazine (DTIC)

Advantages	Disadvantages
Used as single treatment for metastatic melanoma that has spread to other regions of the body.	Non-specific in action – unable to distinguish between tumour cells and healthy cells.
Can be used after surgery to remove lesions if melanoma has spread to the lymph nodes.	Can increase risk of secondary infection due to decrease in white blood cells.
Treatment can result in tumour shrinkage for 7-12 % of patients.	Only offers short term overall survival of 5–7 months.

BRAF: from gene to cancer therapy
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Radiotherapy

Advantages	Disadvantages
Can be used to reduce tumour size and can help to improve the symptoms of advanced melanoma.	The use of radiotherapy can lead to side effects such as fatigue, red sore skin at the sight of treatment and secondary damage to healthy tissue local to the area of treatment.
It can reduce the rate of the local recurrence of lesions.	When used after surgery it does not prolong overall survival for patients compared with surgery alone.

Immunotherapy – ipilimamub (Yervoy)

Advantages	Disadvantages
Stimulates the patient's immune system to destroy cancerous cells.	Causes side effects such as diarrhoea, skin rash, liver failure and inflammation of the intestines.
The drug activates the immune system which results in the T-cells "killing" the tumour cells leaving normal, non-cancerous cells unaffected.	In rare cases it can cause severe adverse effects that can lead to death. Tests are necessary to identify whether treatment is appropriate.
Using ipilimumab in combination with dacarbazine (chemotherapy) increases patient survival compared with dacarbazine and a placebo.	

Targeted BRAF inhibitor – vemurafenib (Zelboraf)

Advantages	Disadvantages
Drug is administered as a tablet.	Side effects include skin rashes and the development of squamous cell carcinoma, skin lesions that can be removed by surgery.
Highly effective at reducing tumour size. It can produce complete regression of tumours in some patients.	Only effective in patients with $BRAF^{V600E}$ mutation.
The drug only targets cells with the $BRAF^{V600E}$ mutation, therefore is more specific in its action than dacarbazine and radiotherapy.	Resistance mechanisms can develop causing relapse of the cancer in patients after treatment.
Treatment can prevent the cancer from progressing further, i.e. the patient lives with it but it doesn't get any worse.	Treatment can prevent the cancer from progressing further in the short term but not long term.

Vemurafenib offers hope for some melanoma patients. Slides 31-33 provide images that show the effects the drug can have and demonstrate how the drug works.

Would all patients require the same treatment?
Melanomas can vary significantly between patients. Not all treatments are therefore likely to be effective for everyone and one size does not fit all. In the case of vemurafenib the drug will not work if you do not have a V600 BRAF mutation.

BRAF: from gene to cancer therapy
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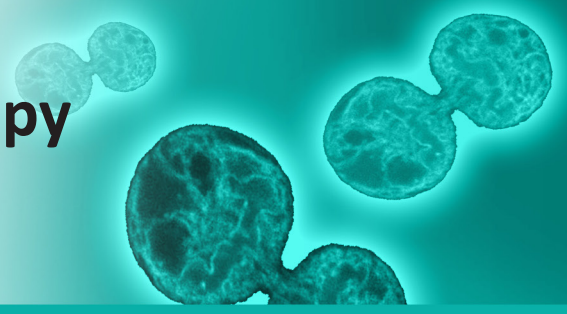
As a comparison you can highlight the example of the breast cancer therapy trastuzumab, commonly known by its commercial name Herceptin®. This drug is only effective on a specific type of breast cancer where there is over expression of the *HER2* gene, which results in the over production of the protein HER2. This is known as being HER2 positive.

HER2 is a receptor found on the surface of cells that binds with human epidermal growth factor. When the growth factor binds with HER2, it starts a signalling cascade that stimulates cell division. Some breast cancers over express the *HER2* gene and therefore have a lot more HER2 receptors. A cancer with more HER2 receptors grows quicker than one with fewer HER2 receptors.

If a patient is not HER2 positive, Herceptin® is ineffective and therefore cannot be prescribed. Herceptin® is an antibody that binds to HER2 receptors on the surface of breast cancer cells. This blocks signalling through the receptors and the cancer cells are therefore no longer stimulated to divide. The binding of the antibody to the receptor also stimulates the body's immune system to destroy the cancer cells.

Ask the students the following question: *Of the 10 samples in their data set how many patients could be prescribed vemurafenib as a treatment?*

As only 50% of the data set has the *BRAF^{V600E}* mutation only five patients would be suitable for treatment with the drug.

How would you determine if treatment with vemurafenib is appropriate?
BRAF mutations are found in approximately 50% of melanomas. Vemurafenib is therefore not a suitable treatment for all melanoma patients. As a result, it is important to identify if the *BRAF^{V600E}* mutation is present in the melanoma of a patient before prescribing vemurafenib.

How do you identify whether a patient has the *BRAF^{V600E}* mutation? There are currently two possible methods; sequencing the entire tumour DNA using next generation sequencing technologies as demonstrated in this activity, or using diagnostic kits that specifically detect the mutation in the BRAF gene.

One diagnostic kit that has been developed to help determine if patients are suitable for treatment with vemurafenib is the **cobas**® 4800 BRAF V600 Mutation Test. This detects the *BRAF^{V600E}* mutation in the DNA from human melanoma tissue. The test takes less than 8 hours from receipt of tissue to diagnosis and in clinical trials was shown to positively identify 97% of samples with the *BRAF^{V600E}* mutation. The cobas® BRAF Mutation Test costs $120-150 per test.

At present sequencing methods are more expensive and take longer than the cobas® BRAF Mutation Test. Advances in technology mean that the cost and time taken to sequence samples is rapidly decreasing and could soon be available in clinical settings.

What happens if the patient develops resistance to the drug or has a relapse?
Resistance to drugs, such as BRAF inhibitors, can pose a problem for the long term survival of melanoma patients. Clinical trials of vemurafenib showed that resistance can emerge anytime between 2 to 18 months after positive treatment responses.

Slide 34 features a video of Sanger Institute faculty member Dr Ultan McDermott discussing the challenges of treating malignant melanoma and some of the processes by which drug resistance can occur in cancer patients.

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Ultan's research focuses on the use of high throughput techniques to characterise the mutations within hundreds of different cancer cells and then screen these cells with pre-clinical and clinical drug compounds. The goal of his research is to be able to predict which mutations each cancer drug works most effectively on.

Slide 35 shows some of the known resistance mechanisms associated with *BRAF^{V600E}* positive malignant melanoma treated with vemurafenib. Researchers have termed these mechanisms as "escape pathways". In these cases the tumour has developed alternative routes for unregulated cell growth through either:

- activating mutations of other proteins downstream of BRAF in the MAPK-ERK signalling pathway
- mutations of proteins in other signalling pathways also involved in the control of cell proliferation or cell survival.

The diagram on slide 35 highlights where these additional mutations can occur in the MAPK-ERK pathway and other signalling pathways. There are four known mechanisms that enable cells to become resistant to vemurafenib:

1. Mutations of the NRAS protein upstream of BRAF in the MAPK-ERK pathway. This "reactivates" the MAPK-ERK pathway leading to unregulated cell division.
2. Over expression (producing too much) of other proteins in the MAPK-ERK pathway, such as CRAF. This overexpression leads to uncontrolled cell division.
3. Mutations of the MEK protein in the MAPK pathway. The MEK protein is involved downstream of BRAF in the MAPK-ERK pathway. Mutation of MEK reactivates the pathway leading to uncontrolled cell division. This is the example given in previous slides.
4. Activation of receptors at the cell surface membrane allows signalling through alternative pathways. This also leads to unregulated cell growth, division and tumour survival.

How do you think you could overcome resistance mechanisms?
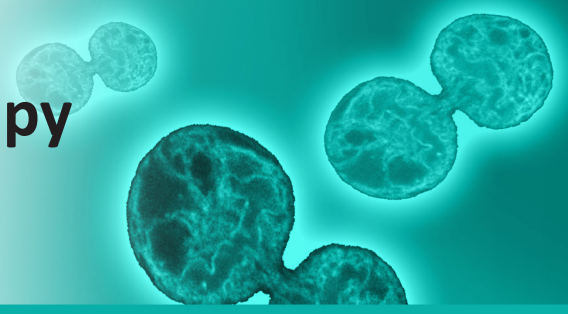Ways to overcome resistance mechanisms could include:

1. Increase the dose of the targeted drug therapy. Increasing the dose of the drug imatinib (Gleevec or Glivec) has proved effective in the treatment of chronic myeloid leukaemia. However higher drug doses could increase the likelihood or severity of adverse effects.
2. Combined, targeted drug therapies target the primary mutation (in this case BRAF^{V600E}) and the bypass mechanism (e.g. MEK1) at the same time. Current research is investigating whether this could be an effective strategy in the treatment of melanoma.

Studies are investigating whether combining BRAF inhibitors with other targeted drug therapies, such as MEK inhibitors, will prevent drug resistance from emerging. . Early clinical trials have shown that combining BRAF inhibitors and MEK inhibitors resulted in a reduction in tumour size and melanoma growth. It is hoped that combining two therapies will prevent resistance developing and prolong patient survival, improving the results seen with current single drug therapies. These studies are ongoing.

Combining radiotherapy and the immunotherapy ipilimumab has also been shown to significantly reduce tumour size in a case study of one melanoma patient.

BRAF: from gene to cancer therapy

Teachers' notes

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Recommended support resources

Websites

The Cancer Genome Project at the Wellcome Trust Sanger Institute uses the human genome sequence and high-throughput mutation detection techniques to identify somatically acquired mutations and genes critical to the development of human cancers. Further information is available online at: http://www.sanger.ac.uk/research/projects/cancergenome/
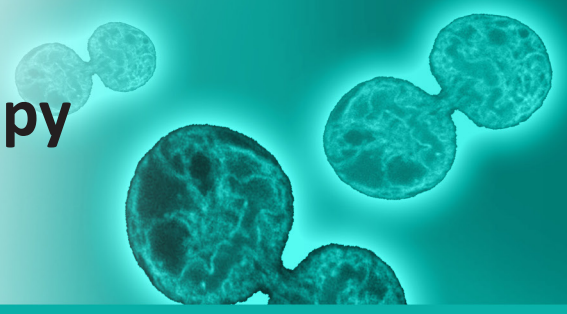
Cancer Research UK provides a wide range of information and statistics on cancer in the UK. http://info.cancerresearchuk.org/

Recent news

Skin cancer drug hopes raised by study – BBC News 23rd February 2012. Available online at: http://www.bbc.co.uk/news/health-17128925

yourgenome.org

New drug for malignant skin cancer – NHS health news 6th June 2011. Available online at: http://www.nhs.uk/news/2011/06June/Pages/vemurafenib-skin-cancer-drug.aspx

Videos

BBC Horizon Defeating Cancer
This clip, taken from a BBC Horizon programme, shows Rosemary, a melanoma patient, who is given a targeted drug (vemurafenib). Available online at: http://www.bbc.co.uk/programmes/p00qrt0w

Cancer and the genome: the issue
This Teachers TV video, filmed in 2006, features Dr Andy Futreal (formerly of the Cancer Genome Project at the Wellcome Trust Sanger Institute and now at the University of Texas, MD Anderson Cancer Center). He talks about how DNA sequencing technologies were used to identify the *BRAF* gene and establish its links to malignant melanoma. It provides a historical perspective on how the *BRAF* gene was discovered and how treatments have evolved since the making of the film. Available online: http://www.tes.co.uk/teaching-resource/Teachers-TV-Cancer-and-the-Genome-The-Issue-6047948/

Further reading

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Lemech C, Infante J and Arkenau HT. The potential for BRAF V600 inhibitors in advanced cutaneous melanoma: rationale and latest evidence. *Therapeutic Advances in Medical Oncology* 2012; 4: 61–73. Available online at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3296083/?tool=pmcentrez

Wagle N, Emery C, Berger MF, *et al*. Dissecting therapeutic resistance to RAF inhibition in melanoma by tumour genomic profiling. *Journal of Clinical Oncology* 2011; 29: 3085–3096. Available online at: http://jco.ascopubs.org/content/29/22/3085.full.pdf