

# Leucocyte-Tumor Cell Hybridization Can Initiate Cancer Metastasis

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## Abstract

In 1911, German gynecologist Prof. Otto Aichel proposed that metastasis occurs following leucocyte-tumor cell fusion and hybrid formation. In this, Aichel not only provided an explanation for metastasis but he also foresaw the science of cancer epigenetics. His idea that a new hybrid cell would form with characteristics of both “mother cells” in today’s terminology would refer to gene expression patterns from both fusion partners in the same hybrid cell. At least some hybrids would express the leukocytic traits of motility, chemotaxis, and homing along with the de-regulated cell division of the cancer cell. This concept has now been confirmed in numerous animal models and, from our group, in three patients with melanoma and two with renal cell carcinoma. Several other labs have now contributed to this rapidly growing field. Here, we review the findings to date as well as provide a contextualization of the works and life of Prof. Aichel.

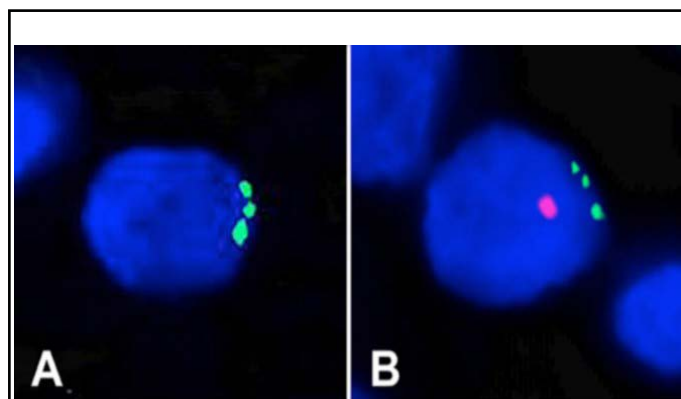
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## Introduction

According to estimates from the International Agency for Research on Cancer, by the year 2030 there will be 22 million new cancer cases and 13 million deaths per year. The main reason for death from cancer is not the initial tumor but it’s metastasis to distant parts of the body, yet this process has remained poorly understood for quite some time. Leukocyte–cancer cell fusion and hybrid formation as an initiator of metastasis was proposed more than a century ago by the German pathologist, Prof. Otto Aichel. This proposal has since been confirmed in many studies in animals and more recently, from our

group in two patients with renal cell carcinoma and three patients with malignant melanoma. Many other labs are now making similar reports. Leukocyte–tumor cell fusion provides a unifying explanation for metastasis. While primary tumors arise in a wide variety of tissues representing not a single disease but many different diseases, metastatic cancer may be only one disease arising from a common, non-mutational [1] event: fusion of primary tumor cells with leukocytes. From the findings to date, it would appear that such hybrid formation is a major pathway for metastasis. Studies on the mechanisms involved could uncover new targets for therapeutic intervention.

Several years ago, our group became attracted to a proposal from 1911 by a German physician Prof. Otto Aichel that metastasis might result from the fusion between leukocytes such as macrophages and cancer cells, with the qualitative differences between chromosomes causing the hybrid to be “thrown out of the path of the mother cells to form what has come to be known as a malignant cell and resulting in an entirely new cell, having the characteristics of both mother cells” [2]. Here Aichel not only provided an explanation for metastasis but he also predicted the science of cancer epigenetics. That is, a new hybrid cell with characteristics of both “mother cells” in today’s terminology would refer to gene expression patterns from both fusion partners in the same cell. At least some hybrids would express the leukocyte traits of motility, chemotaxis, and homing while at the same time have the uncontrolled cell division of the cancer cell. Motivated by Aichel, our group has been studying cancer patients who had previously received an allogeneic bone marrow transplant (BMT), usually for leukemia or lymphoma, and then later developed a solid tumor. By analyzing tumor cells for both donor and patient DNA, we reasoned that such cells were likely to be leukocyte–tumor cell hybrids.



**Figure 1:** FISH analyses of formalin-fixed sections of a primary renal cell carcinoma. The slides were counter-stained with DAPI. Panel A shows a cell with three copies of chromosome 17 (green) but no Y chromosome, indicating that this cell was likely not a hybrid, while Panel B shows a trisomy 17 (green) plus the Y chromosome (red), indicating that the cell was a hybrid between a patient and a male donor cell. [3]. (Used with the permission of the journal Bone Marrow Transplantation).

In our first case, leukocyte–cancer cell fusion and hybrid formation was detected in a renal cell carcinoma (RCC) through the use of fluorescence in

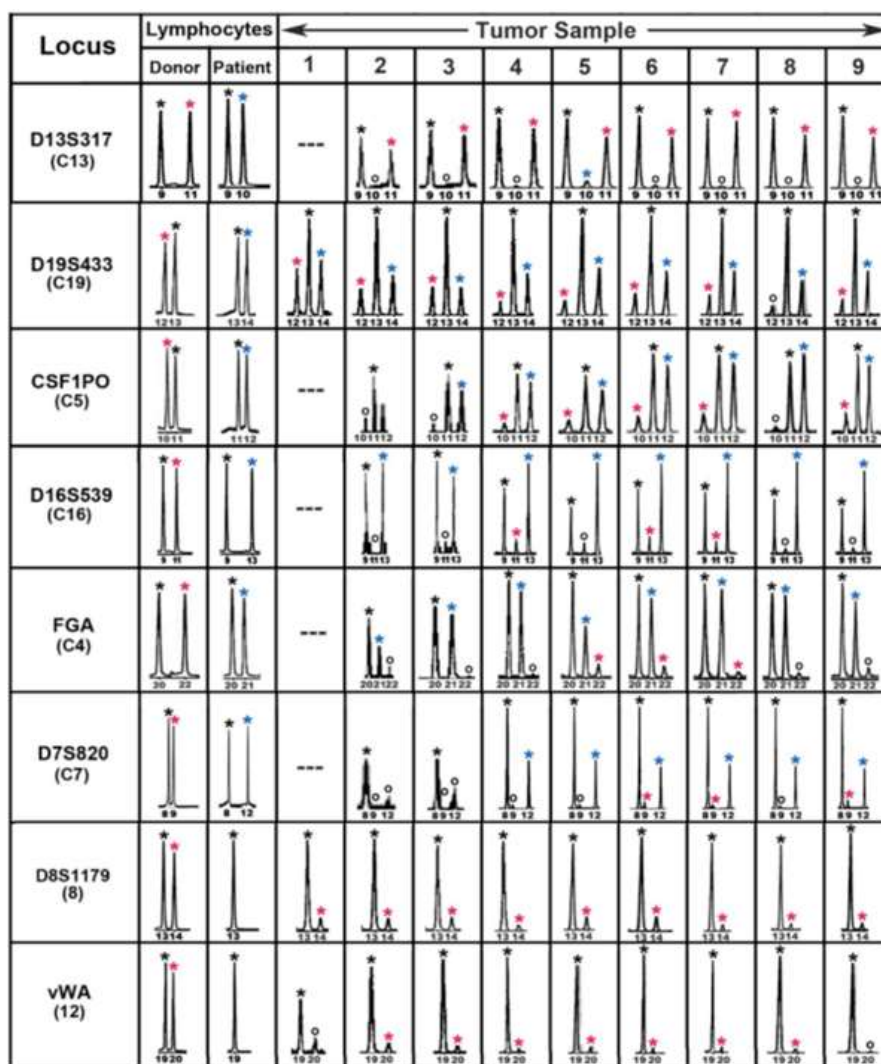
situ hybridization (FISH) [3]. The tumor was from a female patient who two years prior had received a BMT from her son. Due to the male donor–female recipient nature of the BMT, FISH could be used to search for BMT–tumor hybrids [3]. Karyotyping revealed that the tumor cells contained a clonal trisomy 17. Using dual-label FISH, the donor Y and three or more copies of chromosome 17 were visualized together in individual nuclei of carcinoma cells, providing direct genetic and morphological evidence for BMT–tumor hybrids (Figure 1). For example, Panel A shows a cell with three copies of chromosome 17 (green) but no Y chromosome, indicating that this cell was likely not a hybrid, while Panel B shows a trisomy 17 (green) plus the Y chromosome (red), indicating that the cell was a hybrid between a patient and a male donor cell. Such cells were in abundance in an area covering about 10% of the tumor, suggesting a clonal origin of the hybrids.

The first evidence for leukocyte–cancer cell hybrids in a human using DNA genotyping came from our study of a patient who had received an allogeneic BMT for lymphoma and later developed a melanoma brain metastasis with a donor–patient hybrid genome [4]. Tumor cells were isolated by laser micro-dissection and sections were analyzed throughout the tumor, using forensic short tandem repeat (STR) length polymorphisms to distinguish donor and patient genomes. Tumor and pre-transplant blood lymphocyte DNAs were analyzed for donor and patient alleles at 14 autosomal STR loci and the sex chromosomes. Eight of these loci were informative and indicated the presence of donor–patient hybrids. Figure 2 shows these loci with peaks from the electropherograms designated by asterisks with the following colors: black (donor and patient), red (donor only), and blue (patient only). Both donor and patient alleles were present in tumor cells throughout the tumor and the tumor appeared to consist largely if not solely of bone-marrow-derived cell (BMDC)–tumor cell hybrids. Moreover, similar allelic ratios for each locus were seen throughout the tumor, indicating a clonal origin of the metastasis and suggesting that the tumor was generated from a prior fusion event between a single donor BMDC and patient tumor cell. We therefore conclude that the tumor-initiating cell was a BMDC–tumor cell hybrid.

The second forensic evidence for leukocyte–cancer cell hybrids came from a man who, eight years following an allogeneic BMT from his brother for treatment of chronic myelogenous leukemia (CML), developed a nodular malignant melanoma on the upper back with spread to an axillary sentinel lymph node [5]. Combining laser micro-dissection with detection of STR length polymorphisms, we were able to distinguish

donor and patient genomes. Tumor and pre-transplant blood lymphocyte DNAs were analyzed for donor and patient alleles at 15 autosomal STR loci and the sex chromosomes. DNA analyses of the primary melanoma and the nodal metastasis revealed that they exhibited alleles at each STR locus that were consistent with both the patient and donor. The doses varied between these samples, indicative of the relative amounts of genomic

DNA derived from the patient and donor. Table 1 shows genotyping results using short tandem repeats (STRs) at each of the loci of DNA from donor (D), patient (P), primary tumor, and lymph node metastasis. As with the prior cases, the evidence supports fusion and hybridization between donor and patient cells as the initiator of metastasis in this patient.



**Figure 2:** Forensic STR analyses of the melanoma described above along with donor and patient pre-BMT lymphocytes. Shown are loci exhibiting donor- and patient-specific alleles in pre-BMT lymphocytes. Tumor loci are listed in order of relative abundance of the donor-specific alleles (red asterisk) compared to patient-specific (blue asterisk) and shared alleles (black asterisk). Allele peaks, 50 relative fluorescence units were censored as “no call” (open circles). Loci with no detectable alleles after PCR amplification (—) [4]. (Used with the permission of the journal Plos One).

STR Locus	Primary Tumor	Lymph Node	Patient Sample	Donor Sample
D8S117	13,15	13,15	13,15	13
D21S11	28,29,30,30.2	28,29,30,30.2	28,29	30,30.2
D7S820	11,12	11,12,14	12,14	11
CSF1PO	9,11	10,11,12	11,12	9,10
D3S1358	15,16,18	16,18	16,18	15,16
TH01	6,7,9	6,7,9	6	7,9
DS13S317	8,9,12	8,12	12	8,9
D16S539	13	11,13	11,13	13
D2S1338	16,17,18	17,19	17,19	16,18
D19S433	13,15,16	13,15,16	15,16	13,16
vWA	17,18,19	17,18	17,18	18,19
TPOX	8,9,11	8,9	8,9	8,11
D18S51	12	12,20	12,20	15,18
Amelogenin	X,Y	X,Y	X,Y	X,Y
D5S818	9,11,12	9,11,12	11	9,12
FGA	21,22,24	21,24	21,24	22,25

**Table 1:** STR genotyping of DNA from donor (D), patient (P), primary tumor, and lymph node metastasis. STR units: number of tandem repeats of the locus-specific tetranucleotide sequence [5] (Used with the permission of the journal Plos One).

Several laboratories have now extended these findings [6]. For example, Berndt et al. showed that fusion between leukocytes and normal cells can induce aneuploidy and drug resistance. After fusion and hybridization between a leukocyte or fibroblast and a cancer cell, the cell contained two nuclei, one from each fusion partner. When nuclei fused into one, there was a random loss of chromosomes such that individual clones of hybrid cells had different genotypes. Aneuploidy increased with subsequent cell divisions. Also, mutations occurred wherein tumor cells acquired drug and radiation resistance such that when such therapies are applied to the patient, the resistant cells survived and formed more deadly tumors. Today, cancer cell resistance to various therapies remains a central problem in patient survival [7]. Using the Cre-loxP-based method, Searles et al. found that in cell culture, cancer cells can rapidly deliver DNA to macrophages and fibroblasts, producing hybrids. The cells were aneuploid with clonal diversity and showed increased chemo-resistance compared to

non-hybrid cancer cells. Using reporter mice, they also observed the formation of hybrids between B16-GFP-Cre melanoma cells and normal cells *in vivo* [8]. Mohr et al. studied conditions through which plasma membranes between two different cells fuse into a single cell. They found that the pro-inflammatory cytokine TNF- $\alpha$  under hypoxia was a potent inducer of cell fusion in human MDA-MB-435 and MDA-MB-231 breast cancer cells [9]. Sottile et al. studied cell-to-cell interaction between mouse mesenchymal stem cells (MSCs) and embryonic stem cells (ESCs) and found that MSCs can either fuse spontaneously or be invaded by ESCs through entosis. They also found that the ROCK-actin/myosin pathway is required for both fusion and entosis in ESCs but only for entosis in MSCs. They found that MSCs can undergo both fusion and/or entosis [10]. Gast et al. developed methods to detect hybrids in peripheral blood of cancer patients that correlated with disease stage and predicted overall survival, suggesting that hybrids might be used as biomarkers to assess disease progression [9].

## Macrophage Traits in Metastatic Cancer Cells

In addition to genomic evidence for leukocyte–cancer fusion and cancer progression, there are a large number of macrophage-like traits expressed by metastatic cancer cells. Kemény et al. showed that melanoma cells spontaneously fusing with macrophages and fibroblasts *in vitro* can adopt the phenotypes of these cells [11]. Broncy and Paterlini-Bréchet reviewed evidence that circulating cancer cells expressed both epithelial and macrophage-specific markers [12]. These included CD14<sup>+</sup>/CD11c<sup>+</sup> cells of myeloid lineage. B7-H4 is a cell surface antigen encoded by the VTCN1 gene, meaning V-set domain containing T cell activation inhibitor 1 which interacts with ligands bound to receptors on the surface of T cells and has been correlated with tumor progression. CD163 protein is a member of the scavenger receptor cysteine-rich superfamily and is exclusively expressed at the cell surface by monocytes and macrophages. CD146 refers to the melanoma cell adhesion molecule (MCAM) that is expressed in the cytoplasm of adipose and stromal progenitor cells. The CD68 protein is a trans-membrane glycoprotein that is highly expressed by human monocytes and tissue macrophages. CD45 refers to the protein tyrosine phosphatase receptor type C (PTPRC) that is a trans-membrane receptor expressed by mature leukocytes. The CD14 protein is a cell surface antigen expressed on monocytes and macrophages, but also present on other subtypes of myeloid cells such as dendritic cells. CD11b refers to the integrin subunit alpha M (ITGAM) and CD11c to the integrin subunit alpha X (ITGAX) that are both parts of leukocyte-specific integrins. CD133 refers to prominin 1, a trans-membrane glycoprotein which localizes to membrane protrusions and is often expressed on adult stem cells, where it is thought to function in maintaining stem cell properties by suppressing differentiation. CD204 refers to the macrophage scavenger receptor 1 (MSR1) which is a macrophage-specific membrane glycoprotein. CD206 refers to the mannose receptor C-type 1 (MRC1) that is a type I membrane receptor that mediates the endocytosis of glycoproteins by macrophages. Cytokeratins (CK) are intermediate filaments expressed in epithelial tissues and are often used as a specific marker of epithelial cells. The epithelial cell adhesion molecule (EpcAM) is a membrane protein expressed on most normal epithelial cells which functions as a homotypic calcium-independent cell adhesion molecule. Vimentin is a type III intermediate filament protein responsible for maintaining cell shape and integrity of the cytoplasm in mesenchymal cells but has also recently been associated with tumor cells when expressed at the cell surface (i.e., cell surface vimentin, (CSV)) presenting with enlarged nuclei, CD45<sup>+</sup> and

exhibiting cytoplasmic staining by cytokeratins 8, 18, and 19 and epithelial cell adhesion molecule (EpcAM) [13]. Lustberg et al. observed populations of circulating atypical cells expressing cytokeratins 8, 18, and 19, CD45 and CD68 markers in the blood of metastatic breast cancer patients [14]. Recent reports about circulating atypical macrophages have now shed more light on these cells, on the possible mechanism of their formation, and on their relevance in tumor invasion. Earlier studies had pointed out the heterogeneous nature of circulating atypical cells, particularly regarding circulating tumor cells (CTCs), endothelial and epithelial cells, fibroblasts, macrophages, and megakaryocytes [15]. Chen et al. reported circulating macrophages expressing antigens expressed by tumor cells CD68 and B7–H4, in the blood of 56 lung cancer patients, showing that CD68<sup>+</sup> and B7–H4<sup>+</sup> macrophages correlated with tumor size and lymph node metastasis [16]. Adams et al. reported circulating atypical cells with concomitant expression of macrophage-specific and epithelial cell-specific markers. They speculated that cancer-associated macrophage-like cells [CAMLs] might be different stages of myeloid differentiation and/or derive from nonspecific engulfment of epithelial cellular debris. They also described that some CAMLs bind to and migrate in blood attached to CTC [17].

Shabo et al. showed that macrophage traits in cancer cells are induced by macrophage–cancer cell fusion and cannot be explained simply by cellular interactions. They showed that tumor cell expression of the macrophage marker CD163 is related to poor prognosis in patients with breast cancer, colorectal cancer, and urinary bladder cancer [18–22]. Leukocyte–cancer cell fusion as a source of myeloid traits in cancer has also been discussed by Pawelek and colleagues [23–31]. Following macrophage–cancer cell fusion, the resultant hybrid cells acquired new abilities to promote angiogenesis, matrix alterations, motility, chemotaxis, and immune signaling pathways. Macrophage–tumor cell fusion could explain the aneuploidy, plasticity, and heterogeneity of malignant melanoma and it could also account for epidermal–mesenchymal transition in tumor progression since macrophages are of mesodermal origin [23]. There is considerable evidence that fusion between macrophages or other phagocytes and cancer cells causes epigenetic reprogramming. Following fusion *in vitro* between weakly metastatic Cloudman S91 mouse melanoma cells and mouse or human macrophages, more than half of the resulting hybrids were more metastatic than the parental cell line. The metastatic hybrids showed increased expression of a number of macrophage-like molecules including SPARC, SNAIL, MET, MITF; integrin subunits  $\alpha 3$ ,  $\alpha 5$ ,  $\alpha 6$ ,  $\alpha v$ ,  $\beta 1$ ,  $\beta 3$  [28], GnT-V ( $\beta 1,6$ -acetylglucosaminyltransferase-V) and its enzymatic products  $\beta 1,6$ -branched oligosaccharides conjugated to N-glycoproteins

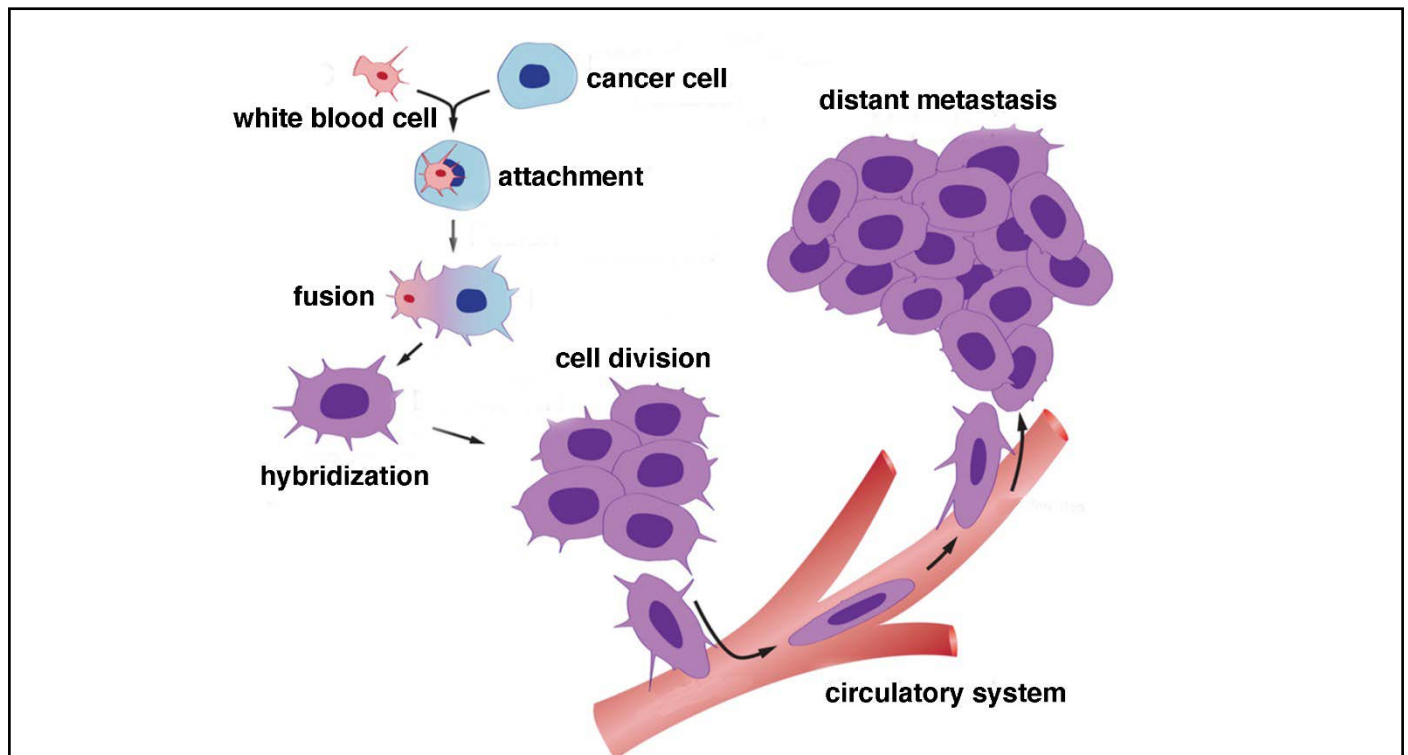
[27-29], cell-surface LAMP1 [30], high levels of autophagy [29], acquired hormone inducible chemotaxis [30], and expression of c-Met pro-oncogene [31]. These traits are all associated with tumor progression and poor outcome in a number of cancers [18-22].

## Discussion and Conclusions

There is now evidence from several sources that fusion and hybridization of phagocytes such as macrophages with cancer cells creates metastatic cells. Our group has demonstrated this in three patients with melanoma and two with renal cell carcinoma. In addition, several labs have made immunological observations that metastatic cancer cells exhibit many macrophage traits. Thus, it seems safe to say that this is at least one mechanism for metastasis. This confirms the century-old proposal of Prof. Otto Aichel that in retrospect was prescient indeed, especially considering that he had only a microscope with which to work. For the first time, we can glimpse the engine that drives metastasis. A scheme for this is shown in Figure 1. This information opens many potential targets for the development of new therapies, for example inhibition of the fusion process itself regarding events such as membrane attachment and heterokaryon

formation; inhibition of the hybridization processes involving integration of parental fusion partner genes into hybrid genomes; and prevention of post-hybridization events involving activation of genes that control cell migration, chemotaxis, intravasation, extravasation, and migration to lymph nodes and distant metastases.

What was Aichel's hypothesis [2]? From a biological viewpoint, the theory is quite simple: on one hand, white blood cells have the ability to migrate around the body to distant tissues and organs, but they rarely divide and have a limited life span. On the other hand, cancer cells have virtually no abilities for migration, but they have de-regulated cell divisions such that they form tumors. When leucocytes phagocytose cancer cells (which can be seen through a light microscope) sometimes instead of digesting the cancer cell, the leucocyte fuses with it, forming a hybrid between the two cells that exhibits both the migratory abilities of the leucocyte and the uncontrolled cell division of the cancer cell. This, proposed Aichel, is the origin of metastatic cells. The model is simple— *white blood cell + non-metastatic cancer cell = metastatic cancer cell*—yet it provides a profound and unifying explanation for metastasis. A schematic diagram of this process is seen below (Figure 3).



**Figure 3:** A modern version of Aichel's leucocyte-cancer cell fusion and hybridization hypothesis. Starting in the upper left, a motile white blood cell approaches a cancer cell. The outer membranes of the two cells become attached. Fusion occurs forming a bi-nucleated cell with a nucleus from each of the fusion partners. The cell goes through the process of genomic hybridization creating a leucocyte-cancer cell hybrid with concomitant deregulated cell division and motility, the genesis of a metastatic cell [4]. (Used with permission of the journal Plos One).

At the time of Aichel's proposal, there was no knowledge of DNA-based genetics and no experimental model to test, and for decades the literature was silent. Today, more than a century later, several laboratories have produced evidence that it was correct [32]. How did Aichel come up with this proposal? In addition to the 1911 paper above [2], we were able to find some additional publications, but only one of them was related to his 1911 paper and appears to be a precursor to it [33]. In this, he asks--how does the cancer cell acquire "foreign" properties? Aichel's answer is the fusion of a leucocyte with any other non-moving somatic cell, but he doesn't specify a cancer cell. Aichel introduces general properties of leukocytes--that they can move through and dissolve tissues. Then he suggests that fusion of a leukocyte with a somatic cell would be similar to the merging of the nuclei of the sperm and egg cells. But in some cases, fusing with a somatic cell could lead to cancer. Besides these works, Aichel described other theories of cancer of the time. In his doctoral thesis, he studied the retina of adult and embryonic teleost fish [34]. He later studied molar pregnancies [35] and in other studies the incidence of tuberculosis amongst twins, gonorrhea in children, the histology of colon cancer, anatomies of various organs such as the frontal sinus of the Gorilla, the anatomy of the jaw and other bones (not shown). The point is that none of these articles provides an explanation for how Aichel came up with the concept of leucocyte-cancer cell fusion and hybridization as an initiator of metastasis.

Given this, we were thus curious to learn more about Aichel's life. Aichel was born in Chile in 1871 as the son of a German diplomat. He studied medicine in Germany and specialized in gynecology. He had great interest in comparative anatomy and anthropology [36]. Aichel was awarded a doctorate in 1896, and a medical degree in 1898. In 1902 he became professor of gynecology at the Universidad de Chile. Interestingly, in 1909 he had to leave Chile for Germany because he was involved in a cover-up of a highly political incident known as "Der Fall Beckert" [37].

Aichel worked as physician in Munich, Halle and Kiel, where he became a full professor. He was deployed as a medic and later chief physician during the first world war [37]. After the war, he returned to Kiel, focusing on comparative anatomy. Historian of medicine, Karl-Werner Ratschko emphasizes Aichel's widespread reputation as an espouser of eugenics in the 1920s [36]. In 1923, Aichel founded a local society for eugenics in Kiel. Aichel became a member of the Nazi Party in 1932. Aichel was a supporter of Nazi eugenics and oversaw the sterilization of "inferiors". However, his insistence on providing a scientific basis for eugenics was not appreciated by many of his Nazi colleagues and higher

political officials, causing an intrigue against Aichel in the last years of his life [36]. Thus, although Aichel's ideology should have ensured him a successful career in Nazi Germany, he was suspended from working [38]. Aichel died in Kiel in 1935 of coronary sclerosis.



**Figure 4:** Professor Otto Aichel in Kiel, Germany, 1913. [39].

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