Should the tumor necrosis factor (TNF) pathway be activated or silenced in the treatment of triple-negative breast cancer?

Sonja Kesselmans.

S2242362.

Bachelorscriptie.

20-06-2014

Supervisor: Marcel van Vugt.

Abstract.

Breast cancers are categorized based on the expression of estrogen, progesterone and her2 receptors. This is clinically important, because these receptors can be therapeutically targeted. If breast cancers do not express any of these receptors, they are called triple-negative breast cancer(TNBC). TNBC accounts for 15-20% of all the breast cancers in the whole world. Because TNBC do not show any receptors there is no specific treatment yet. It is therefore important to find pathways that promote growth and survival of these TNBC cells which may serve as a novel therapeutic.

Tumor necrosis factor(TNF) is a signaling pathway which can result in two different downstream effects. TNF can lead to apoptosis in cells or it could lead to proliferation of the tumor. A relevant question therefore is whether TNF could be used as treatment for TNBC.

There are different studies done which identify TNF as a therapeutically target. Studies show that when TNF-alpha is down-regulated the cells state shifts from the survival to apoptotic state. Also the NF-kB pathway looks to be a good target. Already some anti-TNF-alpha drugs are tested but though it is safe there are no clear effects on the tumor cells. Other studies suggest to activate the TRAIL pathway which also leads to apoptosis. These results indicate that TNF-alpha and NF-kB are good therapeutically targets in the TNBC. Still a lot of investigation is needed to confirm previous results and to get a better understanding of the role of TNF in TNBC and the effects of blocking TNF-alpha and/or the NF-kB pathway and activating the TRAIL pathway on TNBC.

Table of contents.

Introduction.

Although its 5 year survival rate is >90% in the western world, breast cancer is the second most common cause of cancer-death among women world-wide. (World Health Organization 2014). The reason for the poor prognosis is that some breast cancer subtypes can get resistant to the therapies. One of these subtypes is the so-called triple negative breast cancer (TNBC). (Hoeferlin LA 2013)

Tumor cells express a lot of receptors. For breast cancer, only three different receptors are used for determining the breast cancer subtype. These receptors are the progesterone (PR), estrogen (ER) and Her2/neu protein markers. Every breast cancer can be classified as positive or negative for these different receptors. This is clinically relevant, because these receptors can be therapeutically targeted. TNBC is the subtype which is negative for all the three receptors. Because of this reason it is difficult to treat TNBC, while TNBC are not eligible for treatments with anti-HER2 or endocrine therapies. Also these triple negative breast cancers show a pattern of disease which is more aggressive in comparison with other subtypes of breast cancer. Rate of metastatic spread, pattern of spreading and risk-factor profile differs in triple negative breast cancer in comparison with the non-triple negative breast cancer. (Alluri P 2014)

A known signaling pathway in cancer cells is tumor necrosis factor signaling(TNF). TNF is an inflammatory cytokine and belongs to the tumor necrosis superfamily. TNF is involved in many processes inside the cell. These processes include modulation of immune response, proliferation, apoptosis and many other processes. (Balkwill F 2009)TNF can act on a autocrine or paracrine manner. Most of the time TNF acts on the TNFR1, TNFR1 is also the main receptor which is expressed by different human tissues. TNF can act also on TNFR2 which is mostly expressed by immune cells. It is found that TNF plays a role in a variety of human diseases. (Baud V 2001) What is conspicuous is that on first sight it was found that TNF had the ability to induce necrosis in cancer cells. But when this role of apoptosis was researched further they found another role of the TNF signaling. This role was a tumor promoting role. (Balkwill F 2009)

The question now is whether in TNBC the TNF receptor should be activated or silenced in context of therapy. Because nowadays no targeted treatment exists for TNBC patients it is worthwhile checking whether the TNF pathway might serve as a therapeutic target. If the TNF receptor is activated the tumor cell could go in apoptosis, or the activation of the TNF receptor could lead to proliferation and resistance of the tumor. The aim of this review therefore is to look if the TNF pathway should be activated or silenced in TNBC.

I. Breast cancer.

One of the eight women gets breast cancer. Only in the Netherlands, there are 150.000 people which have or have had breast cancer. Although the survival rates are relatively good (80%) there are still 9 people dying every day of breast cancer only in the Netherlands. Breast cancer is the number one cause of death in women between the age of 35 and 55. 23% of all breast cancer patients is younger than 50 years. Although the survival rates have increased, about 1% per year, breast cancer is still a major problem over the whole world. (Integraal kankercentrum Nederland 2013)

In the whole world one million women discover every year that they have breast cancer. Breast cancer can start in the tubes or milk-producing glands. These cells got genetic changes which allow them to proliferate and differentiate unverified. This unverified cell proliferation and differentiation leads to the formation of a breast tumor. These tumor cells can also start to migrate around the body and therefore there can originate metastases. The breast tumor can be detected by examination of the breast or by a mammography. Breast cancer is most of the time treated by debulking surgery. If there are any metastases found the surgery is often followed by chemotherapy or radiotherapy. The therapies don't go without many side effects which can make the patient very ill. (Blows FM 2010)

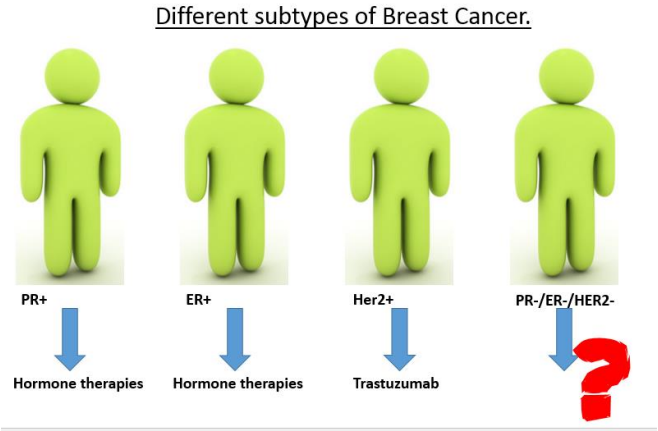

Figure 1. Different Subtypes of Breast cancer and their treatment.

Breast cancer can be classified by pathological and/or clinical features, and is known as a heterogeneous disease. The patient can benefit from the classification due to better targeting of treatment and a better predicted prognosis. Nowadays the human epidermal growth factor receptor-2(HER2), the estrogen receptor(ER) and the progesterone receptor(PR) are used as predictive markers. (Blows FM 2010)

There are several types of breast cancer which originate from the different types of cells in the glands and milk ducts. One subtype is the luminal tumors, which grow slowly. These luminal tumors exist in the duct line cells and glands. Another subtype is the basal-like tumor. These basal-like tumors grow fast and exist in the deeper layers of the glands and milk-ducts. One of the techniques which is used to distinguish between these two different subtypes is the immunohistochemical technique. Breast cancer cells are stained using immunohistochemical techniques with the use of antibodies which recognizes specific proteins. It is important to know the subtype of breast cancer for the treatment specifications. (Blows FM 2010)

Gained genomic abnormalities and other gained genetic variations contribute to the progression and initiation of breast cancer. The copy number aberrations gained from somatic mutations are a dominant feature for sporadic breast cancers. Still the events, which occur during tumorgenesis are hard to explain. These events are especially hard to explain due to all the side effects, which occur randomly as other germ line copy number variations and non-pathogenic co-alterations. (Curtis C 2012)

i. Triple negative breast cancer.

Triple-negative breast cancer(TNBC) is a subtype of breast cancer, which lacks the expression of the progesterone(PR), estrogen receptor(ER), and the protein marker HER2/neu. This type of cancer looks very similar to the basal-like subtype. Mutations of the BRCA gene can be inherited, this mutation can contribute to the initiation of TNBC. The features of TNBC are very important because they can help to develop a good treatment, which is needed. The problem with TNBC is namely that TNBC cannot be treated with anti-HER2 or endocrine therapies, which are treatments which respond well in other types of breast cancer. (Alluri P 2014)

There are two ways to determine the type of breast cancer. The first one is based on the expression of the PR,ER and HER2 receptors. The second one is based on the expression profile of genes. The second one is subdivided into five subtypes. Basal-like breast cancer cells are one of these five subgroups of breast cancer. This Basal-like breast cancer subtype has no overexpression of HER2 and a low expression of ER. Also they express genes which are usually found in myoeptihelial or basal cells in the healthy breast. A lot of cancers fit both the definitions of the triple-negative and the basal-like breast cancer. Around 80% of the TNBC are also basal-like breast cancer, and the other way around. (Weigelt B 2009) Still these two types of breast cancer are two different kinds of cancer and are not synonymous. The differences between TNBC and basal-like subtype are defined by microarrays, clinical and immunohistochemical data. There are different molecular subtypes of breast cancers shown in TNBC. (Foulkes WD 2010) . 60-82% of the basal-like cancers showed on an immunohistochemical analysis the phenotype of TNBC. (Bertucci F 2008) Overexpression of HER2 or expression of the ER was also found in another 20% of the basal-like breast cancer. Looking at the genetic level the basal-like and TNBC are different. (Foulkes WD 2010) Another important gene is the BRCA1 gene. Over 75% of all the women who have a mutation in this BRCA1 gene gets breast cancer with the phenotype of basal-like, triple-negative or both types of breast cancers. (Reis-Filho JS 2008)

Of all the breast cancers in the world 15-20% is related to TNBC. TNBC has a high rate of remote metastases. (Hoeferlin LA 2013) Therefore TNBC is also one of the most invasive breast cancers. Women with TNBC have a reduction of 60% in their 5-year survival rate. Because of this large reduction in the 5-year survival, it is very important to know the mechanisms behind the process of metastasis. These metastases are the main problem because the metastases block the chance of complete destruction of the TNBC. Another problem with metastasized cancer is that most of the time the cancer cannot be removed by surgery.

What is conspicuous is that basal-like and TNBC show cell-surface markers which have the same sections as cell-surface markers of the breast-cancer stem cells. These cell-surface markers are the CD44+CD24- and ALDH1A1. (Morrison BJ 2008) The cells which express those sections are surrounded by cells which have tumorigenic potentials. But still the breast cancer cells with these similar sections do not have to be cancer stem cells. Cancer stem cells can arise from differentiated cells which gained the quality of self-renewal. So they do not have to originate from tissue stem cells. (Visvader JE 2008)

Triple-negative and basal-like breast cancers are likely to be larger than other breast cancers. Most of the time these cancers are high graded due its invasive character. A declining relationship is found in both types of cancers. Namely the declining relationship of the probability of survival and the size of the primary tumor. The mammographic detection is difficult because the disease growths rapidly and mainly occurs in young women. (Foulkes WD 2010) In addition these two types of cancer are more likely to

spread to other organs. Most of the time metastasis are found in brain and lungs, but less metastasis are found in the bones. (Dent R 2009)

Nowadays there is still no good treatment for TNBC patients. This is due to the lack of clear therapeutically targets. A better treatment is badly needed because these patients with TNBC have no good prospects. Possible new target could consist of pathways which are de-regulated and have become essential for the tumor. When such pathways are targeted, the tumor should theoretically decrease. One of the pathways which is very interesting in this context is the tumor necrosis factor (TNF) pathway.

II. Tumor Necrosis Factor.

Tumor Necrosis Factor (TNF) is a gene, which belongs to the tumor necrosis superfamily. The superfamily of TNF consists of 19 ligands and 29 receptors in the human body. TNF-alpha and TNF-beta were discovered as the first members of this superfamily. (Aggarwal BB 2003) TNF is made by myeloid, malignant and other cells in the tumor microenvironment. (Balkwill F 2009) TNF codes for a multifunctional proinflammatory cytokine. TNF is involved in many different processes like endothelial function, modulation of immune response, apoptosis, differentiation, proliferation, induction of inflammation and lipid metabolism. TNF acts most of the time through TNFR1, this is the main receptor for TNF-alpha and is expressed by different human tissues. (Aggarwal BB 2003) TNF can act in a paracrine and autocrine manner. (Balkwill F 2009) TNF can act also through TNFR2, which binds both TNF-alpha and –beta. This receptor is expressed most of the time in immune cells. These two receptors are specific for TNF. TNF is found to play a role in various diseases. Especially in cancer TNF was originally described to play an anti-cancer role. However, in previous studies it was found that TNF and other members of the family can send both death and survival signals to the cells. (Aggarwal BB 2003)

i. <u>Signaling pathways induced by the different TNF Family members.</u>

- *TNF-alpha*

TNF-alpha which is seen as the classic TNF is produced in response to environmental stress, infection and inflammation. TNF-alpha is mainly produced by fibroblasts, lymphocytes and macrophages. TNF-alpha induces, dependent on cell type, different biological responses. TNF-alpha can bind on the cell surface to two different receptors. These receptors are the TNFR1, which binds through p55, and TNFR2 through p75. Minimal three clear-cut effectors are activated due to recruitment of different signal transducers. The effectors lead to activation of two transcription factors, AP-1 and NF-kB, and caspases are also activated. TRADD binds to TNFR1 and recruits FADD,RAIDD, MADD and RIP. (Baud V 2001) TRADD, FADD and MADD contain all a death domain. (HSU H 1996) When TRADD and FADD bind to the TNF-Receptor 1 this complex leads to mobilization and activation of Caspase-8. Caspase-8 induces apoptosis by acting through a proteolytic cascade which also contains Caspases-3, -6 and -7. Caspase-8 also functions in another pathway. Caspase-8 through binding BID acts on the mitochondria. Caspase-8 and BID cause the membrane of the mitochondria to rupture. When the membrane ruptures cytochrome C can escape. Cytochrome C binds then APAF1 which is an apoptotic protease activator. Cytochrome C and APAF1 recruit different caspases which leads also to apoptosis. (Baud V 2001)

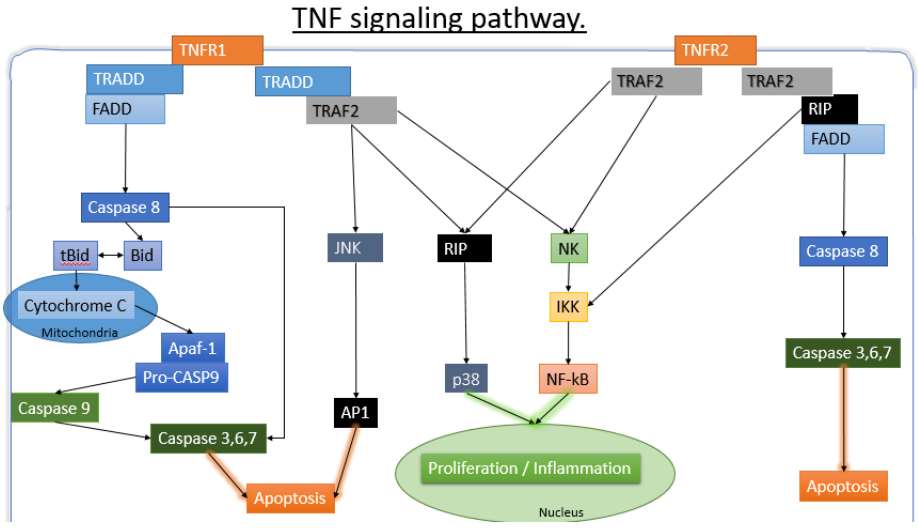

Figure 2 The different pathways of the Tumor Necrosis Factor.

- *TNF-beta*

TNFR2 is the receptor for both TNF-alpha and TNF-betaβ. But for TNF-beta the only receptor to activate. (Balkwill F 2009)TNF-beta is also known as lymphotoxin and is produced by activated lymphocytes. For both healthy and tumor cells TNF-beta can be toxic. (Dorland 2007) Besides activation, TNF-beta can also lead to increase adhesion molecules and elevated expression of MHC. TNFR2 activates a cascade which leads to the activation of NF-kB. Which acts as a transcription factor and on his turn activates cell proliferation and gene expression. (Balkwill F 2009)

- *NF-kB*

NF-kB is a transcription factor from a gene family which contains five members. NF-kB signaling is often hyperactivated in triple-negative basal-like breast cancer cells. The problem is that the origination of the hyperactivation is unclear. NF-kB regulates genes which are included in various hallmarks of cancer. These hallmarks of cancer include survival, invasion, inflammation and proliferation. The subunits of the NF-kB family form hetero- and homo-dimers. One signaling pathway of NF-kB develops via the activation of IkB kinase(IKK) complex. IKK phosphorylates IkB, through this phosphorylation IkB gets ubiquitylated and after this IkB is degraded. When IkB is degraded two other subunits of the NF-kB family , RelA and p50, form a dimer and can enter the nucleus. Inside the nucleus RelA-p50 can regulate the transcription of genes. Above described pathway is the usual pathway NF-kB acts through. The other pathway of NF-kB leads to activation of IKKalpha which phosphorylated p100. P100 is the inhibitor of RelB. If p100 is phosphorylated it does not work anymore so RelB can form RelB-p50 and RelB-p52. RelB-p50 and p52 leads to translocations in the nucleus and therefore DNA can be bind. (Piao H 2014)

TNF Receptor-Associated Factor-2 activates two different pathways. These two pathways lead among other things via the NF-kB inducing kinase to the activation of NF-kB factor. NF-kB is activated by coordinators of the adaptive and innate immune responses. Also NF-kB plays an important role in cancer progression and development. NF-kB is a factor which can cause resistance for apoptosis-based tumor-surveillance mechanisms in malignant and neoplastic cells. Also it is believed that NF-kB can regulate invasiveness and angiogenesis of the tumor. (Michael K 2006) NF-kB has the function of a negative regulatory protein in this whole pathway.

- *TRAIL*

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising factor for cancer therapy. TRAIL is able to distinguish normal and cancer cells in vitro by the expression of different surface markers on cancer cells. TRAIL can kill selectively only cancer cells. TRAIL can bind to two receptors: death receptor 4 and 5 also known as TRAIL-R1 or TRAIL-R2. These receptors are expressed on the cell surface and binding to these receptors leads to attraction of pro-caspase-8 and FADD. Pro-caspase-8, FADD and TRAIL form together the death-inducing signaling complex also known as DISC. DISC activates together with the active form of pro-caspase-8 caspase-3 and 7 which leads to apoptosis. Another pathway which can be activated by caspase-8 is the intrinsic pathway of apoptosis. This is activated by the cleavage of caspase-8 to the protein BID this results in activating caspase-9 which on his turn activates this intrinsic pathway. (Garimella SV 2014) The problem with TRAIL is that in the clinic TRAIL does not work in patients while most of the time the cancer cells get resistant for TRAIL. (Refaat A 2014) Other studies suggest that TRAIL could be a promising agent in combination with other treatments.

ii. Cancer treatment with TNF.

TNF is used as cancer treatment in different research projects. The effect of TNF on tumors is researched in cancer mouse models and the outcomes were variable. One of the research groups showed that low doses of TNF-alpha leaded to an increase of permeability of the tumor vessels. Increase in permeability of the tumor vessels leaded to a solid distribution of liposomes in the tumor. The solid distribution of liposomes helped to get the highest tumor response. Due to a high number of tumor vascular destruction. (Seynhaeve AL 2007) Also there was a lot of research on TNF as a toxic protein. The last results were that TNF was only toxic in combination with metabolic inhibitors. These metabolic inhibitors blocked the downstream of TNF signaling. Therefore TNF-alpha in combination with metabolic inhibitors allowed the cell to proceed to apoptosis. TNF had just a weak toxic effect without the metabolic inhibitors in malignant cells. (Balkwill F 2009) Other studies tested several ligands of the TNF family in already in different stages of clinical research. In these studies several anti-cancer antibodies were found which could be used for cancer therapy. The problem was that systemically given doses had a high toxicity effect in the patient. The challenge now is to find the best dose of TNF-alpha which can be given locally. (Daniel D 2008) During the clinical trials it was found that TNF was also present in the microenvironment in many different kinds of cancers. Because TNF was found to be present in the microenvironment there were doubts if TNF is declining or promoting cancer growth. (Balkwill F 2009)

iii. Is TNF a tumor-promoting agent?

The risk of cancer is increased by mediators and cells of the inflammation response. TNF is found to be a regulator of cancer-related inflammation. This typical inflammation causes among other things stimulation of metastasis, angiogenesis, proliferation and differentiation of malignant cells. The mechanisms of pro-tumor TNF is not all cleared out yet. TNF is produced by malignant cells. (Balkwill F 2009) One of the ways to explain why TNF is produced by malignant cells is that the regulation of this cancer cells is transformed in comparison with healthy cells. The regulation of TNF-alpha is transformed, this was demonstrated in ovarian cancer cell line where TNF-alpha was shown to be regulated differently. Through the differences in regulation there were other cytokines produced which could forward the tumorgenesis. (Szlosarek PW 2006) What should be taken into account are the mechanisms, which are underlying tumor developing conditions, partly are chronic inflammation and carcinogen exposure. The link between tumorgenesis and inflammation is indicated by NF-kB. NF-kB is the essential promoter associated with the inflammation type of cancer. (Pikarsky E 2004) TNF is not only produced in malignant cells but also in myeloid cells. Both these cell types increase production of inflammation-associated tumors. (Balkwill F 2009)

Whether TNF is made by myeloid or malignant cells, both types of produced TNF can cause DNA damage or alter oncogene activation. Not only in malignant cells TNF can cause DNA damage but also in healthy cells. Which means that TNF could also enhance new events of tumorgenesis. Most mutations found in cancer due to TNF were mutations in the MYC or TP53 gene. Also TNF could contribute to the proliferation of tumors by activating the NF-kB factor. Another characteristic of TNF is that TNF increases the risk of metastasis. The increase in risk for metastasis is related to a change from state of the cells into an epithelial-mesenchymal transition state. The epithelial-mesenchymal transition state makes it possible for the cancer cells to disconnect from their environment and travel to another place to create a new niche, which can grow out into a new tumor. (Balkwill F 2009)

In this chapter some of the signaling pathways of TNF superfamily are discussed. It is clear from this previous information that the TNF-alpha pathway should not be activated. Activating the TNF-alpha leads mainly to tumor proliferation. The question now about TNF is, can TNF be used as a treatment or as a target?

III. Triple-negative breast cancer and TNF as a target of therapy.

i. TNF-alpha leads to up-regulation of matrix metalloproteases which promotes tumor invasion.

Malignant tumors exist out of neoplastic and non-neoplastic cells. Non-neoplastic cells contain lymphocytes, endothelial cells, macrophages and fibroblasts which are surrounded by an extracellular matrix (ECM). A crucial interaction for each step of tumorgenesis is the interaction between microenvironment of the tumor and neoplastic cells. Stromal fibroblasts were found to have an important role in differentiation, proliferation and invasion of cancer cells. High concentrations of tumor-associated macrophages (TAM) were found in invasive breast cancer. Normally, macrophages stimulate the antitumor activity, but it was found that the infiltrate from macrophages had a positive correlation with angiogenesis and a negative correlation with prognosis. TNF-alpha is seen as one of the tumor-derived molecules which promotes tumor growth and survival through TAM activities. Before invasion and metastasis can start the biological barriers from the cells need to be broken down. Therefore the important factors are the matrix metalloproteases (MMP). These MMPs are important in metastasis, growth factor release from the ECM and the angiogenesis of the tumor. In invasive breast cancer the subtypes MMP-2 and -9 were found to be correlated with the poor prognosis and decreased survival. Activation and production of MMPs depends on TNF-alpha and other various cytokines. (Hagemann T 2004)

MMP can be up-regulated by TNF-alpha, when MMP is up-regulated it leads to an increase in invasiveness of breast cancer cells. MMPs are not only essential for invasion but also for metastasis and tumor growth. One thing which is still unknown is the interaction between tumor cells and macrophages. This interaction stimulates the production of TNF-alpha and therefore also MMPs, these two factors stimulate the tumor invasion. (Hagemann T 2004) The interaction between tumor cells and macrophages still has to be unraveled because it seems like an important interaction. It is clear that TNF-alpha up-regulates the working of MMPs, which on his turn up-regulates the tumor invasion probabilities. When the interaction between macrophages and tumor cells and the influence on TNF-alpha and MMPs is discovered, and when the interaction also plays an effective role in in vivo models, the MMPs or TNF-alpha could both be good targets. When TNF-alpha or MMPs would be down-regulated the effects of MMPs should also be down-regulated. Which would mean invasiveness, angiogenesis and tumor proliferation in the tumor would decrease. So TNF-alpha is not a good target. But what happens when TNF-alpha is down-regulated?

ii. Down-regulating TNF-alpha promotes tumor cell apoptosis.

TNF-alpha has a role in the evolution from inflammation towards breast cancer. TNF-alpha contains a signaling pathway which leads to the survival of tumor cells. In triple-negative breast cancer(TNBC) TNF-alpha also is linked to a poor prognosis.

TNF-alpha has two opposite roles. One role is that TNF-alpha has a pathway which leads to apoptosis. The other role is that TNF-alpha leads to proliferation and differentiation of the tumor. TNF-alpha is also associated with the proliferation factor NF-kB. Due to NF-kB TNF-alpha production is kept steady. Therefore the continue support of cell growth is going on. This ongoing cell growth also refers to the TNF-alpha which is secreted by the tumor cells. NF-kB moves as a heterodimer to the nucleus where NF-kB acts as a transcription factor and leads to proliferation of the cells. Another characteristic of TNF-

alpha is that TNF-alpha promotes the epithelial-mesenchymal transition which leads to migration and metastases. (Pileczki V 2013)

Neutralizing the TNF activity in cancer patients was done with the use of anti-TNF. These antagonists of TNF showed inhibition of chemokine and cytokine production. Thereby they also inhibited angiogenesis, ECM degradation and attraction of inflammation cells. All these inhibitions are also very useful in cancer patients. TNF-alpha could be a specific target for TNBC patients as TNBC is also one type of cancer which develops in combination with inflammation. There are two similar pathways in tumor promotion and in patients with inflammatory disease. This two pathways could be from high importance, these are the reduction in IL-17 producing T helper cells and the function of T-regulatory cells. (Balkwill F 2009)

When the TNF-alpha gene was silenced in the TNBC cell line there was an inhibition of cell proliferation. Also a decrease in motility and cell growth was observed. When the TNF-alpha was blocked the tumor cells did go into apoptosis. Another thing what happened was that cell migration was inhibited. The NF-kB pathway was also blocked which had influence on the survival of the tumor cell. (Pileczki V 2013)When NF-kB pathway was blocked the JNK pathway was activated, which leaded to state transition of the tumor cells from the survival to the apoptosis state. (Sprowl JA 2012)

The results given prove that TNF-alpha could be an efficient target when it comes to TNBC. Still there is a controversial if TNF-alpha blockage leads to resistance or better response in therapies. The down-regulation of TNF-alpha in TNBC could be a good design for new treatment therapies to slow the cancer down or in best case reduce the cancer. Reducing the growth of cancer cells could be done by blocking the TNF-alpha, this is shown in the results above. Blocking TNF-alpha leads to a shift in cancer cells from proliferation to apoptosis state. (Pileczki V 2013) It looks like TNF-alpha down-regulating is a promising therapy for the feature.

iii. <u>Etanercept as an anti-TNF-alpha agent.</u>

Etanercept is a drug which is already used as an anti-TNF-alpha therapy in rheumatoid arthritis. Etanercept consist of a recombinant dimer of human soluble p75 TNF receptor. Etanercept is a competitive antagonist cause Etanercept blocks the binding of TNF-alpha to the TNF receptor. Through the blocking of TNF-alpha Etanercept neutralizes the biological effects of TNF-alpha. Which could lead to a decrease in tumor promoting factors in breast cancers. In this research it is found that Etanercept was a safe drug which showed to have a biological activity in breast cancer. The problem was that there were almost no clear effects on the tumor proliferation. Most of the patients had a progression of disease. So although Etanercept is a safe drug there has to be investigated in further investigations if higher doses of Etanercept has more effect on the tumor. (Madhusudan S 2004)

iv. NF-kB as target in advanced breast cancer.

NF-kB is also seen as a target in advanced breast cancer because NF-kB plays an important role in the proliferation of tumor cells. NF-kB was shown to be a marker for prediction of resistance to neoadjuvant chemotherapy. So for novel treatment NF-kB inhibitors in combination with chemotherapy should be given. This combination of treatment should be given because the combination of NF-kB inhibitors and chemotherapy could prevent resistance in patients with advanced breast cancer. (Montagut C 2006)

In previous studies it was found that when NF-kB was down-regulated, α-catenin was up-regulated. When α-catenin was up-regulated colony formation and proliferation was suppressed in E-cadherin-negative basal-like breast cancer cells. E-cadherin is an important factor in adherens junctions. Adherens junctions are cell-cell junctions which are intercellular junctions. These junctions are in large amounts present in healthy cells and less present between cancer cells. These junctions are composed of E-cadherin which interacts with β-catenin. β-catenin binds to α-catenin which brings together the actin cytoskeleton with the adherens junctions, therefore α-catenin induces intercellular adhesion. (Piao H 2014) 7% of the gene CDH1 is mutated in human breast cancers. CDH1 encodes for E-cadherin. In breast cancer it was found that loss of E-cadherin promoted metastasis and invasion of cancer cells. (Cancer Genome Atlas Network 2012)

The function of α-catenin may have a tumor suppressor function. It was found that NF-kB signaling was inhibited by α-catenin. Another function of α-catenin may be that α-catenin interacts with IkBα, therefore the ubiquitylation of IkBα is inhibited. If IkBα is hold IkBα cannot interact with proteasome and therefore IkBα is said to be stabilized. Also the function of RelA-p50 is blocked by α-catenin. Therefore RelA-p50 cannot move to the nucleus and leads to no transcription of RelB, so RelB is down-regulated. (Piao H 2014) Taken these results together, it is clear that α-catenin has an inhibiting effect on members of the NF-kB family. Also α-catenin is found to be down-regulated in basal-like breast tumors and NF-kB is found to be up-regulated in these tumors. So overall α-catenin looks to be a good tumor suppressor agent in basal-like breast cancer cells. And because 80% of all basal-like breast cancers also have the triple-negative subtype of breast cancer, α-catenin might also be a good therapeutic agent for treating TNBC.

v. Tumor necrosis factor-related apoptosis-inducing ligand.

TRAIL was the promising factor for cancer therapy. In in vitro studies it showed very promising results. (Garimella SV 2014) The problem with TRAIL is when it was given to patients it did not stop progression of disease. (Refaat A 2014) Nowadays new therapies with TRAIL in combination with other therapies are tested.

In different studies it was found that the majority of cell lines from different types of cancer was resistance to TRAIL. When it comes to breast cancer cell lines it was found that 10 of the 14 TNBC cell lines were sensitive for the apoptosis inducing working of TRAIL. Cell lines which included the ER-positive lines all showed resistance to TRAIL. From the HER2-amplified cell lines two out of eight cell lines responded to TRAIL. (Garimella SV 2014) TRAIL looks to be a promising therapy for patients who suffer from TNBC due to the response of TRAIL in TNBC cell lines.

TRAIL in combination with a mutation or deletion of p53 can bring tumor cells to apoptosis. In TNBC cell lines who are sensitive to TRAIL, p53 is most of the time deficient. Therefore the suggestion is that TRAIL is a better therapeutic agent. (Rahman M 2009) Another observation was that TNBC cell lines were more

sensitive when they had mesenchymal features. (Rahman M 2009) In most of the cell lines one of the two TRAIL receptors was expressed. What was conspicuous was that in the TNBC Cell lines which are sensitive for TRAIL both the receptors were expressed. In breast cancer cell lines which were exposed to TRAIL it was found that the apoptosis was mainly mediated by TRAIL-R2. (Kelley RF 2005)

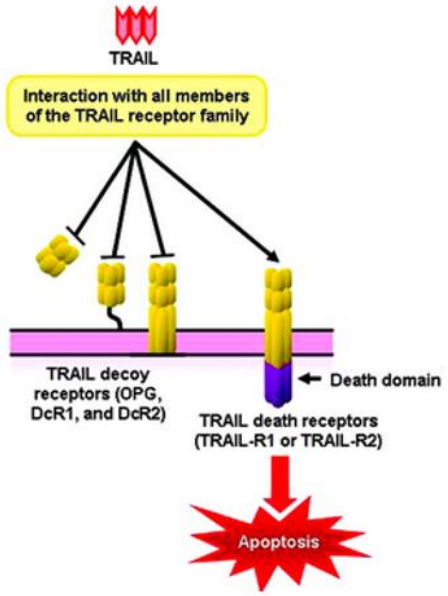

Figure 3 Binding of TRAIL to the TRAIL death receptors. (American Society of Clinical Oncology 2014)

The mechanism of how cells regulate the sensitivity to TRAIL still needs to be partly unraveled. Anti-apoptotic factors like BCL-2 and FLIP were found in high levels of expression when it comes to resistance to TRAIL. Sensitivity to TRAIL was partially correlated by the position of receptors their state of activation and their expression level. (Garimella SV 2014)

Inhibition of the NF-kB which normally protect cells from apoptosis, by increasing anti-apoptotic proteins, leaded to more sensitivity to TRAIL. (Keane MM 2000) Also the inhibition of members of the BCL-2 family leaded to an increased sensitivity to TRAIL. When TRAIL and ABT-737 were used as combined treatment there was more cell death induced in the cancer cells. Because the increasing cell death the BCL-2 family looks to be an interesting goal for further investigation. Another inhibition leaded to an increase in sensitivity to TRAIL. (Garimella SV 2014)This inhibition was the inhibition of SRC with PP2. SRC normally regulates the PI3K/AKT/mTOR pathway which is an cell-survival pathway. (Kim LC 2009) The inhibition of SRC had a large effect in the TNBC cancer cell lines. Which could lead to a new treatment for patients with TNBC. The new treatment should consist of a combination of TRAIL and SRC inhibitors. (Garimella SV 2014)

Till now there are a lot of additional genes which could regulate the working of TRAIL. Two key nodes which may be good points of action would be the BCL-XL and the SRC. Because these two appear to have promising effects on the sensitivity to TRAIL. Also TRAIL induced apoptosis is a promising therapy for people with TNBC cells. Because these cancer cells show overall sensitivity to TRAIL. Maybe in combination with those inhibitors for SRC and BCL-XL the cancer cells could be made more sensitive so the treatment with TRAIL would have larger effects. But the effects of TRAIL and characteristics have to be investigated in feature studies. Studies which were done in patients showed that TRAIL had no effect in the patient. (Refaat A 2014) Still treatments of TRAIL in combination with other therapies could be promising for the future.

Discussion.

In this review some potential targets within the TNF pathway are discussed as treatment start point for patients with triple-negative breast cancer(TNBC). Down regulating the TNF-alpha looks to have promising results in the TNBC cell line. Also NF-kB looks to be a good target for arresting the proliferation of the tumor. The last potential therapeutic target which is discussed is the TRAIL. TRAIL shows in cell lines potential results.

This review was specified to TNBC because patients who suffer from TNBC have no specific treatment yet. Because TNBC cover about 15-20% of all breast cancers it is very important that a good therapy will be developed.

When looking at the tumor necrosis factor alpha (TNF-alpha) it showed promising results by down regulating this pathway. The activity of TNF was neutralized with the use of anti-TNF. This had large effects on the tumor proliferation. The tumor suffered from a decrease in mortality and cell growth and also the proliferation was inhibited. Also the NF-kB pathway was blocked and this leaded to the activation of the JNK pathway. The JNK pathway results in the shift of tumor cells from survival to apoptosis state. Although this results look promising for the future the experiments where done only in one cell line. This cell line was the Hs578T TNBC cell line. But because the experiments are done only in one cell line the results are not that viable. Before acceptance of these results can take place there need to be done more research to prove these results are right. These experiments have to be done in other TNBC cell lines and also in in vivo models before we can really say something about these results. But still the results look promising for the future and look to be an efficient therapeutically target.

TNF-alpha in combination with the MMPs showed another pathway which is still not clear but could be useful as a therapeutically target. Still this research is only done with two breast cancer cell lines which means that it are not very viable results. To confirm these results more research should be done in more cell lines. Also this founded interaction should be verified by the use of in vivo models to look or this interaction also plays a role in the human body or just on the cell lines. If MMPs and TNF-alpha plays a role in the human body than there could be investigation further if the MMPs or TNF-alpha should be silenced down to protect the invasion of tumor cells.

Etanercept appears to be a good agent to silence down the TNF-alpha. It is found that Etanercept is a safe drug which is easily given to the body. The only problem was that with the doses given in this study there were no clear effects on cancer. The disease did go into progression although in rheumatoid arthritis good effects where found when anti-TNF-alpha was given. Because of this low effect of Etanercept on breast cancer there should be investigation about higher doses of Etanercept. Maybe higher doses give more response in tumor tissue. Also a combination of Etanercept with other anti-TNF-alpha agents or combination with other therapies like chemotherapy should be investigated.

The NF-kB also appears to be a good target for patients with advanced breast cancer. To make non-responder patients responders or to make responders more sensitive for chemotherapy, chemotherapy should be given in combination with NF-kB inhibitors. These results are based on real tumor material from the patient. This is a positive point because cell lines always differs some from the real situation in a tumor. The marginal comment on this study is that it is only done by using 51 tumor samples. Which not enough to go with this data to the clinic. Before this treatment is tested in the clinic there should be more research in a lot more patient samples. This research needs to be done to confirm these results. If

in a larger group these tissues derived from patients also gets more sensitive when NF-kB drug is given it should be taken to the clinic. To make this therapy save there should be also a lot of toxicology tests to find the best concentration of drug and the less side effects for patients.

Another drug which is found to inhibit the working of NF-kB and its members of the family is α-catenin. α-catenin shows to work as a tumor suppressor in E-cadherin negative basal-like cancers. Also α-catenin is down regulated in these basal-like tumors when NF-kB is up regulated. This fact makes α-catenin a good target for therapy. Because if α-catenin is up regulated NF-kB is down regulated. When NF-kB is down regulated proliferation of the tumor will decrease and the tumor will be activated to go to the apoptosis. This research is done in basal-like breast cancer subtype but also with the meaning to find a treatment for both basal-like and TNBC. Because around 80% of basal-like breast cancer also have the triple-negative phenotype it could be promising results for both types of cancer. But this target should be investigated also first in TNBC cell lines. When this further experiment show similar results it could be taken to study the NF-kB blocking by α-catenin in in vivo models.

TRAIL should work better in TNBC cell lines because these cell lines are more sensitive than other breast cancer cell lines. This makes TRAIL also a promising agent. TRAIL activates a pathway to apoptosis. TRAIL is investigated already a lot and also in clinical studies. The problem with TRAIL is that in the cell lines it looks to have very promising results but in the clinic TRAIL hardly ever responds in patients. So TRAIL does not look as the promising therapy which researches thought it would be.

The question which was addressed to this review was or the TNF pathway should be activated or silenced in triple-negative breast cancer. With this review several answers came to this question. TRAIL activates the TNF pathway and leads to apoptosis. Only in previous clinical studies it is found that TRAIL does not work that well in patients. Maybe in combination with other therapies TRAIL could be still a promising agent. TNF-alpha and NF-kB should be silenced according to the information given. These pathways have influence on the proliferation and survival of the tumor. By silencing these pathways the tumor should go into apoptosis or at least stop proliferating. So it looks that the TNF pathway should be silenced instead of activated. But further experiments and investigation are needed to confirm the results found and in vivo animal studies can lead to better understanding of the role of TNF in triple-negative breast cancer.

Bibliography.

Aggarwal BB. «Signalling pathways of the TNF superfamily: a double-edged sword.» *Nature reviews Immunology*, 2003: 745-756.

Alluri P, et al. «Basal-Like and Triple-Negative Breast Cancers: Searching for Positives Among Many Negatives.» *Surgical Oncology Clinics of North America*, 2014: 567-577.

American Society of Clinical Oncology. *Journal of Clinical Oncology.* 2014. http://jco.ascopubs.org/content/23/36/9394/F3.expansion (accès le June 16, 2014).

Balkwill F. «Tumour necrosis factor and cancer.» *Nature reviews cancer*, 2009: 361-371.

Baud V, et al. «Signal transduction by tumor necrosis factor and its relatives.» *Trends in Cell biology*, 2001: 372-377.

Bertucci F, et al. «How basal are triple-negative breast cancers? .» *International Journal of Cancer*, 2008: 236–240.

Blows FM, et al. «Subtyping of Breast Cancer by Immunohistochemistry to Investigate a Relationship between Subtype and Short and Long Term Survival: A Collaborative Analysis of Data for 10,159 Cases from 12 Studies.» *PLoS Medicine*, 2010: doi: 10.1371/journal.pmed.1000279.

Cancer Genome Atlas Network. «Comprehensive molecular portraits of human breast tumours.» *Nature*, 2012: 61-70.

Curtis C, et al. «The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups.» *Nature*, 2012: 346–352.

Daniel D, et al. «Tumor necrosis factor: renaissance as a cancer therapeutic?» *Current Cancer Drug Targets.*, 2008: 124-131.

Dent R, et al. «Pattern of metastatic spread in triple-negative breast cancer.» *Breast Cancer Research Treatment*, 2009: 423-428.

Dorland. *Dorland's Medical Dictionary for Health Consumers.* Saunders, 2007.

Foulkes WD, et al. «Triple-Negative Breast Cancer.» *The New England journal of Medicine*, 2010: 1938-1948.

Garimella SV, et al. «Identification of novel molecular regulators of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in breast cancer cells by RNAi screening.» *Breast Cancer Research*, 2014: Issue 2, 21pages.

Hagemann T, et al. «Enhanced invasiveness of breast cancer cell lines upon co-cultivation with macrophages is due to TNF-alpha dependent up-regulation of matrix metalloproteases.» *Carcinogenesis*, 2004: 1543-1549.

Hoeferlin LA, et al. «Challenges in the Treatment of Triple Negative and HER2-Overexpressing Breast Cancer.» *Journal of surgery and science*, 2013: 3-7.

HSU H, et al. «TRADD–TRAF2 and TRADD–FADD Interactions Define Two Distinct TNF Receptor 1 Signal Transduction Pathways.» *Cell*, 1996: 299–308.

Integraal kankercentrum Nederland. *Incidentie borstkanker.* 2013. http://www.cijfersoverkanker.nl/selecties/Incidentie_borstkanker/img534e819a612e4 (accès le June 18, 2014).

Keane MM, et al. «Inhibition of NF-kappaB activity enhances TRAIL mediated apoptosis in breast cancer cell lines.» *Breast cancer research and treatment*, 2000: 211-9.

Kelley RF, et al. «Receptor-selective mutants of apoptosis-inducing ligand 2/tumor necrosis factor-related apoptosis-inducing ligand reveal a greater contribution of death receptor (DR) 5 than DR4 to apoptosis signaling.» *The journal of biological chemistry*, 2005: 2205-12.

Kim LC, et al. «Src kinases as therapeutic targets for cancer.» *Nature Review Clinical Oncology*, 2009: 587-95.

Madhusudan S, et al. «A Phase II Study of Etanercept (Enbrel), a Tumor Necrosis Factor α Inhibitor in Patients with Metastatic Breast Cancer.» *Clinical cancer research*, 2004: 10.1158/1078-0432.CCR-04-0730.

Michael K. «Nuclear factor-kappaB in cancer development and progression.» *Nature*, 2006: 431-436 (25 May 2006) |.

Montagut C, et al. «Activation of nuclear factor-kappa B is linked to resistance to neoadjuvant chemotherapy in breast cancer patients.» *Endocrine-related cancer*, 2006: 607-17.

Morrison BJ, et al. «Breast cancer stem cells: implications for therapy of breast cancer.» *Breast Cancer Research*, 2008: 210-210.

Piao H, et al. «α-catenin acts as a tumour suppressor in E-cadherin-negative basal-like breast cancer by inhibiting NF-κB signalling.» *Nature cell biology*, 2014: 245–254.

Pikarsky E, et al. «NF-κB functions as a tumour promoter in inflammation-associated cancer.» *Nature*, 2004: 461-466.

Pileczki V, et al. «TNF-α Gene Knockout in Triple Negative Breast Cancer Cell Line Induces Apoptosis.» *International Journal of Molecular Science*, 2013: 411-420.

Rahman M, et al. «The TRAIL to targeted therapy of breast cancer.» *Advances in cancer research*, 2009: 43-73.

Rahman M, et al. «TRAIL induces apoptosis in triple-negative breast cancer cells with a mesenchymal phenotype.» *Breast Cancer Research Treatment.* , 2009: 217-30.

Refaat A, et al. «TRAIL combinations: The new 'trail' for cancer therapy .» *Oncology Letters*, 2014: 1327-1332.

Reis-Filho JS, et al. «Triple negative tumours: a critical review.» *Histopathology*, 2008: 108-118.

Seynhaeve AL, et al. «Tumor necrosis factor alpha mediates homogeneous distribution of liposomes in murine melanoma that contributes to a better tumor response.» *Cancer Research*, 2007: 9455-62.

Sprowl JA, et al. «Alterations in tumor necrosis factor signaling pathways are associated with cytotoxicity and resistance to taxanes: a study in isogenic resistant tumor cells.» *Breast cancer research*, 2012: 14(1):R2.

Szlosarek PW, et al. «Expression and regulation of tumor necrosis factor alpha in normal and malignant ovarian epithelium.» *Molecular Cancer Therapie.* , 2006 : 382-90.

Visvader JE, et al. «Cancer stem cells in solid tumours: accumulating evidence and unresolved questions.» *Nature review cancer*, 2008: 755-768.

Weigelt B, et al. «The molecular underpinning of lobular histological growth pattern: a genome-wide transcriptomic analysis of invasive lobular carcinomas and grade- and molecular subtype-matched invasive ductal carcinomas of no special type.» *The journal of Pathology*, 2009: 45–57.

World Health Organization. *Breast cancer: prevention and control.* 2014. http://www.who.int/cancer/detection/breastcancer/en/index1.html (accès le June 6, 2014).