Antiviral Therapy For Progressive Multifocal Leukoencephalopathy

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Master: Molecular and Cellular Life Sciences
Masterthesis – Literature review
01-06-2014 / 31-08-2014
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Laymen's summary

Progressive Multifocal Leukoencephalopathy (PML) is disease of the brain caused by the JC virus (JCV), a polyomavirus. It causes lesions in the white matter of the brain which often leads to cognitive decline, vision loss, impaired speech and paralysis. The majority of cases die. Over half of the general population is infected with JCV. Fortunately, however, PML almost only occurs in people with a compromised immune system. E.g. patients infected with HIV, patients that received an organ transplantation or suffer from diseases like chronic lymphatic leukemia (CLL). With the help of highly active anti-retroviral therapy (HAART), survival chances of PML in HIV infected patients increased to around 50%.

In this review, we have tried to summarize all reports of drugs used to treat PML available in the English literature. To date there is, however, no specific treatment against PML. One of the problems researchers are facing in their efforts to develop a drug against PML is that there is no animal model available. Newly, *in vitro*, developed drugs, therefore cannot be tested on animals. Clinical trials are only approved when drugs have been tested extensively in animal models. The only option is to test already existing and approved drugs used to treat other human conditions. Another challenge is that PML manifests itself in the brains. Therefore, to have any chance of success, drugs have to be able to pass the blood-brain barrier (BBB), the highly selective barrier to the brain. Researchers have searched for different ways to combat PML: drugs that stimulate the immune system (interleukin-2, Interferon-α and Interferon-β), drugs that block viral entry into cells (chlorpromazine, risperdone, mirtazapine) and drugs that target the replication of the virus (cidofovir, CMX001, mefloquine, cytarabine and topotecan). Because PML is so rare, it is nearly impossible to conduct large controlled clinical trials. Most clinical trials are either uncontrolled, not-randomized and/or open-label. Moreover, most studies that have been performed to date are case reports: single patients treatments. In these case reports N=1, in most cases treatment is combined with HAART or other treatment interventions. What is found in the literature is probably also partly biased because

researcher are more likely to write about cases with a good outcome. Case reports, therefore, can never prove whether a drug works. Several clinical trials have been conducted on some of the aforementioned drugs, however we found that none of the tested drugs proved to significantly improve survival chances of PML patients.

We find that, from all drugs tested, CMX001, mirtazapine and risperdone are most promising. These drugs cross the blood brain barrier (BBB), have no severe side effects and show promising results in case reports and *in vitro*. However, none of these drugs have been tested in controlled clinical trials, so further research is needed.

1. Abstract

Progressive Multifocal Leukoencephalopathy (PML) is a demyelinating disease of the brain caused by JC polyomavirus (JCV). PML occurs mostly in people with compromised immune systems like HIV infected persons, patients receiving treatment with immunomodulating monoclonal antibodies like MS patients treated with natalizumab, transplant recipients and patients suffering from hematological malignancies. To date there is no treatment with proven effectiveness for PML. Many different drugs have been tested, however, and in this review we summarize which drugs have been used and to what effect. From all drugs tested CMX001, mirtazapine and risperdone appear to be most promising. These drugs cross the blood brain barrier (BBB), are well tolerated and show promising results in case reports. Because these drugs haven't been tested in controlled clinical trials further research is needed.

2. Introduction

2.1 Progressive multifocal leukoencephalopathy

Progressive Multifocal Leukoencephalopathy (PML) is demyelinating disease of the brain caused by the JC polyomavirus (JCV). Descriptions of the disease can be traced as far back as 1930[1]. The viral nature was postulated in 1959[2], but definite proof that PML was caused by a viral infection was found in 1971 when viral particles were seen by electron microscopic analysis of affected brain tissue [96] .

Symptoms of PML have a sub-acute onset and are highly variable as they depend on the location of the white matter lesions. Typical symptoms are cognitive decline, vision loss, impaired speech and paralysis. Fever is generally absent; involvement of white matter in the spinal cord has rarely been demonstrated. For a thorough review of the clinical picture of PML see [17].

PML is a disease of people with a compromised immune system as could be seen by the 50-fold increased incidence during the HIV epidemic in the 1980s[3]. During the last decade the introduction of several novel immunomodulatory monoclonal antibodies has created a new group of immunocompromised patients that are at risk to develop PML. The monoclonal antibodies that have been mostly associated with an increased risk are Natalizumab[4], Rituximab [5], Efalizumab[6] and/or Alemtuzumab [7]. The risk is, however, not the same for these antibodies. JVC infected patients treated with Natalizumab for 25 to 48 months have risk of 11.2 in 1,000 persons years to develop PML in contrast to only 0.25 in 1,000 person years for people treated with rituximab[8]. Transplant recipients and patients suffering for a prolonged period from hematological malignancies like chronic lymphatic leukemia (CLL) also are at risk to develop PML.

The diagnosis of PML can be difficult and is based on clinical picture, imaging of the brain and laboratory detection of JC virus in cerebrospinal fluid or brain biopsy [8]. See [97] for an overview of the diagnostic criteria.

2.2 JC virus and polyomavirus properties

Just under 20 different polyomaviruses have been discovered in various different mammals and birds. Polyomaviruses have a T = 7 icosahedral symmetry and a diameter of approximately 42nm. The genome consists of closed circular, double-stranded, supercoiled DNA strand with a size of approximately 5100 bp. The length of the DNA strand differs in different variants due to alterations in the non-coding regions. The genome can be divided into three regions: the early viral gene region (EVGR); the late viral gene region (LVGR); and the non-coding control region (NCCR). The EVGR is approximately 2400bp and contains the ORF's of different antigens like large and small T antigen genes (LTag and sTag) and splice variants like T'135,T'136, and T'165. The LVGR is approximately 2300bp and contains the ORF's of structural proteins VP1, VP2 and VP3. The NCCR is approximately 400bp and contains the *ori*, promoter, and enhancer elements. The NCCR is also called hyper variable

regulatory region (HVRR) or regulatory region (RR), because the NCCR changes after infection and many different sequences have been observed[8]

2.3 Pathogenesis of PML

Over half of the general population has been infected JCV and it is generally assumed that all infections are lifelong [9]. Latency of the virus is then established in the kidneys and bone marrow[17]. JCV is excreted in the urine of 19–27% of infected persons [10]. Entry of the virus occurs most likely at the tonsils or the gastrointestinal tract, however it is also possible that infection occurs through kidney epithelial cells[11]. The virions bind to carbohydrate receptors on the surface of the cell. VP1 pentamers, one of the JCV structural proteins, has been shown to bind to lactoseries tetrasaccharide C (LSTc)[12]. LSTc contains a terminal α2,6-linked sialic acid which can attach to a glycoprotein or glycolipid on the surface of the cell[13]. Then, endocytosis is probably mediated via clathrin-coated pits[14]. The virions then travel via the early endosomes, possibly through the late endosome to the endoplasmatic reticulum (ER) and then to the nucleus[15][16]. After release of viral DNA in the endoplasmatic reticulum or nucleus the EVGR is first transcribed followed by transcription of the LVGR.

In PML patients typical gene rearrangements of the JCV genome and specific mutations in the VP1 gene are observed [18]. This mutated JC virus can be found in the brain and other organs and it is thought that these changes determine virulence and viral tropism. It is still not clear at what stage of infection the virus travels to the brain and crosses the Blood-brain Barrier (BBB).

2.3 Antiviral therapies against PML

A well-established effective therapy against PML is not available to date. There is no animal model available to test possible drug candidates before they are used in clinical trials. Therefore, to avoid safety issues, only already existing drugs used for other diseases, can be used in the treatment of PML. Several of these drugs have been tested, some of them show promising results in case reports, although no therapy was proven to significantly increase survival chances for PML patients. One

important property the drugs must have is that is able to pass the BBB, because PML manifests itself in the brain. In this article a literature study was conducted to create an overview of the treatments that have been tried, where the difficulties lie, but foremost, which therapeutic options are most promising and should be pursued in future studies.

3 Methods

We searched the PubMed database for articles published from 1959 until August 2014 using the terms, or a combination of terms: "PML", “progressive multifocal leukoencephalopathy”, "therapy", "antiviral therapy, "treatment", “JC virus”, “Interleukin-2”, “Alpha Interferon”, “beta interferon”, "chlorpromazine", "mefloquine", "cytarabine", "cidofovir”, and " Mirtazapine". Moreover, relevant articles in the references of the articles found on PubMed were included in this review. Studies described are studies wherein PML patients were treated with drugs other than HAART.

4 Results

In our study we found 9 different drugs which have been tested whether they work in the treatment of PML. We will discuss every drug separately below. Furthermore, all studies are listed in tables with specific information for every study.

4.1 Immunomodulating drugs

Interleukin-2 (IL-2) is a cytokine responsible for the activation of leukocytes, mostly lymphocytes. IL-2 binds to IL-2 receptors present on the surface of lymphocytes, thereby activating them. IL-2 is mostly used in the treatment of specific forms of cancer. Three case reports wherein HIV infected patients suffering from PML received interleukin-2 show positive responses of to the treatment [20-22], all can be found in table 4.1. Other immunomodulating cytokines are interferon-α (INF-α) and interferon-β (INF-β), these cytokines interfere with the replication of viruses by activating macrophages and natural killer cells. In 1999 a case control study, involving 53 HIV infected patients

suffering from PML of which 21 were treated with INF-α, showed a significant increase in survival for patients treated with INF-α [23]. However a second, larger, case control study in 2001, involving 97 HIV infected patients of which 36 were treated with INF-α, showed no significant survival benefits for patients treated with INF-α over non-treated patients [24]. Other statistical analyses were used in this second study. Only one case report of PML treatment with INF-β was found, the HIV infected patient was treated for 4 months with INF-β. However, PML progressed and the patient died after those 4 months [25]. All reports can be found in table 4.1.

4.2 Cidofovir

One of the most investigated therapies is treatment with cidofovir. (S)-1-(3-hydroxy-2-phosphonomethoxypropyl)-cytosine (HPMPC, cidofovir) is a acyclic nucleoside phosphonate antiviral shown to be active in lethal aerosol or intranasal cowpox infections [26]. Cidofovir is shown to inhibit viral replication of polyomaviruses, like the Murine polyomavirus and SV40, *in vitro*[27], however another *in vitro* study showed no activity of cidofovir against JCV [28]. Furthermore, cidofovir cannot cross the BBB, which is most likely necessary to be able to be effective against PML[29]. 1998 Taoufik et al. first described a PML case, in an HIV infected patient, where cidofovir, in addition to HAART, was used to treat the patient. This single patient showed significant neurological improvements, moreover, the load of JCV detected in the cerebrospinal fluid dropped significantly during the administration of cidofovir and the load of JCV became undetectable in the patients urine[30]. In the following years 10 different case reports were published, 7 of those patients showed response to the treatment[31-37]. The three patients that did not respond to the treatment were reported in one article and were not HIV infected, two had chronic lymphatic leukemia and one had multiple myeloma. 6 out of the 7 other patients were HIV infected, one had chronic lymphatic leukemia. Moreover, for all three non-responsive patients the diagnoses was based on both MRI scans and the detection of JCV DNA in the Cerebrospinal fluid by PCR. However, on only one of the three patients post-mortem examination was performed and PML was confirmed. On top of those case reports, 6 case control studies were performed [38-43]. In 2008 Di Luca et al. combined the results of these

case control studies in which AIDS-related PML patients received combination antiretroviral therapy with or without cidofovir. They concluded that there was no significant change in PML-related mortality or residual disability [44]. Moreover, cidofovir showed some toxicity in cells of neural origin [43]. All reports, except Bossolasco et al., can be found in table 4.2. Bossolasco et al. can be found in table 4.7.

4.3 CMX001

Nevertheless, because cidofovir showed positive results as a treatment for several viruses, like the herpes virus and in the inhibition of replication of polyomaviruses *in vitro*, research on this compound continued. 1-O-hexadecyloxypropyl-cidofovir (CMX001), a hexadecyloxypropyl lipid conjugate of cidofovir, has been shown to also inhibit replication of polyomaviruses. More specifically, the replication of the JCV can be inhibited with the use of such small dose CMX001 *in vitro* that it will not cause any significant side effects *in vivo*[44][45]. There are also indications that CMX001 is able to pass the BBB, which makes it a possible candidate drug in the treatment of PML. One case report on CMX001 was published, after initial ineffective treatment with cidofovir, mefloquine and risperdone, interleukin-7 and CMX001 was administered. The idiopathic CD4+ lymphocytopenia patient responded to this treatment and survived (table 4.5)[46]. Because several different drugs were administered, it is impossible to attribute the survival of the patient to CMX001.

4.4 Mefloquine

Mefloquine is a drug used as a prophylaxis as well as a treatment of *Plasmodium falciparum* malaria in cases where chloroquine is not useful due to resistance [47]. A large *in vitro* screen in 2009 of possible compounds brought mefloquine to the surface as a possible therapy against PML. Starting off with approximately 2000 candidates, the first test was inhibition of the viral infection rate in a human fetal astroglial cell line (SVG-A) infected with a modified form of JCV, namely JCV(M1/SVEΔ). The 67 compounds that inhibited the JCV infection rate by ≥20%, and doing so without causing significant cell toxicity, were tested again with different concentrations in the same assay. Of the 14

compounds that inhibited the JCV infection rate by ≥50%, again without causing significant cell toxicity, only mefloquine was able to pass the Blood-brain barrier (BBB)[48][49]. Mefloquine was then tested on a different, more relevant, cell line, human fetal astrocytes. Furthermore, its effect against a known pathogenic form of JCV, JCV(Mad4), was tested. Results of both sets of experiments were similar as the results in the initial screening. Further experiments on mefloquine indicated that it inhibits the infection rate at one of the steps involved in viral DNA replication and that it effectively inhibits replication in already infected cells.[49] From 2010 till 2014 a total of 10 case reports, with patients with various underlying diseases, where published where mefloquine was the only treatment, besides HAART [50-58]. All but 2 patients responded to the therapy, however these two patients, one with Idiopathic CD4+ lymphocytopenia and one with Waldenström macroglobulinemia, started mefloquine treatment relatively late, namely 2 months and 5 months after onset of PML [57]. Because these were all single patients that were treated it is impossible to say with certainty that the improvement was due to mefloquine. Nevertheless, these results, together with the *in vitro* results where promising. However, a larger clinical trial with a total of 37 patients in 2013 was terminated prematurely, because the Data Safety Committee predicted a small probability of showing a significant difference in the outcome of the different groups in this study. Until the termination of the study, no activity of mefloquine was observed[59]. However, the number of patients was very limited. Moreover, the patients had various different underlying diseases and therefore different survival chances. These facts combined, make a significant result unlikely, even if the study had not been terminated. All reports can be found in table 4.3.

4.5 Cytarabine

Cytarabine or cytosine arabinoside (z-B-D-arabino-furanosylcytosine) is chemotherapy agent used mainly as treatment for hematological malignancies such as acute myeloid leukemia (AML) and non-Hodgkin lymphoma. It affects the replication of DNA and can therefore be a candidate for PML treatment[60]. Cytarabine can, however, cause severe side effects, especially immune suppression. Eight case reports, dating back as far as 1989, have been published. Four of these patients were HIV

infected. Out of these 8 patients, 7 showed response to the treatment with cytarabine. [61-68]. The patients that did not respond had chronic lymphatic leukemia [62]. However, a study with 4 HIV infected patients treated with cytosine arabinoside showed no effect of the treatment, all 4 patients died within 71 days after the start of the therapy[69]. Moreover, two larger studies showed no effect of cytarabine on patients suffering from PML. The first controlled study included 57 patients, all HIV infected and suffering from PML. These patients were randomly assigned to one of three treatments, antiretroviral therapy alone (15 patients), antiretroviral therapy plus intravenous cytarabine (18 patients), or antiretroviral therapy plus intrathecal cytarabine (16 patients). 42 patients died over the course of the trial, divided perfectly over the three groups (14 in each group). Therefore suggesting that cytarabine is not effective against PML in combination with the specific antiretroviral therapy (zidovudine plus either didanosine or stavudine)[70]. A second study, in which all 19 non-HIV infected patients received intravenous cytarabine treatment, 36% of these patients (7 out of 19) survived and showed improved neurological functions[71]. This survival rate is higher than expected for PML (10%), however, the number of patients is still very small. Moreover, due to selection bias, the time between the onset of neurologic symptoms and the initiation of treatment was delayed to at least 1 month. Therefore, it is very difficult to draw any solid conclusions from these results. Other clinical trials also did not show significant increase of survival chances while treated with cytarabine[72-75]. All reports can be found in table 4.4.

4.6 Drugs which activate serotonin receptors

Mirtazapine is an antidepressant which activates serotonin receptors in the brain and is able to cross the BBB. It has been suggested that JCV uses those serotonin receptors to infect the central nervous system, therefore, mirtazapine can be a suitable drug in the treatment of PML [98]. One case report, a polycythemia vera patient, and one case series of 4 HIV infected patients have been published, all with positive outcome. All 5 patients responded to the therapy [76][77]. However no clinical trials have been reported on. Another drug which activates serotonin receptors and was suggested as a possible treatment of PML is the antipsychotic Risperdone[78]. The only report on the use of

risperdone is a case report, the non-Hodgkin lymphoma patient in this report responded to the treatment [79]. All reports can be found in table 4.5.

4.7 Topotecan

Topotecan is a chemotherapeutic agent primarily used in the treatment of cancer. Topotecan inhibits topoisomerase I and thereby DNA replication[80]. The administration of topotecan via an implanted ventricular reservoir can overcome the inability of topotecan to cross the BBB [99]. One non-controled clinical trial with 11 HIV patients where topotecan was used showed a possible effect of topotecan on lesion size and survival time. However, although survival time seemed to increase, only 3 patients survived (table 4.6)[81].

4.8 Studies with combination therapy

Several case reports where PML patients have been treated with combination of at least one of the aforementioned drugs have been published.

One PML patient was treated with cidofovir in combination with chlorpromazine. Chlorpromazine is a drug that may be prescribed for a wide range of symptoms, i.e. to control vomiting and nausea, to manage psychotic disorders and as a complement in the treatment of tetanus. *In vitro* experiments show that Chlorpromazine, at low dose, in combination with neutralizing anti-JCV antibodies, is able to inhibit viral spread. Most likely because chlorpromazine is able to inhibit clathrin-dependent endocytosis by inhibiting the disassembly of the clathrin at the plasma membrane as well as inhibiting receptor recycling there. At higher doses the same results can be achieved without the antibodies. However, there are significant side effects at higher doses[82]. Chlorpromazine might be able to cross the BBB[83]. The chronic lymphatic leukemia patient treated with chlorpromazine and cidofovir showed no response to the treatment [84].

Two reports of combination therapy with IL-2 have been published, one in combination with cytarabine and one in combination with cidofovir. The patient treated with cytarabine responded,

this patient had underwent autologous bone marrow transplant. The mediastinal thymic B-cell non-Hodgkin lymphoma patient treated with cidofovir did not [85-86].

Four reports on the combination of cidofovir with cytarabine have been published, all patients responded to the treatment. One of the patient had chronic lymphatic leukemia, the other three were HIV infected [43, 87-88].

One report on the combination of cidofovir with mirtazapine has been published, the sarcoidosis patient responded to the treatment[89].

Three reports on the combination of mefloquine with mirtazapine have been published, all the patients responded to the treatment. Two of the patients were also suffering from multiple sclerosis and one was HIV infected [90-92].

One report on the combination of cytarabine and mirtazapine has been published, the dermatomyositis patient responded to the treatment[93].

Two reports on the combination of cytarabine and INF-α have been published, both patients responded to the therapy. One patients had Cröhn desease the other one sarcoidosis [94-95].

One report on the combination of mefloquine, mirtazapine and cytarabine (both intravenously and intrathecally) was published, this chronic lymphatic leukemia patient did not respond to the treatment [96].

Most of reports show a responds of the patient to the different therapies. All reports can be found in table 4.7.

Reports on immunomodulating drugs in the treatment of PML.

Author (ref.)	Year	Underlying disease	No. of patients	Type of study	Drug	Treatment (time, dose, administration)	Other treatments	Treatment response	Other relevant details
Przepiorka et al [20]	1997	ABMT	1	CR	IL-2	4 weeks, 0.5 MIU / m2 / day, continuous infusion		1 (100%)	
Buckanovich et al. [21]	2002	HL	1	CR	IL-2	6 weeks, 0.5 MIU / m2 / day, continuous infusion Plus an additional several days, 0.5 MIU / m2 / day, continuous infusion		1 (100%)	20 days between IL-2 therapies, duration of second therapy not mentioned.
Kunschner et al. [22]	2005	MDS	1	CR	IL-2	5 weeks, 0.5 MIU / m2 / day, continuous infusion Continued by 1 week, 1.0 MIU / m2 / day, continuous infusion		1 (100%)	
Huang et al. [23]	1998	HIV	53 (21 treated with INF-α)	CCS / OL	INF-α	>20 days, 3 or 5 MU SQ 3 times a week.		7 treated with INF-α (33%)	Treated patients lived significantly longer
Geschwind et al. [24]	2001	HIV	97 (36 treated with INF-α)	CCS / OL	INF-α	>20 days, 3 MU SQ 3 times a week.	HAART (32 patients)	Not mentioned	No significant additional survival benefits.
Nath et al. [25]	2006	HIV	1	CR	INF-β	4 months, 44 μg, 3 times per week, subcutaneously	HAART	0 (0%)	Interruption of treatment during 1 week

Table 4.1. Underlying disease: ABMT = autologous bone marrow transplant, HL = Hodgkin's lymphoma, MDS = myelodysplastic syndrom, Type of study: CR = case report, CSS = case control study OL = open label

Reports on cidofovir use in treatment of PML.

Author (ref.)	Year	Underlying disease	No. of patients	Type of study	Drug	Treatment (time, dose, administration)	Other treatments	Treatment response	Other relevant details
De Luca et al. [44]	2008	HIV	370 (185 treated with CDV, 50%)	Variable	Cidofovir	variable	Variable	182 (49%)	No significant decrease or increase in survival rate for people treated with CDV
Gasnault et al. [38]*	2001	HIV	46 (24 treated with CDV 52%)	CCS / OL	Cidofovir	1-30 doses, 5 mg / kg / week for the first 2 weeks, then 5 mg / kg / 2 weeks, intravenously	HAART (all patients)	23 (50%) 12 treated with CDV (50%)	
De Luca et al. [39]*	2001	HIV	43 (16 treated with CDV 37%)	CCS / OL	Cidofovir	5 mg / kg / week for the first 2 weeks, then 5 mg / kg / 2 weeks, intravenously	HAART (all patients)	19 (44%) 10 treated with CDV (63%)	CDV treatment showed a trend towards a reduced risk of death.
Marra et al. [40]*	2002	HIV	24 (24 treated with CDV 100%)	NCCT / OL	Cidofovir	24 weeks, 5 mg / kg / week, intravenously, followed by infusions every 2 weeks with the dose adjusted for renal function	HAART (all patients)	13 (54%)	CDV did not cause neurological improvements
Berenguer et al. [41]*	2003	HIV	118 (44 treated with CDV 37%)	CCS / OL	Cidofovir	3-11 doses, 5 mg / kg / week, intravenously	HAART (all patients), Corticosteroid therapy (8 patients)	75 (63%) 28 treated with CDV (37%)	No significant decrease or increase in survival rate for people treated with CDV

Antinori et al. [42]*	2003	HIV	101 (32 treated with CDV 32%)	CCS / OL	Cidofovir	Not mentioned	HAART (all patients)	57 (56% 19 treated with CDV 60%)	CDV treatment showed a trend towards a reduced risk of death
Taoufik et al. [30]	1998	HIV	12 (1 treated with CDV 9%)	NCCT / OL	Cidofovir	First 2 weeks: 11 times 3.0 mg / kg , then in the next 18 weeks: 2 times 3 mg / kg, 6 times 5 mg / kg, and 1 one of 3 mg / kg, subsequently, 1 infusion / 2 weeks, intravenously	HAART (all patients)	5 (42%) 1 treated with CDV (100%)	
De Luca et al. [31]	1999	HIV	1	CR	Cidofovir	Indefinite, 5 mg / kg / week for the first 2 weeks, then 5 mg / kg / 2 weeks, intravenously	HAART	1 (100%)	
Dodge [32]	1999	HIV	1	CR	Cidofovir	>1 year, 5 mg / kg / week for the first 2 weeks, then 5 mg / kg / 2 weeks, intravenously	HAART	1 (100%)	
Meylan et al. [33]	1999	HIV	1	CR	Cidofovir	Indefinite, 5 mg / kg / week for the first 2 weeks, then 5 mg / kg / 2 weeks, intravenously	HAART	1 (100%)	
Portilla et al. [34]	2000	HIV	1	CR	Cidofovir	12 doses total, 5 mg / kg / week for the first 2 weeks, then 5 mg / kg / 2 weeks, intravenously	HAART	1 (100%)	34 year old man
Portilla et al. [34]	2000	HIV	1	CR	Cidofovir	12 doses total, 5 mg / kg / week for the first 2 weeks, then 5 mg / kg / 2 weeks, intravenously	HAART	1 (100%)	28 your old woman
Torgovnick et al. [36]	2006	HIV	1	CR	Cidofovir	5 mg / kg / week for the first 2 weeks, then after 2 weeks 1 time 5 mg / kg, intravenously	HAART	1 (100%)	
Herrlinger et al. [35]	2003	MM	1	CR	Cidofovir	2 times 5 mg / kg / week, intravenously		0 (0%)	
Herrlinger et al. [35]	2003	CLL	1	CR	Cidofovir	2 times 5 mg / kg / week, intravenously		0 (0%)	
Herrlinger et al. [35]	2003	CLL	1	CR	Cidofovir	4 times 5 mg / kg / week, intravenously	Mitoxantrone, 2 times, 8 mg / m2 / day, intravenously Chlorambucil, 3 times, 3 mg / m2 / 2 days, orally	0 (0%)	
Sanchez-Quintana et al. [37]	2013	CLL	1	CR	Cidofovir	3 doses, 5 mg / kg / week, intravenously	HAART	1 (100%)	After second stem cell transplantation patient relapsed, treatment with mefloquine was unsuccessful, patient died.

Table 4.2. *Studies included in the analysis of De Luca et al.[44]

Underlying disease: CLL = chronic lymphatic leukemia, MM = multiple myeloma

Type of study: CR = case report, CS = case series, CSS = case control study, OL = open label, NCCT = Non-controlled clinical trial

Reports on mefloquine use in treatment of PML.

Author (ref.)	Year	Underlying disease	No. of patients	Type of study	Drug	Treatment (time, dose, administration)	Other treatments	Treatment response	Other relevant details
Clifford et al. [59]	2013	Various	37 (30 treated with mefloquine 81%)	RCCT / OL	Mefloquine	8, 12 or 16 weeks, 250 mg / day for 3 days, then 250 mg / week	Standard-of-care therapy for PML (all patients)	12 (32%) 12 treated with mefloquine (40%)	Study was terminated prematurely upon recom-mendation by the Data Safety Committee
Kishida et al. [50]	2010	AML	1	CR	Mefloquine	275 mg / day for 3 days, then 275 mg / week, orally		1 (100%)	
Naito et al. [52]	2012	HIV	1	CR	Mefloquine	275 mg / day for 3 days, then 275 mg / week, orally		1 (100%)	
Young et al. [55]	2012	HIV	1	CR	Mefloquine	250 mg/day for 3 days, then 250 mg / week, orally		1 (100%)	
Christakis et al. [56]	2013	HLD	1	CR	Mefloquine	250 mg / day for 3 days, then increasing dose 250 - 500 mg / week, orally Mirtazapine 30 mg / day, orally		1 (100%)	
Kobayashi et al. [57]	2013	ICL	1	CR	Mefloquine	275 mg / day for 3 days, then 275 mg / week, orally		0 (0%)	
Shin et al. [58]	2014	IN	1	CR	Mefloquine	2 months, 275 mg / day for 3 days, then 275 mg / week, orally		1 (100%)	
Gofton et al. [51]	2011	SARC	1	CR	Mefloquine	500 mg 2 times / week, then 250 mg / week, orally		1 (100%)	
Hirayama et al. [53]	2012	SARC	1	CR	Mefloquine	275 mg / day for 3 days, then 275 mg / week, orally		1 (100%)	
Beppu et al. [54]	2012	SLE	1	CR	Mefloquine	1100 mg / day first day, then 275 mg / week, orally		1 (100%)	
Kobayashi et al. [57]	2013	WM	1	CR	Mefloquine	275 mg / day for 3 days, then 275 mg / week, orally		0 (0%)	

Table 4.3. Underlying disease: SARC = Sarcoidosis, AML = acute myelocytic leukemia, SLE = Systemische Lupus erythematodes, HLD = Hyperlipidemia, ICL = Idiopathic CD4+ lymphocytopenia, WM = Waldenström macroglobulinemia, IN = immunoglobulin-A (IgA) nephropathy

Type of study: CR = case report, RCCT = Randomized controlled clinical trial

Reports on cyterabine use in treatment of PML.

Author (ref.)	Year	Underlying disease	No. of patients	Type of study	Drug	Treatment (time, dose, administration)	Other treatments	Treatment response	Other relevant details
Antinori et al. [69]	1994	HIV	4 (4 treated with cytarabine 100%)	CS	Cytarabine IV + IT	1-3 cycles, 2 mg / kg / day, for 5 days every 4 weeks intravenously, plus 2-8 cycles, 50 mg / m2 / week, intrathecally	Zidovudine, 1000 mg / day G-CSF, 3 µg / kg / day, days 6-19	0 (0%)	
Moreno et al. [70]	1996	HIV	13 (8 treated with	CCS / OL	Cytarabine IV	1-6 cycles, 2 mg / kg / day, for 5 days every 4 weeks, intravenously	HAART (3 patients)	3 (23%) 3 treated with cytarabine	Cytarabine treatment has no

			cytarabine 62%)					(38%)	significant impact on the survival rate
Hall et al. [71]	1999	HIV	57 (20 treated with Intravenous-cytarabine 35%, 19 treated with Intrathecal-cytarabine 33%)	RCCT / OL	Cytarabine IV + IT	Group 1: 24 weeks, 4 mg / kg/ day for 5 days, then 16 days only HAART, cycle repeated, Intravenously. Group 2: 24 weeks, 50 mg / week for 4 weeks, then 50 mg / 2 weeks for 8 weeks, then 50 mg / 8 weeks for 12 weeks, intrathecally.	HAART (all patients)	15 (26%) 6 treated with Intravenous-cytarabine (30%) 5 treated with Intrathecal-cytarabine (26%)	Cytarabine administered either intravenously or intrathecally does not improve the survival rate in combination with this specific HAART treatment
Enting et al. [72]	1999	HIV	35 (19 treated with cytarabine)	CCS / OL	Cytarabine IV	Various cycles, 2 mg / kg / day for 5 days each month, intravenously	HAART (all patients)	7 (20%) 6 treated with cytarabine 32%	Cytarabine treatment has no significant impact on the survival rate
Aksamit [73]	2001	Various non-HIV	19 (19 treated with cytarabine 100%)	NCCT / OL	Cytarabine IV	5 days, 2 mg / kg / day, intravenously		7 (37%)	Cytarabine treatment has no significant impact on the survival rate
Montes Santiago et al. [74]	2002	HIV	12 (2 treated with cytarabine)	CCS	Cytarabine	Unknown*	HAART (all patients) Interferon with zidovudine (2 patients treated with cytarabine) zidovudine and lamivudine (1 patient treated with cytarabine)	0 treated with cytarabine 0%	Cytarabine treatment has no significant impact on the survival rate
De Truchis et al. [75]	1993	HIV	16 (8 treated with cytarabine)	CCS / OL	Cytarabine IV	1-6 cycles, 2 mg / kg / day, for 5 days every month, intravenously 1-6 cycles, 50 mg / m2 / month, intrathecal		1 (6%) 1 treated with cytarabine 13%	Cytarabine treatment has no significant impact on the survival rate
Yokoyama et al. [62]	2008	CLL	1	CR	Cytarabine IV + IT	5 days, 2 mg / kg / day, intravenously, then 20 mg / day, intrathecal		0 (0%)	
Vulliemoz et al. [63]	2007	DM	1	CR	Cytarabine IV	2 cycles, 2 mg / kg / day, for 5 days two weeks apart, intravenously		1 (100%)	
Portegies et al. [67]	1991	HIV	1	CR	Cytarabine IV	6 cycles, 2 mg / kg / day, for 5 days every 1-4 weeks, intravenously		1 (100%)	
Portegies et al. [67]	1991	HIV	1	CR	Cytarabine IV	Indefinite, 2 mg / kg / day, for 5 days every 4 weeks, intravenously	Zidovudine 1000 mg / day	1 (100%)	
Hervas Laguna et al. [65]	1994	HIV	1	CR	Cytarabine IV	2 mg / kg / day, for 5 days every 4-6 weeks, intravenously		1 (100%)	

Garrels et al. [64]	1996	HIV	1	CR	Cytarabine IV	Not mentioned	1 (100%)
Langer-Gould et al. [61]	2005	MS	1	CR	Cytarabine IV	2 cycles, 2 mg / kg / day, for 5 days four weeks apart, intravenously	1 (100%)
O'Riordan et al. [66]	1989	NHL	1	CR	Cytarabine IV	6 cycles, 2 mg / kg / day, for 5 days every 3 weeks, intravenously After 3 cycles, 6 cycles, 50 mg / m2 / week, intrathecal	1 (100%)

Table 4.4. *Article unavailable, information from abstract. Underlying disease: CLL = chronic lymphatic leukemia, NHL = non-Hodgkin lymphoma, MS = Multiple sclerosis, DM = dermatomyositis. Type of study: CR = case report, CS = case series CSS = case control study, OL = open label, NCCT = Non-controlled clinical trial, RCCT = Randomized controlled clinical trial

Reports on drugs which activate serotonin receptors in treatment of PML.

Author (ref.)	Year	Underlying disease	No. of patients	Type of study	Drug	Treatment (time, dose, administration)	Other treatments	Treatment response	Other relevant details
Verma et al. [76]	2007	PV	1	CR	Mirtazapine	1 month, 15 mg / day, orally	HAART	1 (100%)	
Cettomai et al. [77]	2009	HIV	4	CS	Mirtazapine	Variable, 15 mg / day, orally		4 (100%)	
Focosi et al. [79]	2006	NHL	1	CR	Rispedone	10 days, 8 mg / day, orally		1 (100%)	

Table 4.5. Underlying disease: NHL = non-Hodgkin lymphoma, PV = polycythemia vera. Type of study: CR = case report, CS = case series.

Report on topotecan use in treatment of PML.

Author (ref.)	Year	Underlying disease	No. of patients	Type of study	Drug	Treatment (time, dose, administration)	Other treatments	Treatment responds	Other relevant details
Royal et al. [81]	2003	HIV	11 (11 treated with topotecan)	NCCT / OL	Topotecan	38 cycles total for all patients, 0.3-0.6 mg / m2 / day, intravenously		3 (27%)	Topotecan does not significantly improve chances of survival

Table 4.6. Type of study: NCCT = Non-controlled clinical trial, OL = open label

Reports on combinations of drugs used in treatment of PML.

Author (ref.)	Year	Underlying disease	No. of patients	Type of study	Drug	Treatment (time, dose, administration)	Other treatments	Treatment response	Other relevant details
Patel et al. [46]	2010	ICL	1	CR	Cidofovir Rispedone Mefloquine	Cidofovir, twice, 5 mg / kg / week, Intravenously Risperidone, 2 weeks, 2 mg / 12 h, orally Mefloquine, 2 weeks, first 3 days 250 mg / day, then		1 (100%)	Patient did not respond to cidofovir, risperdone and mefloquine. However the

					interleukin-7 CMX001	250mg / week, orally CMX001 and interleukin-7 not mentioned			patient did respond after initation of interleukin-7 and CMX001.
Re et al. [85]	1999	ABMT	1	CR	IL-2 Cytarabine	IL-2, 4 weeks, $9x10^6$ IU every other day, subcutaneous Cytarabine, 3 weeks, 40 mg / week, intrathecal		1 (100%)	Refused treatment after 4 weeks, still showed improvement
Goldberg et al. [86]	2002	TMBL	1	CR	Cidofovir IL-2	Cidofovir, not mentioned IL-2, not mentioned		0 (0%)	
Pöhlmann et al. [84]	2008	CLL	1	CR	Cidofovir Chlorpromazine	Cidofovir, 5 mg/kg/week, intravenously Chlorpromazine, in a period of 30 days, 12 times 200mg, 12 times 400mg		0 (0%)	
Blick et al. [87]	1998	HIV	1	CR	Cidofovir Cytarabine	Cidofovir, indefinite, 5 mg / kg / week for the first 2 weeks, then 5 mg / kg / 2 weeks, intravenously Cytarabine, first 5 days, 5 mg / kg / day, repeat every 14 days	HAART	1 (100%)	
Terrier et al. [88]	2007	CLL	1	CR	Cidofovir Cytarabine IV	Cidofovir, 6 cycles, 5 mg / kg / 2 weeks, intravenously Cytarabine, 6 cycles, 2 mg / kg / day, for 5 days every 3 weeks, intravenously		1 (100%)	
Bossolasco et al. [43]*	2005	HIV	61 (21 treated with CDV 34%, 5 treated with cytarabine 8%)	CCS / OL	Cidofovir Cytarabine IV + IT	Cidofovir, 5 mg / kg / week, intravenously Cytarabine, not mentioned	HAART (38 patients)		
Blick et al. [87]	1998	HIV	1	CR	Cidofovir Cytarabine	Cidofovir, indefinite, 5 mg / kg / week for the first 2 weeks, then 5 mg / kg / 2 weeks, intravenously Cytarabine, first 5 days, 5 mg / kg / day, repeat every 14 days	HAART	1 (100%)	
Park et al. [89]	2011	SARC	1	CR	Cidofovir Mirtazapine	Cidofovir, 3 weeks, 5 mg / kg / week for the first 2 weeks, then after 2 weeks 1 time 5 mg / kg, intravenously Mirtazapine, 3 weeks, 15 mg / day, orally		1 (100%)	
Schröder et al. [90]	2010	MS	1	CR	Mefloquine Mirtazapine	Mefloquine, 250 mg / day for 3 days, followed by 250 mg / week, orally Mirtazapine, 60 mg orally daily	Plasma exchange (5 courses)	1 (100%)	
McGuire et al. [91]	2011	MS	1	CR	Mefloquine Mirtazapine	Mefloquine, 250 mg /day for 3 days, then 250 mg / week, orally Mirtazapine, 15 mg / day, orally		1 (100%)	
Moenster et al. [92]	2012	HIV	1	CR	Mefloquine Mirtazapine	Mefloquine, 275 mg / day for 3 days, then 275 mg / week Mirtazapine, 30 mg / day, orally		1 (100%)	
Di Pauli et al. [96]	2014	CLL	1	CR	Mefloquine Mirtazapine Cytarabine IV Liposomal cytarabine IT	Mefloquine, 16 days (1-16), 250 mg / day, orally Mirtazapine, day 1-16, 30 mg / day, orally Cytarabine, day 17-21, 2 mg / kg / day, intravenously Liposomal cytarabine, day 17-21, 50 mg / day, intrathecal		0 (0%)	
Vulliemoz et al. [93]	2007	DM	1	CR	Cytarabine Mirtazapine	Cytarabine, 2 mg / kg / day, for 5 days, intravenously Mirtazapine, 30 mg/day, orally		1 (100%)	

Garrels et al. [94]	1996	CD	1	CR	Cytarabine IFN-α	Cytarabine, not mentioned IFN-α, not mentioned	Globulin-γ	1 (100%)	
Steiger et al. [95]	1993	SARC	1	CR	Cytarabine IFN-α	Cytarabine, not mentioned IFN-α, not mentioned		1 (100%)	Respons of patient only after IFN-α treatment

Table 4.7. *Studies included in the analysis of De Luca et al.[44] Underlying disease: ICL = Idiopathic CD4+ lymphocytopenia , ABMT = autologous bone marrow transplant, CLL = chronic lymphatic leukemia, TMBL = mediastinal thymic B-cell non-Hodgkin lymphoma, SARC = Sarcoidosis, MS = Multiple sclerosis, CD = Cröhn desease, DM = dermatomyositis

Type of study: CR = case report, CSS = case control study, OL = open label

5 Conclusion

To date there is no effective therapy for PML. Several drugs show promising results in case reports, but for the drugs that were evaluated in clinical trials, such as IFN-α, cidofovir, cytarabine and mefloquine, no significant effect could be shown. Cidofovir, is not effective against PML, probably because it cannot pass the BBB. However, an improved cidofovir drug, CMX001, shows promising results *in vitro* on cell lines, it can pass the BBB and in the only case report wherein CMX001 was used, among other drugs, the patient responded to treatment. Mefloquine also shows promising results *in vitro*, and even *in vivo* case reports look promising, however a clinical trial with 37 patients was terminated prematurely because no effect of mefloquine was measurable. Cytarabine is widely tested in many case reports, many of the patients responded to the drug. However, in clinical trials, no significant effect was measurable. Immunomodulating drug IL-2, shows promising results in case reports. However, it is not tested in clinical trials, therefore we cannot conclude treatment with IL-2 is effective. INF-α has been shown to not significantly improve survival benefits. In the only case report on INF-β, the patient did not respond to treatment. Chlorpromazine has only one case report in which the patient did not respond to the treatment. Drugs which activate serotonin receptors, risperdone and mirtazapine, show promising results in case reports. Moreover, mirtazapine shows even more promising results in case reports where it is used in combination with other drugs. Again, the drugs were not tested in clinical trials. In most other case reports on a combination of several drugs the patients responded to the treatment, however none of the combinations where tested in clinical trials, therefore we cannot conclude a combination of drugs to be effective against PML. Only one clinical trial reported the use of topotecan. Although only 3 out of 11 patients survived, there was a possible increase in survival time and possible decrease of lesion size due to the topotecan.

6 Discussion

PML is demyelinating disease of the brain caused by the JC virus (JCV), it has a devastating effect on patients who suffer from it and the mortality rate is very high. Although the disease is fairly rare, very specific groups, namely groups with compromised immunesystems, are a hit by it. For instance, JVC infected patients treated with Natalizumab for 25 to 48 months have risk of 11.2 in 1,000 persons years, which is very much higher than the average person with an estimated risk of 0.1 in 100,000 person years. To date, there is no effective therapy found to treat or prevent PML. Difficulties in finding such a therapy lie in a range of causes. First, there is no animal model in which new drugs can be tested because JC virus cannot replicate or cause PML in non-human primates. Therefore, to avoid safety issues, only already existing drugs that have been approved for other human conditions can be used in the treatment of PML. Second, PML is a rare disease, therefore it is nearly impossible to conduct thorough studies with a large number of patients and control group. This makes it almost impossible to prove that a drug is effective. Another problem in clinical trials is that patients have different backgrounds, their immune system can compromised by various causes like MS treatment with Natalizumab, Rituximab, Efalizumab, and/or Alemtuzumab; HIV infection; and organ transplantation. The course and outcome of PML differ for each group of patients. To be able to draw solid conclusions in a clinical trial one should have a group of patients with the same background and underlying disease and start treatment in the same stage of disease. A third issue is that research on therapies for PML have only recently became more popular. Before 1995 a very limited research was performed on JCV, PML and the treatment of PML. What makes treatment of PML more difficult is that the drug should be able to cross the BBB. It apparently has taken much time before it was realized that cidofovir is hardly able to cross the BBB. Data on the penetration in the brain is not available for many drugs. What makes the cidofovir derivative CMX001 promising is that it most likely does penetrate the brain and that in vitro inhibition of JC virus is accomplished at very low dosages. Other drugs, like mefloquine, chlorpromazine, risperdone and mirtazapine also have been proven to be able to cross the BBB. Only immunomodulating drugs can lack the ability to cross the

BBB. Although there are several promising results in case reports, like IL-2, immunomodulating drugs cannot be used in the treatment of PML in patients that just underwent a transplantation, because a boost to the immune system may lead to rejection of the transplanted organ.

The drugs that can cross the BBB, show promising results in case reports and *in* vitro studies and that have not yet been tested in clinical trials are CMX001, mirtazapine, risperdone, chlorpromazine and IL-2. Treatment with chlorpromazine and IL-2 can however, cause severe side effects. Therefore, I would recommend controlled clinical trials for the use of CMX001, mirtazapine and risperdone.

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