

## MICROBES, ONCOGENES AND CANCER

### Introduction

The subject of the presentation of *Prof. Dr. med. Andreas Neubauer* (Philipps-Universität Marburg and University Hospital Gießen and Marburg, Department of Haematology, Oncology and Immunology, Baldingerstraße 1, 35043 Marburg, Germany) was “microbes, oncogenes and cancer”.

Cancer is a readout of chronic inflammation. Normally, cancer diagnosis is an easy task: the pathologists will tell if it is cancer tissue or if is inflammatory tissue, but sometimes it is not that easy. When it comes to the hallmarks of cancer, one does not think of inflammation but of proliferation, of the loss of the capacity to differentiate. There is a bidirectional relationship between cancer and chronic inflammation. Chronic inflammation can lead to cancer by damaging cell

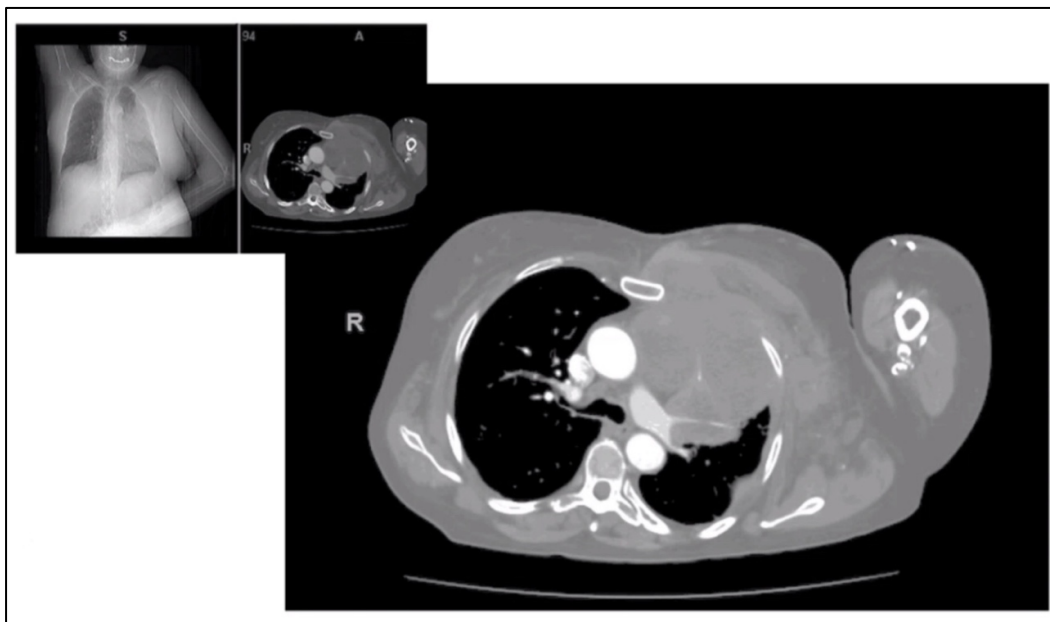
DNA and promoting tumour growth, and cancer itself can trigger or perpetu-

ate chronic inflammation, creating a cycle that fuels the disease's development, progression, and spread. This complex interaction occurs at almost every stage of cancer, involving processes like immune suppression, tissue remodelling, DNA damage, and increased cell proliferation. That is the normal view of how a cell transforms to a cancer cell, which is due to mutagenic changes in the genome. The classical hallmarks of cancer are proliferation, oncogenic changes, and the absence of differentiation.

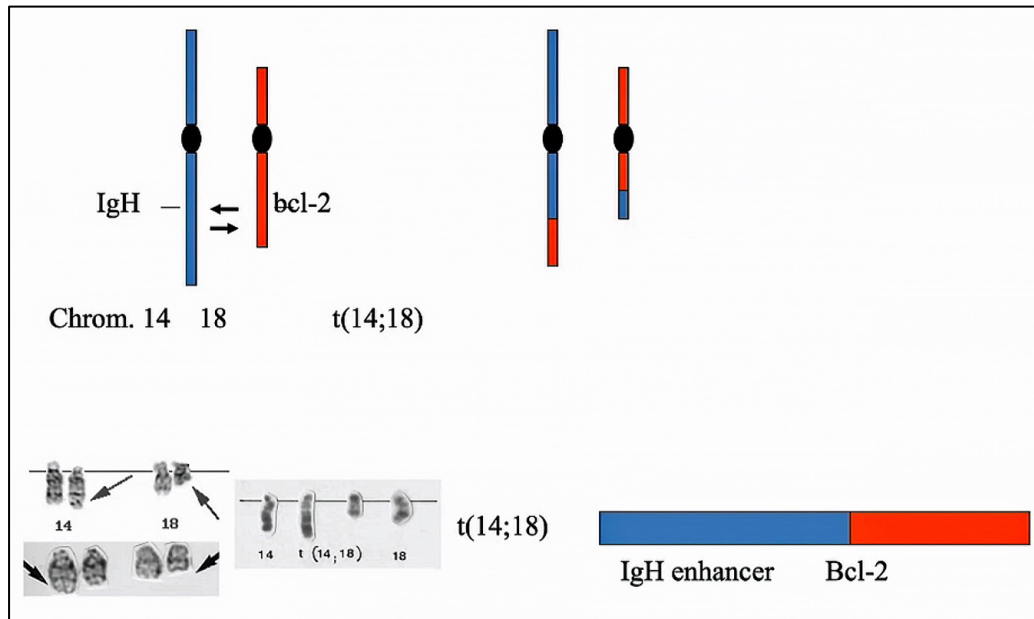
### The 14-18 translocation

Dr. Neubauer presented a 50-year-old female patient who came to the emergency room because of chest pain.

The CT-scan in Figure 1 shows a huge tumour which is infiltrating the ribs and the chest wall and the tumour is also infiltrating the heart. The patient was a non-smoker. The diagnosis of the



**Figure 1:** CT-scan of a 59-years old female with chest pain showing an massive tumour infiltrating the chest wall and probably the heart. Later, histology showed that this was a follicular B-cell lymphoma.



**Figure 2:** 14;18 translocation leading to promotor / enhancer exchange.  
(Composition figure from A.N. and Atlas of cytogenetics [lower left panel]).

pathologist was “indolent” follicular B-cell lymphoma (according to the American classification). “*dolere*” in Latin means “to suffer pain”, so indolent means that there is no pain to suffer. Lymphomas do not typically cause pain itself, but symptoms like pain can arise when swollen lymph nodes press on nearby nerves and tissues and that is what is happening in this patient. Histology showed an increase of follicles in the biopsy with quite regular appearance, but finally the diagnosis of a follicular lymphoma was made.

What was also found in this patient with the follicular B-cell lymphoma was a 14;18 translocation. The 14;18 translocation (or t(14;18)) is a specific genetic abnormality that involves the joining of the IgH promotor and enhancer on chromosome 14 to the Bcl-2 gene on chromosome 18 (which is the most important anti-apoptotic protein which prevents Caspase-9 to be recruited to the nucleus and start apoptosis), leading to the over-expression of the Bcl-2 gene (Figure 2).

This translocation is considered the hallmark genetic event in follicular lymphoma and is found in about 80-90% of cases. It can also be present in healthy individuals at low levels and is associated with an increased risk of developing follicular lymphoma, especially with increasing age. However, when performing a very sensitive PCR on normal tonsils from regular people, this translocation can be found without the presence of cancer.

### Philadelphia chromosome

Prof. Neubauer presented another patient. She was a 19-year-old patient with leukaemia. She had a blood count of 500,000 leukocytes and died. The post-mortem histology showed a bone marrow full with white cells and a left-shift, which indicates the presence of immature white blood cells (like band neutrophils, myelocytes, and meta-myelocytes) in the peripheral blood, which is characteristic of chronic myeloid leukaemia (CML). In CML, this signifies abnormal myeloid cell

production, where the bone marrow releases these immature cells prematurely due to the clonal proliferation of myeloid cells caused by the Philadelphia chromosome.

The Philadelphia chromosome is a genetic abnormality found in the bone marrow cells of almost all CML patients. This abnormality occurs when a part of chromosome 9 breaks off and fuses with chromosome 22, creating a new gene (BCR::ABL1) that causes immature white blood cells to grow uncontrollably. The term highlights the strong diagnostic link between CML and the Philadelphia chromosome, named for the city where it was discovered in 1960. But here again, if you do a sensitive PCR in normal bone marrow, you find this translocation also in a certain percentage of healthy people.

These are 2 cases where an oncogene is involved in the development of cancer, but these oncogenes can also be found in healthy persons without any signs of disease. Apparently, just carrying an oncogene is not enough to develop cancer, so the question is: What drives cancer? In addition, in both cases, the cells under the microscopy did not look transformed at all, the cells appeared rather regular, or inflamed. These cases were presented to open up a discussion as to whether there may be a border after which one could call a tissue a cancer-tissue, but that there may be a gradual, stepwise progression to cancer where one sometimes cannot define the correct border.

Oncologists focus on oncogenes and on some tumours, but forget all the other things. Persons suffering from primary or secondary immunodeficiency have a more probability of developing cancer in their lifetime. Another important (and long-time neglected) influence on the development of cancers is “infection”.

In 2020 the WHO calculated 19,2 million new cases of cancer and 9.9

million mortalities from cancer. Increasingly, apart from lifestyle, diet, and genetic predisposition, infections caused by pathogenic microorganisms and parasites have also been linked to cancer (14% of all cancers worldwide).

### **The Axl gene**

In the 1990-ies, prof Neubauer worked in the laboratories of Dr. E.T. Liu (Lineberger Comprehensive Cancer Center, University of North Carolina in Chapel Hill, USA). There, together with Drs. John O'Brien and Patty Coxwell, he worked on leukaemia with the key question: what actually turns the chronic phase to the acute phase, to the plastic phase, where all the patients at that time died from?

After almost 2 years of hard work, using a sensitive transfection-tumorigenicity assay, they isolated a novel transforming gene from the DNA of two patients with chronic myelogenous leukaemia (*O'Bryan et al., 1991*). This gene, that they named Axl, is a tyrosine kinase receptor that promotes cancer development by increasing proliferation, survival, invasion, and migration in cancer cells. Axl also contributes to the development of resistance to chemo-, radio-, immune- and targeted therapy in many cancer types. They showed that Axl is expressed in cells of the myeloid lineage in both normal and malignant states, and that in normal haematopoietic cells Axl is expressed predominantly in myeloid precursors and in stromal cells (*O'Bryan et al., 1995*). What was also found is that Axl can transform CML into a more aggressive blast phase (or blast crisis), which is a type of acute leukaemia (*Neubauer et al., 1994*).

Axl is a gene also causing inflammation and recently it was shown in mice by Takehiko Shibata and colleagues that Axl/GAS6 prevents immunity against *Streptococcus pneumoniae* and that blocking the Axl/GAS6 fully restored

the antibacterial immunity (Shibata et al., 2020). Axl/GAS6 is preventing immunity because it polarizes macrophages.

The AXL gene encodes for a receptor tyrosine kinase (RTK) that, when activated by its ligand Gas6, promotes cell survival, proliferation, and invasion, playing a significant role in cancer progression and metastasis. In the context of *Helicobacter pylori* (*H. pylori*) infection, studies have shown that elevated levels of Gas6 and Axl are associated with increased gastric cancer survival and invasiveness, with Axl contributing to the malignant phenotype. Therefore, Axl represents a potential therapeutic target for *H. pylori*-associated gastric cancers.

With regard to the relation between inflammation and cancer, Neubauer and colleagues were the first to prove that curing the *H. pylori* infection in humans can lead to complete regression of gastric MALT-lymphomas (Bayerdörffer et al., 1994; Bayerdörffer et al., 1995; Neubauer et al., 1997; Thiede et al., 1997; Thiede et al., 2001; Wündisch et al., 2003; Wündisch et al., 2005). They later performed the largest phase II trial that firmly established this antibiotic therapy as first line therapy (Wündisch et al., 2012). They observed, using B-cell clonality PCR, that there were patients where lymphoma could not be detected histologically, but that PCR showed monoclonal disease. So they called this a monoclonal gastritis.

Vice versa, in a follow-up study they found that 14 of 52 analysed lymphoma patients reaching complete histologic remission showed ongoing B-cell monoclonality which was associated with a higher risk of relapse (Wündisch et al., 2005).

Consequently, the WHO called infection with *H. pylori* in 1994 the “carcinogen” for gastric cancer (IARC Monograph on the Evaluation of

*Carcinogenic Risks to Humans Volume 61*, 1994).

### The Plcg2 gene

Prof. Neubauer and colleagues used BALB/c mice with a gain-of-function mutation in the Plcg2 gene (Ali5) to analyse its role in the development of gastric MALT lymphoma.

Heterozygous BALB/c Plcg2Ali5/+ and wildtype (WT) mice were infected with *Helicobacter felis* (*H. felis*) and observed up to 16 months for development of gastric MALT lymphomas. Plcg2Ali5/+ mice developed MALT lymphomas less frequently than their WT littermates after long-term infection of 16 months (Gossmann et al., 2016). Infected Plcg2Ali5/+ mice showed downregulation of proinflammatory cytokines and decreased *H. felis*-specific IgG1 and IgG2a antibody responses. Plcg2Ali5/+ mice harboured higher numbers of CD73 expressing regulatory T cells (Tregs), possibly responsible for impaired immune response towards *Helicobacter* infection. Plcg2Ali5/+ mice may be protected from developing gastric MALT lymphomas as a result of elevated Treg numbers, reduced response to *H. felis* and decrease of proinflammatory cytokines.

### TET-1 and TET-2 genes

TET-1 and TET-2 are genes that code for Ten-eleven translocation (TET) proteins, which are enzymes that catalyse DNA demethylation by converting 5-methylcytosine to 5-hydroxymethylcytosine, playing a crucial role in epigenetic regulation. Different mutations in TET-1 and TET-2 can lead to distinct phenotypes, such as TET-2 loss promoting increased stem cell self-renewal and myeloid transformation, and TET-1 loss being associated with B-cell lymphoma.

While frequent in haematological malignancies like leukaemia, their

functions are also emerging in solid tumours. Combined TET-1 and TET-2 loss can promote B-cell malignancies (Zhao et al., 2015).

### **t(10;11) translocation**

A t(10;11) translocation is a rare chromosomal abnormality where parts of chromosomes 10 and 11 are exchanged, most commonly seen in paediatric and young adult acute myeloid leukaemia (AML). This translocation creates an MLL-TET-1 gene fusion, which can occur in various cancers like T-cell lymphoblastic lymphoma and AML.

The specific breakpoints are often written as t(10;11)(p12;q23), indicating the locations on the short (p) arm of chromosome 10 and the long (q) arm of chromosome 11.

It is associated with distinct clinical features and can lead to complications like diffuse intravascular coagulation and tumour lysis syndrome. Patients with t(10;11) are often classified into high-risk groups for treatment protocols and have an unfavourable prognosis.

The t(10;11) translocation, specifically t(10;11)(p12;q23), occurs in about 8-9% of AML cases and is particularly relevant in childhood AML.

### **IDH mutation**

An IDH mutation is a genetic change in the isocitrate dehydrogenase (IDH) gene that leads to the production of an abnormal substance called 2-hydroxyglutarate (2-HG). This process can promote cancer by disrupting the function of other enzymes involved in DNA and histone methylation, which affects cell growth and development (Prensner and Chinnaiyan, 2011). These mutations are common in brain tumours, such as gliomas, and also in other cancers, including AML.

There is no leukaemia with a t(10;11) translocation as well as an IDH mutation. These mutations run the same epi-

genetic pathway. The IDH mutations, IDH-1 or IDH-2, can either be in mitochondria or in the cell cytoplasm. Both mutations actually create the wrong metabolism. The 2-hydroxy glutamate (2-HG) inhibits TET-1 and TET-2, so it wouldn't make any sense for a cancer cell to have a mutation in TET as well as a strong epigenetic mutation.

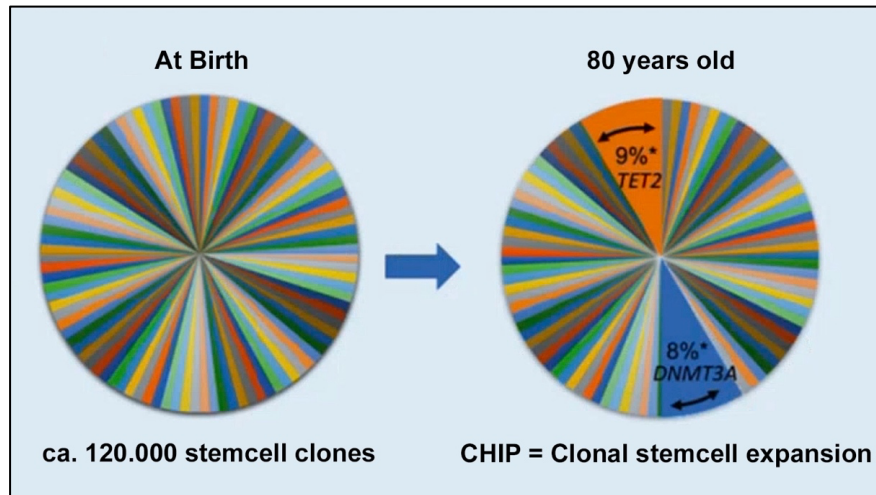
When over 60 persons are being sequenced, 3-5% clonal haematopoietic cells carrying mutations are being found. They don't play a big role, except maybe that these persons have a higher frequency of myocardial infarctions and strokes.

Prof. Dr. Andreas Burchert (University Hospital Giessen and Marburg) published an article about pulmonary inflammation, which is now called inflammageing, because this inflammation is increasing by age (Burchert, 2022).

In a normal situation there are millions of different progenitor cells. Mice can be transplanted with one single bone marrow stem cell. They are then reconstituted and can live a normal life. But we have millions of blood-forming stem cells of which a small number can acquire mutations and expand in number. "CHIP" refers to Clonal Haematopoiesis of Indeterminate Potential, a condition where a small number of blood-forming stem cells acquire mutations, get a growth advantage and expand in number, increasing the risk of developing blood cancers or cardiovascular disease (Figure 3).

### **TET-2 deficiency, bacterial translocation and preleukaemic proliferation**

Meisel and colleagues (Meisel et al., 2018) used TET-2 deficient mice. TET-2 deficiency (TET<sup>-/-</sup>) leads to severe myeloproliferation, extramedullary haematopoiesis and splenomegaly that



**Figure 3:** Clonal haematopoiesis and epigenetics are closely linked with age-related epigenetic changes. (Figure from *Burchert, 2022*).

mimic preleukaemic myeloproliferative disorders. Figure 4 shows the difference in self-renewal of haemopoietic stem cells (HSC) between  $TET2^{+/+}$  mice and  $TET2^{-/-}$  mice. However, preleukaemic myeloproliferation (PMP) occurs in only a fraction of  $TET2^{-/-}$  mice. This suggests that extrinsic non-cell autonomous factors are required for disease onset. Meisel and colleagues showed that bacterial translocation from the small intestines is critical for the development of PMP in  $TET2^{-/-}$  mice (*Meisel et al., 2018*). This translocation is the result of dysfunction of the small intestine barrier. In symptom-free  $TET2^{-/-}$  mice, PMP can be induced by disrupting the intestinal barrier integrity, or in response to systemic bacterial stimuli such as the toll-like receptor 2 antagonist. PMP was reversed by antibiotic treatment and did not develop in germfree  $TET2^{-/-}$  mice, illustrating the importance of microbial signals in the development of PMP.

#### **Antibiotics, microbiota diversity and allogeneic stem cell transplantation**

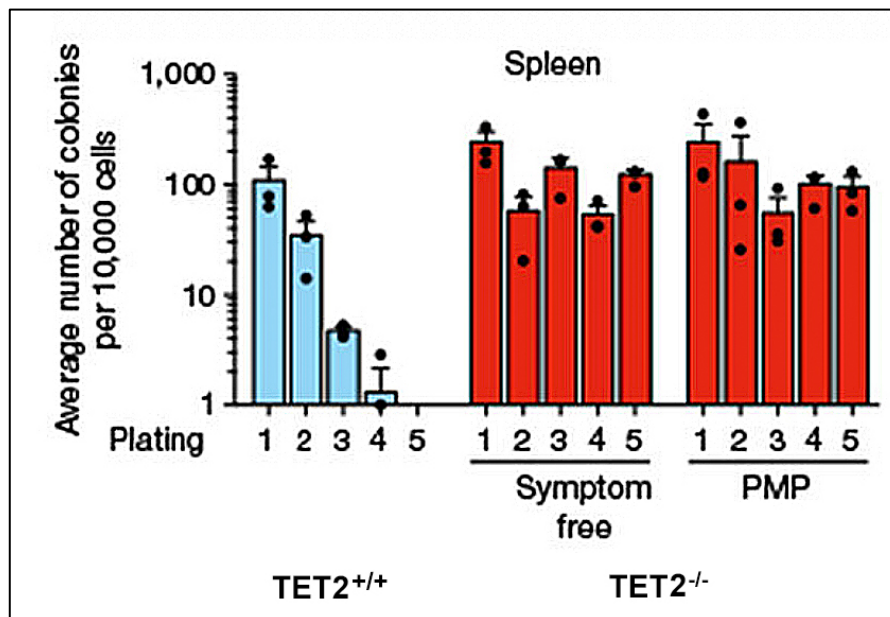
Six or seven years ago the clinic in Marburg stopped giving antibiotics as infection prevention during allogeneic stem

cell transplantation, based on studies from four centres in the USA that reported a reduction in microbiota diversity after allogeneic hematopoietic stem cell transplantation for leukaemia and found that lower microbiota diversity was associated with higher mortality after transplantation. Higher diversity of intestinal microbiota at the time of neutrophil engraftment was associated with lower mortality (*Peled et al., 2020*).

When haematopoietic stem cell transplantations were started in Marburg in 1985 it was common use to eradicate the colonization microbiota in order to prevent infections in those immunocompromised patients. At present, based on the publication of Peled and colleagues, the Marburg transplantation team tries to prevent antibiotic treatment as much as possible in order to preserve the microbiome of the patient.

#### **Therapeutic role in cancer of bacteria and bacterial products**

The concept that bacteria or their products play a therapeutic role in cancer is not new; in 1891, Coley used the toxins from *Streptococcus erysipelas* and



**Figure 4:** *In vitro* HSC self-renewal colony forming assay of haematopoietic progenitors in mice. Mean  $\pm$  s.e.m. (Figure adapted from Meisel et al., 2018).

*Serratia marascens* to treat inoperable sarcoma (Hoption Cann et al., 2003).

### Cancer prevention by vaccination

Another important issue is vaccination. A Study among 1,672,983 girls and women who were 10 to 30 years of age from 2006 through 2017 were included in a study to he efficacy and effectiveness of the quadrivalent human papillomavirus (HPV) vaccine in preventing high-grade cervical lesions. The conclusion was that among Swedish girls and women 10 to 30 years old, quadrivalent HPV vaccination was associated with a

substantially reduced risk of invasive cervical cancer at the population level (Lei et al., 2020).

### Summary

Cancer is not a box that opens up and then you have cancer, yet cancer develops over years, sometimes decades. One important reason may be infections, such as *H. pylori*. That curing the infection can clear malignant lymphomas remains a wonderful example of the gradual and still “reactive” process of malignant transformation.

*This paper was reviewed Prof. Dr. med. Andreas Neubauer before publishing.*

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