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Amanita Phalloides Avoids Tumor Growth of Leukocytes with Philadelphia Chromosome: Case Report

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Case Study

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ABSTRACT

Background: Treatment failure in patients who have chronic myeloid leukemia (CML) using tyrosine kinase inhibitors is common due to development of secondary mutations. *Amanita phalloides* (Amanita) contains low dose of alpha-amanitin (amanitin),which inhibitis RNA polymerase II (RNAP) leading to slowing down of the growth of tumor cells without affecting normal cells.
Aim: To determine if Amanita inhibits the growth of Philadelphia chromosome (Ph1) carrying leukocytes in CML.
Methods: A patient with leukocytes carrying a Ph1 and loss of Y chromosome was treated with Amanita as the only tumor specific therapy. Pre-treatments with the tyrosine kinase inhibitors Imatinib and Nilotinib had to be terminated because of severe side effects. Monitoring was performed with blood cell count and quantitation of *bcr-abl* fusion transcripts.
Results: The disease state could be stabilized for nearly two years until now with Amanita alone. Whereas leucocyte´s tumor growth was inhibited, and cell count remained low, the percentage of *bcr-abl* fusion transcripts rose. Although the relative amount of lymphocytes decreased transiently, it remained stable in the range of 1.5/nl [0.6-4.7/nl] blood. Compared to the initial phase of CML

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diagnosis in 2008, leukocyte count is 5 fold reduced due to the Amanita therapy. **Conclusions:** Amanita can inhibit tumor growth of cells carrying Ph1. However, the cells with Ph1 and loss of Y chromosome have growth advantage over the cells without these mutations. The percentage of potential tumor cells increased, but without complications.

Keywords: Amanita phalloides; philadelphia chromosome; chronic myeloid leukemia.

1. INTRODUCTION

Ph1, a translocation between chromosomes 9 and 22 was first described in 1960 and associated with leukemia [1]. 95% of chronic myelogenous leukemia cells and 25% of acute lymphocytic leukemia cells carry the Ph1 chromosome. Ph1 is as well involved in acute myelogenous leukemia.

The gene *abl* at the translocation site was identified by Heistercam et al. in 1983 [2]. Canaani *et al*. found a year later, that this gene was transcribed as a new 8 kb RNA transcript, indicating a direct involvement in the development of leukemia as oncogene [3].

Most cells in patients with CML and the Ph1 chromosome contain a new *abl* transcript, now called *bcr-abl*. It codes for a protein-tyrosine kinase. In 1996 first publications opened a new way for therapy: inhibition of the BCR-ABL tyrosine kinase [4].

Nilotinib is a BCR-ABL tyrosin kinase-inhibitor. In 2007 it was first admitted in Switzerland to treat CML, followed by admissions in the USA and EU. However, chromosome aberrations can be induced by the drug, and resistance is often achieved after a short period of time [5]. Today several optimized treatment schedules including combination strategies try to elongate treatment possibilities [6,7].

CML is frequently first diagnosed as chronic leukocytosis with severe infections. For therapy rapid monitoring RT-PCR of the RNA is possible since 2015 [8]. It raises as parameter the percentage of the *bcr-abl* transcript related to the not mutant *abl* transcript. It is still in discussion, whether this parameter adds information for the therapeutic regimen.

A genetic and molecular study identified the central potential targets for therapeutic intervention in tumor cells: switch genes. In tumor cells the switch genes are over-expressed [9, review, 10]. This switches the growth of tumors to ON. All switch genes belong to the class of HOX genes, and use RNAP for their action. If the switch genes are over expressed, RNAP is used to full extent, creating a bottleneck for tumor growth. The extract of Amanita contains amanitin, among other active peptides, that lead amanitin into the cellular nucleus, where RNAP works [11,12]. RNAP in somatic cells has lower levels of activity. Inhibition of 50% of RNAP has no effect on normal cells, but inhibits tumor growth of cells. Growth inhibited tumor cells can be recognized by the immune system and digested. Through this approach it is possible to stabilize the state of disease for years.

Dilutions of Amanita phalloides are used since 300 years, the classical indication is fear of death. After anamnesis the patient is treated with Amanita phalloides (zert. Riede) D2 [Herbamed AG, CH], at an average dose of 4 x 10 drops per day, resulting in an average uptake of 50 ml per month. The daily dose contains 50 molecules of amanitin per cell. With 100 ml of this drug, about 50% of all RNAP molecules in all cells are inhibited [9]. Usually no side effects occur. Degradation of cells is monitored with lactatedehydrogenase (LDH) levels in serum. Successful treatments of mammary-carcinoma, thyroid cancer, colon cancer, prostate cancers or B cell leukemia have been described [9]. In all patients, in addition to Amanita as the only tumor specific drug, the oral uptake of essential fatty acids is indicated. Essential fatty acids enhance the fluidity of cellular membranes, and decrease the risk of autoimmunity. For monitoring of the therapy, the regular measurement of blood cell count and LDH is performed here.

2. CASE PRESENTATION

The patient was born in 1939, no genetic disposition, no other risk for tumor induction could be identified. In September 2008 he suffered from a diverticulitis with elevated levels of leukocytes (56.5/nl; Fig. 1, red star). I.v. antibiosis could reduce leucocyte level to 41,4/nl only. Too many myelocytes and metamyelocytes and less lymphocytes (12%, Fig. 1, green star) were diagnosed. Lymphocytosis concerned T-

cells and NK-cells and resulted in an overexpression of IgA. Bone marrow analysis revealed an elevated granulopoesis of 97.5%, elevated filaments and less fat cells. Erythropoesis was reduced to 2%, indicating an affected red blood cell system.

On Okt-7-2008 leukocyte level was at 46.5/nl, revealing a duplication time of seven months since the antibiotic treatment. On Okt-16-2008, leukocyte level was at 53.6/nl, indicating a duplication time of two months since the last measurement.

Further investigations led to the diagnosis of CML. As chromosome aberrations loss of the Y chromosome and translocation t(9;22)(q34;q11) were found, the *bcr-abl* rearrangement was confirmed *in situ* hybridization with a *bcr-abl* probe. A *bcr-abl* fusion transcript was identified by RT-PCR. Additional loss of the Y chromosome was confirmed by *in situ* hybridisation with probes against centromers in X and Y chromosomes. No mutation of V617Fin JAK2 gene was identified, a gene involved in other myeloproliferative syndromes [13].

Therapy followed with 400 mg Imatinib per day, a tyrosine kinase inhibitor. This reduced tumor growth for 18 months considerably by a factor of 2.9. Gastrointestinal and skin side effects led to a reduction to 200 mg daily. Further incompatibility and leukocytosis followed. In November 2011 Imatinib was replaced by Nilotinib, 200 mg/day. Tumor growth was reduced by a factor of 4.1 to 7.2/nl leukocytes. In September 2015 *bcr-abl* transcripts only represent 0,0008% of *abl* transcripts. In October 2015 suddenly *gamma*-Glutamyltransferase (gGT), a liver enzyme, raised to 1200 U/l. Abstention of Nilotinib led to a reduction of gGT.

Infusions with vitamin C and curcuma were applied. Amygdalin reduced circulating epithelial cells by 60%, determined by maintrac [14]. Maintrac is not specific for a tumor process, but functioning as a tumor marker test once a tumor is diagnosed. It counts epithelial cells in the blood, showing the activity of the tumor process.

In January 2015 a problem with the right kidney lead to the diagnosis of an ureter carcinoma. The tissue was ulcerating, partially necrotic and infiltrating the muscle. It was classified pT3 pN0(0/3) L1 V1 G3, and operated in March 2015. At that time leukocytes were at 6.4/nl. The patient treated himself until today with Infi Ononis and Cysto Hevert, homoeopathic combination drugs, and prostagutt, a phytotherapeutic drug. He applied sodium-bicarbonate daily until the urine got a pH 7.8. Until today there are no complications or side effects from this type of tumor.

In February 2016 the patient began the Amanita therapy with 4 x 10 drops of Amanita phalloides (zert. Riede) D2 per day (Fig. 1). Diagnosed were in addition active *Borrelia burgdorfferi* by ITT® culture (6.8 [normal range is given in brackets; <1 pg/ml]). ITT shows active T-cells producing antibodies against Borellia in an immuneblot. To treat the infection, Terebinthina laricina D1 in 2 x 10 drops per day were applied in addition to Amanita. With Terebinthina, Borrelia or other spirochaetes can be eliminated within months efficiently [15]. In addition, virae of the herpes family were present: varicella zoster virus (IgG 4597 [<1 U/ml]; IgM negative; IgA 4); Epstein Barr virus (ITT 7 [<1 pg/ml]) and herpes simplex virus1/2 (IgG 2.2 [<1 U/ml]; IgM and IgA negative).

In the beginning of the Amanita therapy, the patient became tired and tried to overcome it with sports. Because of low iron levels, a diet was recommended and iron supplements and infusions were given. In June 2016, the LDH level increased, together with an increasing *bcr-abl* transcript. The leukocyte level remained in the normal range (Fig. 1, Table 1). Creatinine levels increased (1.54 [0.7-1.3 mg/dl]) indicating a kidney insufficiency. The patient was motivated to drink more, and to resign from eating white sugar. He took less Terebinthina because of the kidney problem, resulting in an overall uptake of 100 ml in September 2016. CRP levels increased during this period, and decreased afterwards. The patient took another 100 ml Terebinthina between January 2017 and June 2017. The erythrocyte sedimentation rate decreased from April 2016 until February and June 2017 indicating a decreasing inflammation process. gGT increased at this time, an MRT of the liver showed no metastases. gGT levels decreased from that time on into the normal range. IgA levels remain stable about 1.7 fold over the normal range. Thrombocytes remained in the normal range, indicating no affection in the bone marrow.

Table 1. Laboratory parameters

		2008	2015	2016					2017				
Parameter	Normal range		12	2	4	6	8	10	12	2	4	6	8	Sep-14
Maintrac (1)	150-8000		1000									150		750
ESR/hr (2)	<20				40					28		24		
CRP	<0.3 mg/dl	127			<0.3		0.61	0.89			0.77	0.59		
Creatinine	0.7-1.3 mg/dl					1.35	1.54	1.52		1.30		1.35		1.50
gGT	<60 U/l	209		77	81	51	59	101		80		53		55
IgA	0.7-4 g/l	6.04		6.98						6.89	6.86	7.09		6.68
Monocytes	2-12%	2.2			11.7		10.2			8.4		7.0		7.3
Granulocytes	40-75%				63.6		69.5			71.4		74.2		77.5
Basophiles (3)	100-800/µl													1400
Monocytes	250-850/µl													900
Thrombocytes	140-400/nl	508			265					243		254		281
LDH		545		166	165	255				152	165	166		198

(1) Maintrac. With this parameter circulationg epithelial cells are detected;
(2) Erythrocyte Sedimentation Rate per Hour;
(3) Basophile Granulocytes

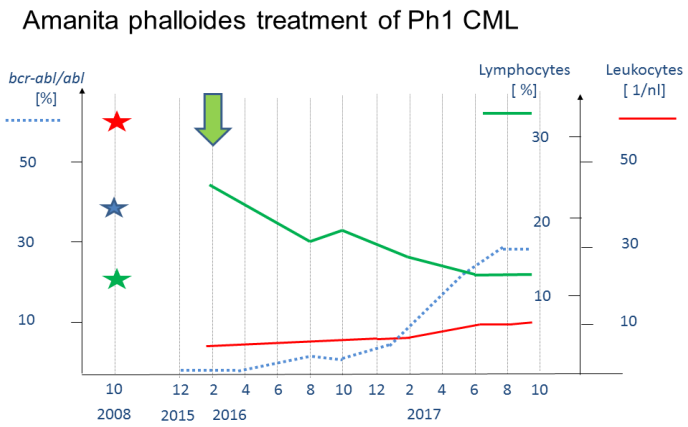

Fig. 1. Amanita inhibits leukocytosis

Stars represent parameters at the time of diagnosis in 2008: leukocytes (red; initially 56.5 [3.5-9.8/nl]); lymphocytes (green; initially 12 [20-45%]) and quotient of bcr-abl transcripts over not mutant abl transcripts (blue; initially 39 [0%]). Amanita therapy started in February 2016 (arrow). During the therapy the relative amount of lymphocytes decreases to a level of 12%. bcr-abl transcripts increase, due to a growth advantage of cells with Ph1 chromosome and lost Y chromosome to a level of around 30%. The percentage of bcr-abl transcripts is in the range of the initial diagnosis in 2008. It would be expected that with this number of mutant cells, leukocytes would be in the same range than 2008. Leukocytes increase from 6/nl to 12.4/nl, five fold less than 2008, leukocytosis fails to appear

From August 2016 to October 2016 the frequency of the *bcr-abl* transcript decreased once, but then increased further until today (Fig. 1). Anyhow the percentage of the *bcr-abl* transcript seems to stabilize at around 30%. Actually 29,9% *bcr-abl* transcripts are present, a similar level than at the time of diagnosis in 2008. In the initial phase of CML diagnosis in 2008, 40% of *bcr-abl* fusion transcripts compared to *abl* transcripts occurred (Fig. 1 blue star). Leukocytes now are at 12.4/nl, compared to 56.5/nl at the time of diagnosis. Therefore leukocyte count is 5 fold reduced due to Amanita. Lymphocyte levels are actually at 12.2%, compared to 12% in 2008. This means, that the tumor cells with the Ph1 chromosome overgrew the not mutant leukocytes, but did not induce excessive proliferation. Amanita successfully interrupted the tumor growth of cells.

A maintrac was measured in December 2015 and in June 2016. It decreased by a factor of 6.5 (Table 1) to 150 cells per slot, a range not indicating tumor growth. This means that in addition to the inhibition of tumor growth of the leukocytes, the ureter tumor cells are inhibited as well by the therapy. Amanita inhibits tumor growth of most tumor cells, regardless of the tissue they originate.

To decrease the amount of *bcr-abl* transcripts, end of September 2017 an interval with additional Nilotinib started. It is planned to omit Nilotinib again after some months.

3. DISCUSSION

Here the successful Amanita treatment of a patient with a Ph1 chromosome is shown. Whereas tumor growth of leukocytes could be inhibited, the number of cells carrying the *bcr-abl* mutation increased. Obviously they have a growth advantage, which is possibly supported by the additional loss of the Y chromosome. Less DNA has to be replicated in a cell cycle, which is advantageous.

Measuring the *bcr-abl* transcript as monitoring parameter is useful in the treatment with tyrosine kinase inhibitors, as this parameter in the functional chain of the drug is affected. For the Amanita therapy in this case, blood cell count and LDH are basically sufficient to monitor tumor growth, and to minimize the dosage. A maintrac, measuring circulating epithelial cells, can help to confirm the successful inhibition of tumor growth. In the case presented here, no specific tumor marker for the existing ureter tumor cells is known. The decrease of the maintrac shows that as well these tumor cells are inhibited by

Amanita. The maintrac parameter can be used to monitor treatments of most sorts of tumors, where no specific tumor marker is known. However, it is not a parameter specific for tumor growth, like LDH, and can be elevated as well in patients without any disease.

Tyrosine kinase inhibitors cause mutations, and can add chromosome aberrations in cells. It is possible, that the ureter carcinoma in this patient occurred from such a mutation. Tumor therapy today often uses measures that cause severe side effects and complications, including induction of new mutations and new tumors. However, without inhibition of the tumor growth an increasing leukocytosis would lead to anemia very soon.

The Ph1 chromosome is found in acute and in chronic forms of leukemia. This indicates that it is possibly not the only mutation involved. It is suggested that in acute forms an additional proliferative gene mutation is involved, inducing a central replication origin, causing faster replication starts and shorter cell cycles.

It is evident that infections support tumor growth of cells. In one case, Borrelia seemed to actively induce leukemia: eliminating the Borrelia eliminated the leukemia [16]. From 16 cases with lymphocytic disorders, Borrelia infection was diagnosed in 11 cases, identified by antibodies against Borrelia, a mean of 70%. In many cases seroconversion was absent or incomplete (own unpublished results). In the case presented here, antibiosis was able to reduce the leukocyte level to certain extent. Treating with Terebinthina to eliminate Borrelia could reduce the inflammation processes: CRP levels and the erythrocyte sedimentation decreased. It seems to be important to reduce the infectious burden, to give the immune system room to degrade the tumor cells.

Not all patients can be cured by eliminating the infectious burden by Borrelia. Probably the missing seroconversion shows, that they can interact with the DNA of the immune cells and hinder the full possibilities of the immune system. IgM antibodies, as the first immune response, should switch to IgG antibodies, which can interact with the more effective cellular immune system. If this shift is prohibited, Borrelia cannot be eliminated. In that they have a selective advantage. Interacting with the DNA of the cells, they might switch as well the tumor formation on. This can be reversible in some cases, or irreversible and established in others.

A strategy in tumor therapy should consider that all tumor cells are human somatic cells. They all have the same human cell structure. There are no cell structures in tumor cells – like in bacteria – that are not present in other somatic cells. A tumor therapy should not use drugs or physical methods that also destroy normal body cells. A tumor therapy should therefore use weak selective pressure to avoid resistance development. Only one drug at a time, in a minimal dose, for a maximal duration, should be used. This approach requires monitoring of the disease state. In case of resistance or severe side effects, an interval with another drug treatment that focuses on a different biochemical target should be used. In this interval the resistance or side effects against the first drug can disappear. These intermitting interval strategies seem to be the solution of the cancer problem in future [6]. And Amanita will be a potent drug in the years to come.

4. CONCLUSION

The treatment of this patient with CML with Amanita occurred without side effects, the quality of life was unaffected. Amanita reprograms the tumor cell and reduces tumor cell activity. This therapy can as well be given in advance of other tumor therapies.

CONSENT AND ETHICAL APPROVAL

Only existing drugs in usual doses are used during this study. No new drug was applied. Therefore no ethical permission is necessary in this case. Informed consent of the patient was obtained to document, evaluate and publish data in anonymous form.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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