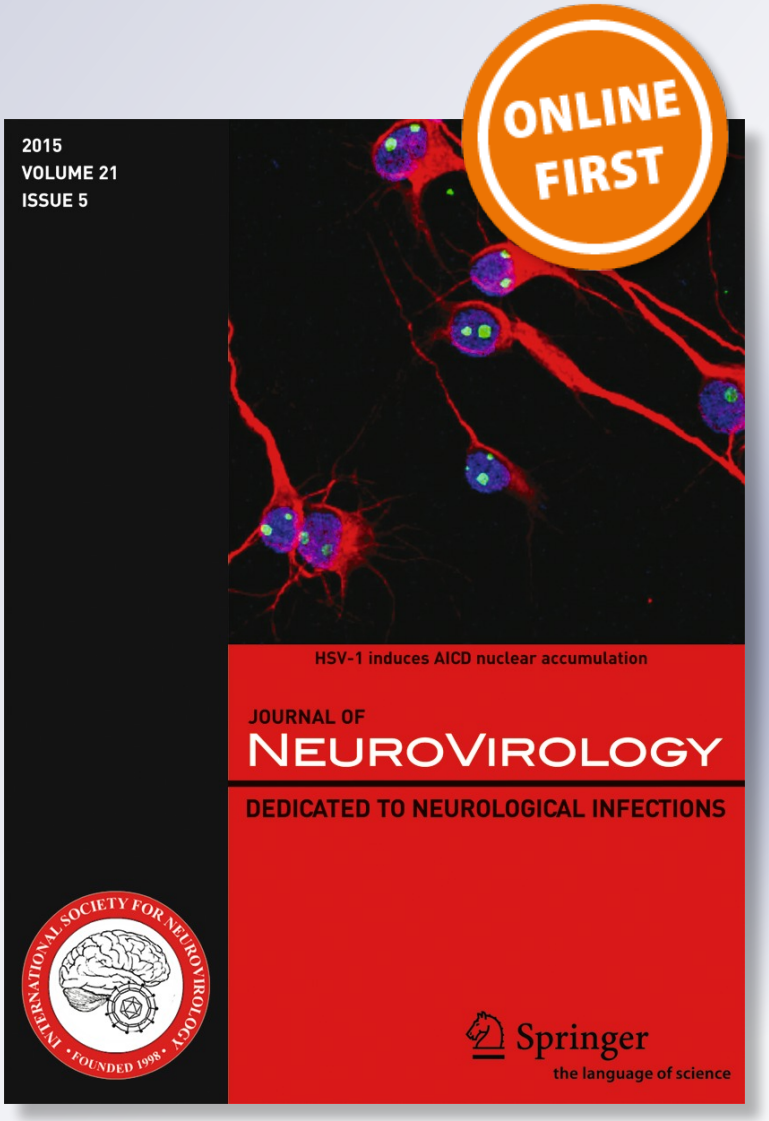
2nd International Conference on Progressive Multifocal Leukoencephalopathy (PML) 2015: JCV virology, progressive multifocal leukoencephalopathy pathogenesis, diagnosis and risk stratification, and new approaches to prevention and treatment

on behalf of the PML Consortium

Journal of NeuroVirology

ISSN 1355-0284

J. Neurovirol.
DOI 10.1007/s13365-015-0392-5



Springer

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MEETING REPORT

2nd International Conference on Progressive Multifocal Leukoencephalopathy (PML) 2015: JCV virology, progressive multifocal leukoencephalopathy pathogenesis, diagnosis and risk stratification, and new approaches to prevention and treatment

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Received: 25 September 2015 / Accepted: 5 October 2015

Conference aims

Progressive multifocal leukoencephalopathy (PML) is a rare but serious demyelinating disease of the brain that can result in severe disability or death. PML is caused by infection of oligodendrocytes by the neuropathogenic form of JC polyomavirus (JCV). There is a high prevalence of chronic but asymptomatic JCV infection in the general population. However, the incidence of PML is relatively low due to the requirement for transformation of an otherwise non-pathogenic virus to a neuropathogenic form, which is a slowly evolving process typically occuring in individuals with compromised immune system.

Notable progress has been made in defining risk factors for PML, but advances in diagnosis, accurate susceptibility prediction, prevention, and treatment lag. The "2nd International Conference on Progressive Multifocal Leukoencephalopathy, " held in Mölndal, Sweden, on August 25–26, 2015, brought together scientists, clinicians, and regulatory experts from academia, industry, and government to address emerging scientific and clinical questions, current challenges in diagnosing and treating PML, and future directions for research. This conference was sponsored by the PML Consortium, a not-for-profit pharmaceutical collaboration intended to advance research and methods to enable prediction and prevention of PML associated with immunomodulatory and immunosuppressive treatments.

Session topics included JCV virology, PML pathogenesis, diagnosis and risk stratification, and clinical trials with new approaches to treatment and prevention. Each of the 2 days

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Published online: 26 October 2015

concluded with a roundtable addressing key points from presentations, outstanding questions and knowledge gaps, and future directions. These discussions are summarized herein.

Session 1: Virology

The virology session sought to provide fundamental biologic perspectives from several viral systems that might be applied to understanding the replication and pathogenesis of JCPyV.

Lynn Enquist (Princeton) gave a keynote lecture on how alpha herpes viruses infect neurons. The technological tools he described for transfection of neurons and visualization of virus trafficking could apply directly to the study of JCPyV pathogenesis. In addition, his work suggested that the proteome change in CNS cells after infection may reveal important pathogenic mechanisms.

Viral entry and cellular trafficking were discussed for BKPyV by Michael Imperiale (University of Michigan) and JCPyV by Daniel DiMaio (Yale University). These pathways are likely conserved among the polyomaviruses and will point to key cellular components required for JCPyV trafficking. In particular, Dr. DiMaio's shRNA screen has identified specific kinases and phosphatases affecting JCV infection, which may prove excellent targets for small molecule antivirals.

Walter Atwood (Brown University) described the distribution of polyomavirus receptors in the brain and kidneys. Discussion centered on the etiology of PML-associated mutations in VP1 that have reduced infectivity against their usual target cells due to loss of affinity for the LSTc glycan motif. Ursula Neu (Max Planck) provided a detailed structural basis for the loss of cell surface glycan binding by these PML-associated VP1 mutants. These VP1 mutations are remarkably consistent across patient populations, indicating a positive selection either for affinity to a new receptor or an entirely different function. During the roundtable discussion, the biologic relevance and source of these mutations was identified as an area of high interest for future research. The roundtable discussion also underscored the current lack of clear targets for direct antiviral therapies against JCV, and how development of small molecule inhibitors of virus replication would be a welcome addition to PML therapy.

Session 2: Pathogenesis

Richard Ransohoff (Biogen Idec) reminded the audience of the recent rediscovery of lymphatic vessels in dura mater draining the walls of all main supratentorial sinus walls into deep cervical lymph nodes. He described the differences between leuko-cortical, sub-pial, and intracortical lesions in multiple sclerosis.

Hans Hirsch (University of Basel) discussed the testing of JC and BK PyV antibody responses. The seroprevalence of BKPyV (~80 %) and JCPyV (~58 %) is the best characteristic of the 12 human polyomaviruses. Curiously, the seroprevalence of BKPyV peaked between 20 and 30 years of age, whereas JCPyV seroprevalence continued to increase with age. The important role of BKPyV and JCPyV antibody testing has led to widespread use in both organ transplantation and neurology. BKPyV antibody-negative kidney transplant recipients of a BKPyV antibody positive donor show a significantly elevated risk of graft rejection as compared to BKPyV-negative donors and recipients. Rarely, primary JCPyV infection has been associated with nephropathy and kidney rejection in a BKPyV antibody negative transplant recipient. With regard to JCPyV, the antibody response has been recognized to increase with the development of PML. The intrathecal humoral IgG response to VP1-VLPs can be diagnostic for PML in cases negative for JCPyV DNA by PCR.

Dorian McGavern (NINDS, NIH) discussed how the development of two-photon laser scanning microscopy (TLSM) in vivo has led to very elegant studies of lymphocytes, monocytes, and granulocytes trafficking through blood vessels in dura mater, meninges, and brain in viral, bacterial, and parasitic infections of the brain. The LCMV model shows recruitment of these cells to the meninges. Cytotoxic T lymphocytes (CTLs) scan infected meninges. Active damage happens via synchronous extravasation of CTL-recruited monocytes and neutrophils. Neutrophils cause severe vascular leakage, the monocyte-derived macrophages attack extravascularly.

Yukiko Shishido-Hara (Kyorin University, Tokyo) described how elegant, classical light and electron microscopic studies have revealed JCPyV inclusion development. With JCPyV infection, the cell nuclei enlarge and the cell cycle is activated. Developing promyelocytic leukemia nuclear bodies (PML-NBs) appear and enlarge with cell cycle transition from S to G2. JCPyV virions replicate in PML-NBs with dot-shaped and growing inclusions, ultimately dispersing through the entire nucleus forming the well-known lattice-like structures.

Session 3: Diagnosis and risk management

The third conference session focused on the occurrence of PML and its pathogenic characteristics by investigating host factors that involve the humoral and cellular immune response to JCV infection and molecules that arise in patients with or at risk for PML.

Roland Martin (University Hospital Zurich) described the importance of T cell-mediated response to JCV infection in PML patients. He noted that CD4+ cells are found in high numbers in brains of MS patients treated with natalizumab. These CD4+ cells correlate with HLA class II expression and

are associated with clearing virus from the brain. The topic of HLA class II in patients who develop PML was discussed further by Tomas Olsson (Karolinska Institutet, Sweden). Initially using GWAS screens, HLA haplotype DRB1*15 was negatively associated with JCV serological status while DQB1*06:03 was positively correlated.

Ole Lagatie (Janssen Diagnostics, Belgium) presented multiple approaches identifying biomarkers for JCV infection. These technical approaches included viral peptide antibodies to VP2 167-15mer, deep sequencing of JCV nucleotides from CSF of MS patients with PML showing each patient has unique signature of viral regulatory region reflecting the prototype virus, and finding miRNAs that arise during infection that may have physiological roles in controlling virus. Raija Lindberg (University Hospital Basel) further defined miRNA 126 function in upregulating transcription factor SpiB in a time-dependent manner consistent with the development of PML in MS patients and binds the JCV promoter. miRNA 17 was found downregulated in MS patients treated with natalizumab but upregulated during MS relapses.

The increased incidence of PML associated with natalizumab, rituximab, infliximab, and small molecules was discussed by Joseph Berger (University of Pennsylvania). Therapies for underlying diseases like MS, malignancies, and rheumatic diseases could be classified as having a high (natalizumab) to low (infliximab and mycophenolate mofetil) risk for PML. Eamonn Molloy (St. Vincent's University Hospital, Ireland) added evidence of PML in patients with rheumatic diseases showing the very low incidence of PML in rheumatoid arthritis but a higher incidence in systemic lupus erythematosus (SLE) with the observation that B cell activation is higher in those patients. The session ended with a talk by Tilman Schneider-Hohenforf (University Clinic of Muenster) that returned to immune responses and discussed how the high incidence of PML in MS patients treated with natalizumab also showed a high seroconversion of patients from seronegative to seropositive status while on treatment.

Session 4: Clinical trials; new approaches to prevention and treatment

David Clifford (Washington University, St. Louis) began with an overview of approaches to PML treatment. Older randomized controlled clinical trials in HIV-PML failed to show efficacy of either cytosine arabinoside or cidofovir. He noted the most recent trial of mefloquine was equally disappointing with no effect on either CSF viral load or patient disability. 5-HT2 inhibitors such as mirtazapine remain in frequent use despite the lack of convincing evidence for efficacy in retrospective studies. He emphasized that the most effective treatment of PML is immune reconstitution, as was clearly established when use of cART dramatically improved outcome in HIV-PML. Whenever feasible, this approach remains the mainstay of current therapy. Promising new drugs worthy of further study include human recombinant IL-7, either alone or in combination with vaccination against JCV VP1 protein. PML-IRIS seems to invariably accompany immune reconstitution in JCV PML. Steroids are the mainstay of treatment, but the optimal timing, dose, and duration of therapy remain uncertain. Drugs such as the CCR5 antagonist maraviroc have also shown benefit in case reports.

Avindra Nath (NINDS) discussed preliminary work to establish the antiviral potential of JCV DNA oligonucleotides in treatment of PML. Surprisingly, initial studies of intracerebroventricular administration by Alzet osmotic pump in rats showed unexpected toxicity, with animals dying of multiorgan dysfunction of unknown mechanism involving the heart, kidney, and lungs. Interestingly, the oligonucleotides tested in this system were preferentially localized to neurons rather than oligodendrocytes or astrocytes, which would be problematic for PML therapy.

Milton Werner (Inhibikase Therapeutics, Atlanta) noted that his company has been exploring the antiviral effects of inhibiting host cell Abelson kinases. He discussed strategies to "clear" JCV from sites of latent and/or persistent infection. He reviewed approaches to develop an optimal assay to study JCV load in people to monitor drug efficacy. He noted that the optimization of urinary JCV assays suggests that JCV urinary shedding may be more prevalent than generally recognized with viral loads that are individually consistent and that correlate with the magnitude of serum antibody indices.

The final talk was given by Christopher Buck (NCI Center for Cancer Research), who discussed JCV vaccines. He emphasized that antibody responses against JCV may be more important in control of infection than generally recognized. Patients often develop neutralization escape mutants that may have altered binding characteristics. He noted that the holes in host immunity that allow these mutants to exist can be eliminated by JCV VLP vaccines. The VLP vaccine approach has been extremely successful in prevention of HPV infection and can provide long lasting immunity, with booster immunizations serving to broaden antibody diversity. He indicated that this may be a promising approach to prevent JCV infection as well.

Conclusions

The "2nd International Conference on Progressive Multifocal Leukoencephalopathy" provided the opportunity for researchers and clinicians in the JCV and PML fields to review current and emerging knowledge about JCV virology, PML pathogenesis, diagnosis and risk management for PML, and approaches to prevention and treatment as well as clinical trials. The conference also identified priority areas for future

research, including the biologic relevance of VP1 mutations and targets for antiviral therapies, among other topics, which may be taken up in the future. For those wishing to learn more, recordings of the conference talks are available on the PML Consortium website (www.pmlconsortium.org).

Acknowledgments The authors would like to acknowledge the Progressive Multifocal Leukoencephalopathy Consortium's Board of Directors for its support of the research conference; the conference speakers and attendees who contributed to the discussions presented herein; Drs. Len Calabrese and Marianne Gerber, who also contributed to organization of the conference; and the PML Consortium Secretariat and AstraZeneca for their organizational support.

Compliance with ethical standards

Conflict of interest AstraZeneca/MedImmune, Pfizer, and Roche are PML Consortium member companies. D. Pavlovic and M. Gerber are members of the PMLC Clinical Working Group. A. Patera, S. Butler, and R. Elston are members of the PMLC Research Working Group. Dejan Pavlovic and Andriani Patera are employees of MedImmune, the global biologics research and development arm of AstraZeneca, and own stock in AstraZeneca. Robert Elston and Marianne Gerber are employees of and own stock in F. Hoffmann-La Roche AG. Scott Butler and Anne Ryan are employees of and own stock in Pfizer Inc.

David B. Clifford, Robert L. Garcea, Eugene O. Major, Kenneth L. Tyler, and Thomas Weber are members of the PML Consortium Scientific Advisory Board. Kenneth Tyler has served as a scientific consultant or scientific advisory board member for Hoffman La Roche, Biogen, Genentech, Johnson & Johnson, and Pfizer.

Paola Cinque is a PML Consortium grantee and has received funding from the Consortium to support her research.

Ilse Peterson is an employee of Drinker Biddle & Reath, LLP, which serves as the PML Consortium Secretariat, and has no conflicts of interest to report.

Related online content

Since work described herein is primarily unpublished, titles of presentations are referenced below to allow readers to view the recorded presentations posted on www.pmlconsortium.org.

Atwood W. Human polyoma virus receptor distribution. Session 1

Berger J. PML associated with neurologic therapies. Session 3

Buck C. Developing vaccines against JC polyomavirus. Session 4

Clifford D. PML treatment/IRIS. Session 4

DiMaio D. Cellular factors required for infection by small DNA viruses. Session 1

Enquist L. Axons, the front line sensors of alpha herpes virus PNS invasion. Session 1

Hirsch H. Testing JC and BK PyV antibody responses: why and how. Session 2

Imperiale M. Trafficking of BKPyV to the nucleus. Session 1

Legatie O. Biomarkers for JCV infection. Session 3

Lindberg R. Molecular markers for a PML risk in natalizumab treated MS patients. Session 3

Martin R. T cell immunity in PML and MS. Session 3

McGavern D. T cell surveillance of the acute and persistently infected brain. Session 2

Molloy E. PML associated with autoimmune and autoinflammatory diseases. Session 3

Nath A. Antisense to JCV. Session 4

Neu U. Polyomavirus structure, receptor interactions. Session 1

Olsson T. Host genetics in the control of JCV infection. Session 3

Ransohoff R. Blood brain barrier, CNS immune surveillance. Session 2

Schneider-Hohendorf T. Natalizumab treatment is associated with increased anti-JCV antibody seroconversion. Session 3

Shishido-Hara Y. JC viral inclusions in PML: PML-NBs. Session 2

Werner M. Host Abelson-kinase inhibitors. Session 4