let´s go on a DRUG HOLIDAY

Reading '*drug holiday*', you were most likely thinking of some sort of vacation to Amsterdam with much cannabis and alcohol usage, but probably not about a possible new treatment for melanoma patients. However, in the next few pages you´ll be reading about a medical breakthrough on drug holidays instead of drug usage during holidays.

Luckily there is anti-cancer medication these days. But, unfortunately, when cancer patients are treated with a single anti-cancer drug, eventually drug resistance occurs in the tumours. Resistant tumours will grow unrestrained, even though medication is used. In these situations, most doctors will stop the treatment. Prospects for these patients are infaust. However, it is known that in some situations the tumour-cells stop dividing and sometimes the tumours even shrink after ceasing the medical treatment. Until recently this phenomenon was a true medical wonder. Rene Bernards investigated this so called 'drug holiday'. Together with his team, he unravelled the underlying mechanism of the 'drug holiday' in certain melanoma patients[i].

Melanoma

The study from Rene Bernards was performed on melanoma patients. A melanoma is a form of cancer that begins in melanocytes (cells that make the pigment melanin in the skin). Melanocytes can undergo changes. Mostly, these changes are harmless. But sometimes they can cause uncontrolled cell division. The cause of this is explained in figure 1. Cells can only survive if they receive a survival signal from somewhere else in the body. But this signal is not transferred to the cell directly, but through different steps (here represented by gears). Once gear 1 is turning, gear 2 will be turning, then gear 3 will be turning, etc.. Eventually, when gear seven is turning, the signal is passed on to the target cell. The survival signal will be noticed by a receptor, which urges the cell to cell division.

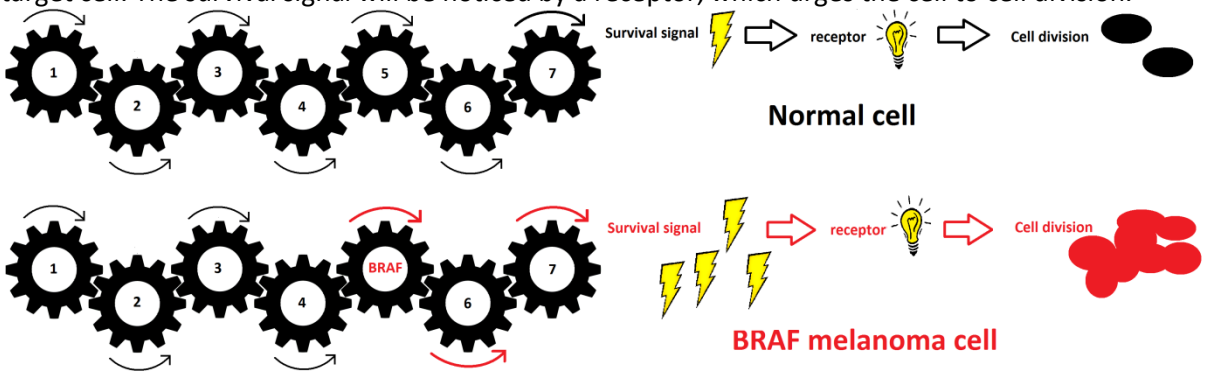

Fig.1 Changes in a BRAF melanoma cell

However, in melanoma cells, some of these steps differ from the steps in normal cells. For example in BRAF melanoma cells; in these cells, the "BRAF gear" has replaced gear 5 and does not execute his function normally. This gear will turn much more rapidly than usual, which will make gear 6 and 7 turn much more rapidly as well. Eventually, the cell will receive much more survival signals than the

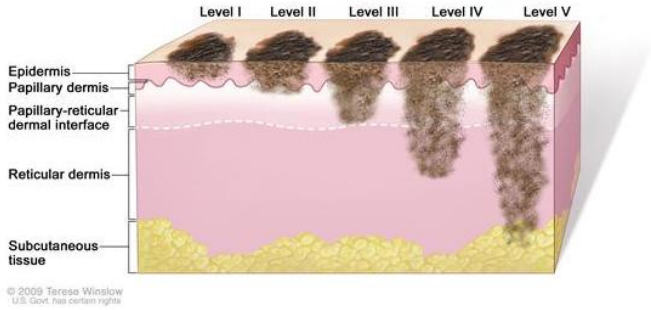

Fig. 2 levels of melanoma development

normal tissue cell does. This is noticed by the receptor, making the melanoma cell divide very rapidly and uncontrolled. Once this happens, a tumour will grow. Tumour growth can be staged in different levels. *(figure 2)* Once a melanoma has grown so big that it invades other tissues, it can cause tumours to grow in other places of the body. At this point, the melanoma has become a deadly disease.

Current treatment of melanoma patients

It is advised to avoid too much UV-light in the sunlight and at the solarium, because this light is the most important risk factor for the development of melanomas. If, despite of the prevention, a melanoma has developed, doctors have to look into the stage of the tumour. A surgical procedure can be used to remove the melanoma. However, if the tumour is in a later stage, a more drastic therapy has to be used.

In this case, most BRAF melanoma patients are treated with medication (vemurafenib[ii]). This will inhibit the operation of the "BRAF gear" (fig. 3). Once the "BRAF gear" stops working, gear 6 and 7 in the figure will also stop. As a result, there will hardly be any survival signal delivered to the cell. The receptor will not detect any survival signal, so the cells will stop dividing.

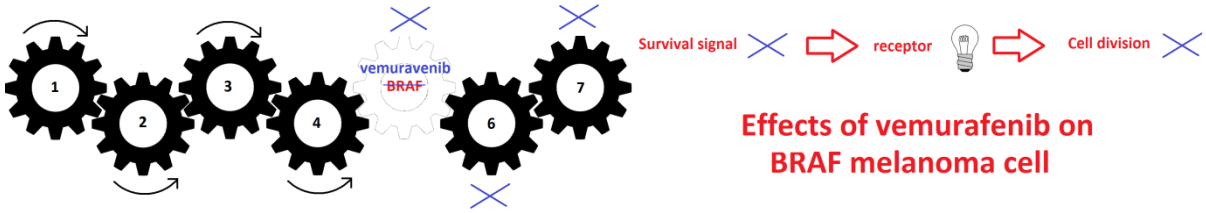

Fig. 3 Effects of vemurafenib on BRAF melanoma cell

The downside of this therapy, is, besides side-effects, that eventually the effect of the medication fades away; the melanoma cells again start dividing in large numbers. Mostly the doctor stops the treatment in these situations. Sometimes, miraculously, after stopping the treatment the cancer cells stop dividing. This small medical wonder was then named "the drug holiday", but was never really investigated...just until recently!

The research

Rene Bernards, a biomedical geneticist, is a leading professional in cancer research. In earlier research he, together with his team, found out that a new combination of drugs resulted in less tumour growth than the usual treatment[iii]. This and many other research results are clinically very useful. In his newest research, he investigated the underlying mechanisms of the drug holiday. His goal was to be able to understand why the same medication started working after stopping the anti-cancer treatment for a certain amount of time.

His research was published in the science journal Nature on the 26th of March 2014[iv]. In the article, he explains his findings.

There are many different sorts of cancer cells. Tumour cells are categorised by the location where the tumour is found and the difference between the tumour-cells and normal tissue cells. However, within the group of melanoma cancers, there are many more types of cancer cells. The type of cancer

cell is determined by the change in DNA (comparable to gears as in the preceding example), which can make the cell divide very rapidly. The BRAF mutant cells (used in the example) can be treated. The same kind of treatment is used in BRAF mutant colon cancers as in BRAF mutant melanoma cancers. Earlier research has proven that colon cancers are intrinsically resistant to this medication, due to a feedback mechanism[vvi]. Bernards suggested that the same feedback mechanism is seen in melanoma cells; the cancer cells produce more receptors for the survival signal so that they can survive with just a little of the signal. *(figure 4)*

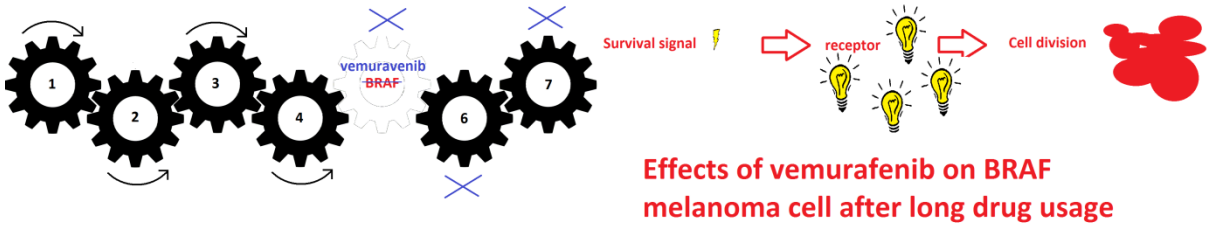

Fig. 4 Effects of vemurafenib on BRAF melanoma cell after long drug usage

To prove his suggestion, Bernards took samples from the melanomas of 16 melanoma patients. The biopsies were taken before the patients were treated with the medication and after the development of drug resistance. The results are shown in figure 5; looking at the changes between the post-treatment biopsies, six out of twelve gained notable receptor expression, as judged by immunohistochemistry[vii].

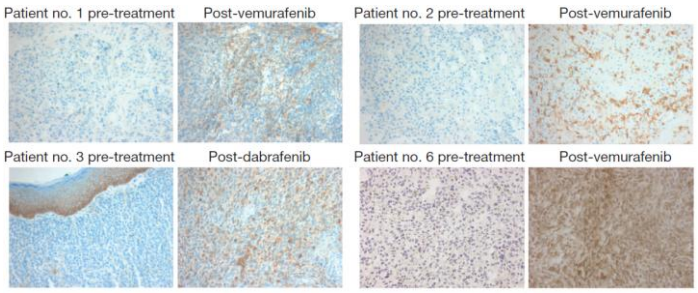

Fig. 5 Results from melanoma biopsies pre-treatment and post-vemurafenib

But Bernards went even further; he wanted to know which gene regulated the changes during drug holiday (increase of receptors) and how these changes were made. In order to find the gene responsible for the effects of the drug holiday, a list was made with genes that were most likely to be involved in the changes. First, all genes were tested at once by knocking them down in cells in vitro, but no relevant information was found. Only when the genes were knocked down and the cells were placed under drug selection for vemurafenib, cells showed a significant increase in receptors. Cells with high levels of receptors are apparently not favourable in normal situations, only when the patient is using this anti-cancer mediation. This is probably the main reason why the medication does not work after drug resistance development, but does work after a drug holiday; cells with many receptors are not favoured, so they will disappear. Relatively more cells with less survival signal receptors will appear. These cells are not resistant to the medication.

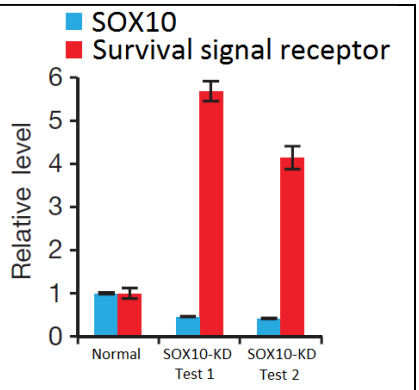

Fig. 6 Results from melanoma biopsies pre-treatment and post-vemurafenib

Further tests were done to determine which gene of those tested was responsible for the changes. Only suppression of the SRY (sex determining region Y)-box (SOX10) gene induced prominent receptor expression. The tests where the SOX10 gene was knocked down (SOX10-KD) were executed in two different ways. Both showed an enormous increase in receptors *(figure 6)*; when SOX10 is knocked down, on average, the level of survival signal receptors quintuples.

Relevance

Because the underlying principle of the drug holiday is now partially known, a stimulus was given for further research. This research showed how it is possible that after a drug holiday, the same medication can be effective again, but not why the tumour growth stops when patients stop their medication. It is thought that this is because of an overload of the stimulus. *(figure 7)* However, this has never been researched.

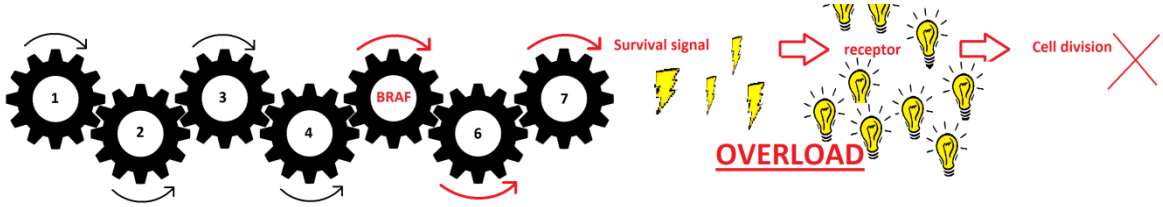

Fig. 7 possible consequence of drug holiday on the cell

The more we now about the drug holiday, the more it can be used clinically. Now, every cancer cell eventually develops resistance to anti-cancer drugs. But, by using the drug holiday in treatments for melanoma patients, it will be possible to extend the lives of cancer patients. This research has brought us closer to making cancer a chronic disease, instead of a lethal one.

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i Bernards, R. et al. Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. Nature doi:10.1038/nature13121
ii http://www.farmacotherapeutischkompas.nl/preparaatteksten/v/vemurafenib.asp
iii http://www.umcutrecht.nl/zorg/patienten/zorgverleners/B/bernards/
iv http://www.nature.com/nature/journal/vaop/ncurrent/full/nature13121.html
v Prahallad, A. et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 483, 100–103 (2012).
vi Corcoran, R. B. et al.EGFR-mediated re-activation ofMAPKsignaling contributes to insensitivity ofBRAFmutant colorectal cancers toRAF inhibitionwith vemurafenib. Cancer Discov. 2, 227–235 (2012)
vii Bernards, R. et al. Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. Nature doi:10.1038/nature13121