Summary of the Merkel Cell Interest Group Meeting at the IID 2013

Merkel Cell Carcinoma: New Insights & Emerging Therapeutics

Tuesday, May 7, 2013

Edinburgh, Scotland

Summaries courtesy of Victor Huang, MD
Edited by Paul Nghiem, Jayasri Iyer & the Speakers; revised Aug 7, 2013

Speakers/Topics (detailed in following pages):

1) Merkel polyomavirus in MCC: Mechanisms of transformation and progress toward a new gold standard for viral detection (Keynote)
James DeCaprio, MD

2) CD8+ T cell epitopes from Merkel polyomavirus oncoproteins elicit anti-viral T cell responses exclusively in Merkel cell carcinoma patients (Invited speaker)
Sine Hadrup, PhD

3) Defective expression of class I antigen presentation pathway members in Merkel cell carcinoma may underlie low MHC I expression and contribute to immune evasion
Victor Huang, MD

4) Merkel cell carcinoma-specific T cells fluctuate with tumor burden and express therapeutically targetable PD1 and TIM3 exhaustion markers
Paul Nghiem, MD, PhD

5) Screening for cellular binding partners of the Merkel cell polyomavirus-small T antigen by yeast two-hybrid system
Harrison Nguyen

6) UISO cell line is not representative of Merkel cell carcinoma
Isaac Brownell, MD, PhD

7) Tumour-associated immune cells in primary Merkel cell carcinoma
Rachel Wheat, MSc

8) Improving Guidelines and Developing Trials for MCC in the UK
Neil Steven, MD

9) Towards immune-stimulating clinical trials for MCC
Isaac Brownell, MD, PhD

1) **Merkel polyomavirus in MCC: Mechanisms of transformation and progress toward a new gold standard for viral detection**

- James Decaprio, MD

Twelve human polyomaviruses have been discovered to date, including the well characterized JC and BK viruses. Infection is characterized by persistent, lifelong infection and prevalence in the general population is high (60-80%). Merkel cell polyomavirus (MCPyV) is unique in its association with carcinogenesis in humans, mediated by the expression of the viral T antigen that is alternatively spliced into large T, small T, and a 57 kD protein. Since its characterization in 2008, MCPyV has been associated with ~80% of MCC tumors by several groups. The development of new, more sensitive antibodies suggest that MCPyV infection may be present in nearly 100% of MCC tumors that are CK20-positive. The mechanisms of transformation are also unique. MCPyV large T antigen binds to Rb, but not to p53 (in contrast to SV40). p53 mutations are found in <10% of MCC tumors, suggesting a minor role for it in carcinogenesis.

2) **CD8+ T cell epitopes from Merkel polyomavirus oncoproteins elicit anti-viral T cell responses exclusively in Merkel cell carcinoma patients**

- Sine Hadrup, PhD

As a tumor associated with MCPyV infection, epitopes from virally derived proteins ought to be presented on MHC I molecules. A high throughput method for identifying these epitopes that minimizes the amount of required primary patient material would be ideal for the development of MHC-peptide tetramer reagents. *In silico* prediction of epitopes in VP1, large T, and small T antigens was undertaken. Confirmation of epitope binding to MHC was undertaken using UV-sensitive conditional MHC ligands, revealing binding of ~40% of predicted epitopes. By using combinatorial tetramer staining of peripheral T cells from patients, it was possible to identify MCPyV specific T cells. Virus specific T cells were identified in 22/38 patients with MCC compared to 6/30 healthy patient controls. Interestingly, T cell reactivity to the T antigen oncoproteins was only found in MCC patients and not in control subjects. Longitudinal examination of patients showed that these T cells remained present in the circulation for years after tumor excision. Functional killing with T cells isolated with this technique was confirmed with K562 cells transfected with HLA and large T antigen. Utilizing these methods, virus-specific T cells can be isolated and are being used in adoptive cell transfer therapy of MCC.

3. **Defective expression of class I antigen presentation pathway members in Merkel cell carcinoma may underlie low MHC I expression and contribute to immune evasion**

- Victor Huang, MD

Initial studies aimed at epitope discovery revealed that not only are viral epitopes missing from the surface of MCC cell lines, but there is an absence of epitopes from ubiquitous housekeeping genes as well. Further investigation revealed downregulation of surface MHC I in 3 of 4 verified MCPyV positive cell lines. Reduced MHC I expression has been noted in MCC primary tumors representing a potential mechanism for immune evasion. Transcription of MHC I genes was noted to be variably reduced in 3 of the 3 cell lines with reduced surface MHC I expression. The dramatic and uniform decrease in surface MHC I expression in these cell lines, however, suggested additional mechanisms at play. Examination of members of the peptide loading complex revealed that transcription of TAP1, TAP2, and tapasin were downregulated as well. Treatment with IFN-gamma rescued surface MHC I expression completely, while only partially increasing MHC I transcription. On the other hand, transcription of TAP1, TAP2, and tapasin were dramatically increased. Comparison of clinically available IFNs revealed that IFN-gamma was much more potent than type I IFNs in rescuing MHC I surface expression.

4. **Merkel cell carcinoma-specific T cells fluctuate with tumor burden and express therapeutically targetable PD1 and TIM3 exhaustion markers**
   - Paul Nghiem, MD, PhD

   MCPyV-specific CD8 T cells were detected from the peripheral blood in 7 of 11 patients with MCPyV positive tumors and HLA-A24. The number of these T cells increased with tumor recurrence and disease burden. Evaluation of surface markers for activation and exhaustion phenotypes was undertaken. Higher levels of PD1 and TIM-3 (markers of T cell exhaustion) were observed compared to T cells specific for EBV and CMV in these patients. Moreover, as the amount of CD8 tumor infiltration increased, the amount of PD-L1 produced in MCC tumors increased. Taken together, MCPyV specific T cells can be used along with anti-MCPyV antibodies as markers for tumor burden. Moreover, PD1 and TIM3 represent important exhaustion markers that may be used as therapeutic targets in order to overcome the immune suppressive tumor environment.

5. **Screening for cellular binding partners of the Merkel cell polyomavirus-small T antigen by yeast two-hybrid system**
   - Harrison Nguyen

   A yeast two-hybrid system was used to explore potential binding partners to MCPyV small T antigen. “Prey” proteins were derived from the Universal Human Normalized library and Bone Marrow library. PP2A, a serine/threonine phosphatase, was identified as a small T binding partner confirming previous reports. In addition, beta-hemaglobin and cereblon (CRBN) were identified as having small T interactions. CRBN is required in development for proper limb development and is a component of E3 ubiquitin ligase.

6. **UISO cell line is not representative of Merkel cell carcinoma**
   - Isaac Brownell, MD, PhD

   UISO is atypical of most MCCs. Isolated from the thigh of a 46-year-old woman, it was epidemiologically unusual. It also stained only focally with NSE and CAM5.2. Finally it grows in culture as an adherent layer unlike other MCC cell lines. However, UISO remains a commonly used cell line in MCC studies. Using whole transcriptome gene expression arrays, UISO was compared to MKL-1, WaGa, and primary MCPyV positive and negative MCC tumors. It was demonstrated that UISO represents a phenotype that is distinct from typical MCCs and is more genetically similar to small cell lung cancers than MCC. In addition, UISO cells grown as xenografts in immunocompromised mice demonstrated a distinct histology and immunophenotype compared to WaGa xenograft tumors.

7. **Tumour-associated immune cells in primary Merkel cell carcinoma**
   - Rachel Wheat, MSc

   Histologic examination of MCC tumors revealed that CD8+ cells are usually present in MCC tumors. However, they are often restricted to the outer periphery of tumors within fibrovascular septae that contain CD34+ vessels and D240+ lymphatics. Poor penetration into the body of MCC tumors was observed in 18/20 tumors investigated. In addition, CD8+ cells were also found to be functionally compromised with decreased CXCR3 and granzyme B expression, regardless of whether they were infiltrating or restricted to fibrovascular septae. CXCL12 was found in these septae and hypothesized to be responsible for restriction of CD8+ cells to the periphery, however, the T cells were negative for the cognate receptor of CXCL12, CXCR4.

8. Improving Guidelines and Developing Trials for MCC in the UK

- Neil Steven, MD

The evidence for determining appropriate treatment of MCC is sparse and largely based on anecdotal reports and expert consensus. A survey of dermatologists in the UK revealed a wide degree of variability in treatment practices, particularly when it came to sentinel lymph node biopsy and adjuvant XRT, suggesting that a standard of care has yet to be established. A proposal from the UK NCRI (Natl Cancer Research Inst) CSG (Cancer Studies Groups) was outlined for a phase III trial to randomize patients to XRT alone, excision and XRT, and excision alone for the treatment of primary MCC. This is intended to begin to address the lack of data as well as establish a biobank for translational research. Decisions on adjuvant treatment will be left to primary teams taking care of the patients to maximize the therapeutic flexibility available within the trial. Concerns remain, however, in that decisions on adjuvant therapy, sometimes based on SLNB, will drive primary treatment making randomization impossible. The trial design is currently being revised to address these concerns.

9. Towards immune-stimulating clinical trials for MCC

- Isaac Brownell, MD, PhD

A review of immune therapy based trials that are currently or will be recruiting patients was undertaken. Caveats to trials were discussed including the possibility that delayed responses to immunotherapies may make it difficult to see benefit in the setting of rapidly progressive disease. Adoptive T cell transfer with IL-2 given after XRT or IFN-beta is currently recruiting at the Fred Hutchinson Cancer Research Center. IL-12 gene therapy using electroporation to introduce exogenous plasmid is recruiting as a partnership between the Fred Hutchinson Cancer Research Center and UCSF. The Medical University of Graz in Austria is investigating the potential of F16-IL2, an anti-Tenascin C antibody-IL-2 fusion protein, for the treatment of MCC. Anti PD-1 antibody is being explored through the Cancer Immunotherapy Trials Network. Finally, a trial of ipilimumab will be underway at the NIH recruiting patients with measurable disease. 10mg/kg doses are planned q 3 weeks for 4 cycles.

Goals of the Merkel cell carcinoma Multi-center Interest Group (MMIG)

- Promote communication and collaborative studies on MCC
- Enhance access to patient data and specimens
- Expand evidence-based care for MCC

The homepage for MMIG contains photos, meeting summaries and more at:
http://merkelcell.org/MMIG.html

MMIG is funded in part by donations from Merkel cell carcinoma patients.

Please note that in some cases these summaries reflect unpublished data and are provided to help MMIG members manage their patients and give an overview of what is being done at different centers for care and research.

Attendees at Edinburgh Meeting, May 7, 2013

David Blackbourn	University of Birmingham (UK)
Christophe Blanchetot	arGEN-X (Belgium)
Isaac Brownell	NCI/NIH, Bethesda, MD (US)
Rachael Clark	Harvard/Brigham & Women's Hospital (US)
James DeCaprio	Dana-Farber Cancer Institute, Harvard Medical School (US)
Jennifer Garioch	Norfolk & Norwich University Hospital (UK)
Sine Reker Hadrup	CCIT Herlev Hospital, Copenhagen (Denmark)
Catherine Harwood	Queen Mary, London (UK)
Christoph Hoeller	Medical Univ. of Vienna (Austria)
Victor Huang	Harvard/Brigham & Women's Hospital (US)
Hiromi Kimura	Saga University (Japan)
Rikke Lyngaa	CCIT Herlev Hospital, Copenhagen, Denmark
Jerry Marsden	University of Birmingham (UK)
Laurent Misery	University of Brest, (France)
Marc Moncrieff	Norfolk & Norwich University Hospital (UK)
Kotaro Nagase	Saga University (Japan)
Paul Nghiem	University of Washington, Seattle (US)
Harrison P. Nguyen	Baylor College of Medicine (US)
Lalit Pallan	University of Birmingham (UK)
Keyoumars Soltani	University of Chicago (US)
Neil Steven	University of Birmingham (UK)
Monique Verhaegen	University of Michigan (US)
Rachel Wheat	University of Birmingham (UK)