

**Molecular Imaging Approaches to the Evaluation of Infectious Diseases – A Prospective
Assessment**

Thomas M. Bocan, PhD¹

¹Ke'aki Technologies LLC
Molecular and Translational Sciences
US Army Medical Research Institute of Infectious Diseases
1425 Porter Street
Ft. Detrick, MD 21702

Running Title: Molecular imaging of viral infections

Keywords: *In Vivo* Imaging, PET, SPECT, CT, Optical, VEEV, Ebola, Viruses

Address Correspondence to:

Thomas M. Bocan, PhD
US Army Medical Research Institute of
Infectious Diseases (USAMRIID)
1425 Porter Street
Ft. Detrick, MD 21702
Phone: 301-619-2647
Email: Thomas.m.bocan.ctr@mail.mil

Address Reprint Requests to:

Thomas M. Bocan, PhD
US Army Medical Research Institute of
Infectious Diseases (USAMRIID)
1425 Porter Street
Ft. Detrick, MD 21702
Phone: 301-619-2647
Email: Thomas.m.bocan.ctr@mail.mil

ABSTRACT

Molecular imaging, primarily defined as nuclear and optical imaging have been used extensively in neuroscience, cardiovascular, inflammation and oncology research to evaluate disease progression and drug intervention. Opportunities exist to extend the use of the tools developed for the above disease areas into infectious diseases caused by lethal and debilitating BSL3/4 pathogens such as alphaviruses and filoviruses. Virus distribution and replication can be monitored in animal models preclinically using reengineered pathogens containing nuclear or optical bioreporters. Use of the bioreporters will establish the tissue burden of virus, provide a means of assessing the effect of direct antiviral agents on pathogen replication and identify reservoirs of virus that may appear late in the disease that are left unaffected by drug therapy. Measuring tissue viral levels by imaging more directly assesses the degree of infection and when related to tissue levels of a PET radiolabeled drug, a relationship between plasma and tissue drug and virus levels can be established to better direct therapeutic intervention. The consequences of infection can be assessed dynamically using specific PET/SPECT tracers developed to assess inflammation, hypoxia, metabolism, perfusion, apoptosis, hemorrhage and end organ damage. Colocalization of radiolabeled drug, virus and changes in tissue function can be used to define the stage of disease progression, monitor drug efficacy and allow for a greater mechanistic understanding of the viral infection. Given the sensitivity and specificity of molecular imaging approaches, coupling measures of viral load, tissue response and proteomic and genomic biomarker approaches may better define markers of BSL3/4 infection and disease progression.

Introduction

While molecular imaging has been used to characterize disease progression and evaluate drugs in the areas of neuroscience, cardiovascular, inflammation and oncology, application of imaging in infectious disease is limited [for review see reference 1]. Imaging approaches have the potential to more fully and dynamically characterize the disease process in relation to tissue viral load rather than plasma viral load in the presence and absence of drug therapy. Establishing stages of infection allows one to better define the appropriate pharmacologic regimen, e.g., direct antiviral vs anti-inflammatory agent, and to select cohorts of animals at relatively the same stage to study disease mechanisms and reduce variability upon pharmacologic treatment. Current approaches to the study of infectious diseases involves reduced mortality or extended time to death as the efficacy endpoint, plasma viremia as an index of infection and serial necropsies to establish the consequences of infection. In contrast to the current approaches, imaging allows one to track the progression of the infection in the same animal, to perform mechanistic studies with defined molecular probes, to initiate pharmacologic therapy at disease-driven time points and to explore novel mechanisms of drug action.

Imaging tools developed for studies in neuroscience, cardiovascular, inflammation and oncology can be adapted for use in the study of infectious diseases. The main objective of this manuscript is to review molecular imaging approaches that are amenable for use in evaluating processes involved in viral infection that are defined by histologic assessment but from a dynamic rather than static perspective. The focus of the paper will be on the alphavirus, Venezuelan equine encephalitis virus, and the filovirus, Ebola virus, and on what molecular imaging approaches can be applied preclinically and potentially, clinically to the study of these viral infections.

Pathogenesis of alphavirus and filovirus infection The genus alphavirus from the family, *Togaviridae*, contains RNA viruses that cause disease in animals and humans. The old-world alphaviruses include Chikungunya, O'nyong-nyong and Ross River viruses which result in polyarthrititis. New world alphaviruses consist of Venezuelan (VEEV), eastern (EEEV) and western equine encephalitis (WEEV) and cause overt encephalitis. VEEV is classified as a Biosafety Level-3 (BSL3) pathogen which is of concern as a bioweapon because of ease of production, potential for aerosolization, stability, high infectivity and ability to cause debilitating encephalitis. VEEV is lethal in adults (10%) and children (35%) who demonstrate neurological symptoms [2] and there are no small molecule therapies for treatment. Vaccines such as TC83, a live attenuated virus vaccine, are currently under evaluation.

In mouse and non-human primate (NHP) models of VEEV infection, the pathologic process is amenable to evaluation by molecular imaging [3]. Post-intraperitoneal or subcutaneous (sc) infection, virus appears in draining lymph nodes, spleen, thymus and bone marrow. In mice, virus appears in the exocrine pancreas, hepatocytes and dental pulp at later time points. Neuro-invasion is noted 36-48 h post-sc infection and within 16 h post-nasal infection in mice. Neuro-invasion is also noted in NHP but the timeframe for appearance varies. Following infection, inflammatory infiltrates of macrophages, dendritic cells, astrocytes are noted in the infected organs and in regions of the brain such as the thalamus. Lymph node necrosis followed by hyperplasia is observed in both mouse and NHP models. In general, virus replication and a robust inflammatory response are noted in a broad range of organs post-VEEV infection.

Ebola virus (EBOV) is a lethal virus which causes hemorrhagic fever and it is of global impact as evidenced by the recent outbreak in West Africa in 2014/5. Ebola virus (EBOV) is a

negative-stranded, enveloped RNA virus of the *Filoviridae* family. EBOV is classified as a BSL4 pathogen. Rodents, primarily mice, and non-human primates are the main models for evaluating countermeasures against filoviruses. Except for neonatal, SCID, or Type I IFN receptor knockout mice, adult immunocompetent mice are not susceptible to wild-type EBOV and require several *in vivo* passages of the wild-type EBOV to generate a mouse adapted strain [4]. Non-human primates (NHP), including rhesus or cynomolgus macques, are sensitive to wild-type EBOV, recapitulate lethal disease and exhibit the coagulation disorders and lymphocyte apoptosis noted with the progression of the human disease course [4]. In the NHP Cynomolgus macaque model of Ebola virus disease as in humans, extreme alterations in liver enzymes, thrombocytopenia and lymphopenia, elevations in d-dimers consistent with disseminated intravascular coagulation, organ hypoperfusion and multiple organ failure leading to death are noted. Plasma viremia of 10^4 pfu/ml by Day 3 leading to rapid expansion to 10^7 pfu/ml by Day 6, i.e., time of death, is observed. Involvement of inflammatory cells appears by Day 2 in spleen and lymph nodes but progresses to other organs such as liver, adrenal, kidney, bone marrow, tonsil, gastrointestinal track by day 4 [5,6] By day 6, infection of tissue parenchymal cells appears and significant end organ damage is noted in lymph node, spleen, gastrointestinal tract, liver, and adrenal glands. Thus like VEEV, dissemination of virus initiates the infection process and a major inflammatory reaction occurs in response to the infection which culminates in end organ pathology leading to death or incapacitation of the animal.

Classical approaches to the study of BSL3/4 infections involve serial necropsies, tissue dissection, plaque counts and immunohistochemistry to demonstrate the distribution of pathogens and degree of infection, drug extraction from tissues as a measure of drug exposure and mortality as endpoints for assessing drug efficacy. However, numerous other questions

requiring a dynamic assessment of infection and pathophysiology remain unanswered. In addition to knowing that an animal has been exposed to an aerosolized dose of a pathogen one needs to determine the dose of pathogen received within the lung and the relationship of breathing kinetics to dose. Since distribution of the pathogen in the tissues and organs may differ acutely post-infection versus late in infection, it is important to map the differences so as to insure proper drug exposure late in infection. While the degree of viremia and plasma drug levels may be good measures to demonstrate infection and the potential for drug efficacy, respectively, correlating measures of tissue pathogen and tissue drug concentrations may be more important to link efficacy with drug dose. In some instances like with VEEV, one must know both the time course and route of brain infection and whether the therapeutic agent under development reaches the brain in sufficient quantities to result in a beneficial effect. In cases of EBOV infection, it is important to assess whether reservoirs of virus remain in immune privileged organs and if therapeutics under development distribute to those organs. In all cases noted above, dynamic real-time measurements can provide important data to track infection, host response, time course for intervention and to assess drug efficacy rather than rely on mortality as the sole endpoint. By using dynamic measures of drug efficacy such as molecular imaging, one might be better able to discern potential mechanisms for efficacy and potential targets/target organs to refine the drug discovery approach and/or compound.

Monitoring virus replication and distribution and drug biodistribution Fundamental processes following viral infection are amenable to evaluation by molecular imaging. Upon infection, the amount of infectious agent, tissue distribution and time course of infection are important in understanding the natural history of the virus. Assessment of viral replication dynamically allows one to establish the tissue burden of virus, provide a means of assessing the

effect of direct antiviral agents on pathogen replication and identify reservoirs of virus that may appear late in the disease that are left unaffected by drug therapy.

Optical imaging approaches have been developed to directly assess viral replication and track virus distribution in animal models of disease. Luciferase which requires exogenous luciferin administration or the lux operon containing both luciferase and luciferin has been transfected into murine herpesvirus-4 [7], varicella zoster [8], Venezuelan equine encephalitis [9], Chikungunya [10], cowpox [11] and murine cytomegalovirus [12] viruses. Incorporation of the optical reporter in the above viruses was shown to not markedly affect growth rate, survival and virulence and functioned similarly to the wild type pathogen. Factors influencing the expression of the bioreporter were the choice of promoter, size of the amino acid flanking regions and whether the construct was inserted randomly or site-specifically. The bioreporters allowed for whole body detection of pathogens, assessment of the time course of infection and the bioluminescent signal was highly sensitive with the signal intensity correlating to pathogen number.

Refinements in the placement of the optical bioreporter and the type of bioreporter construct used have created VEEV constructs which more closely mimic the authentic parental virus [13]. Insertion of the small nanoLuc (nLuc), i.e., approximately 500 nucleotides, reporter gene between the capsid and PE2 as an autocleaving element or as a nonstructural protein 3 (nsP3) fusion virus has resulted in a stable expression system which remains intact *in vitro* and *in vivo* through multiple rounds of replication. Insertion of the larger, 1650 nucleotides, firefly luciferase (fLuc) in similar locations resulted in transient expression that was lost over time which is indicative of the reporter being excluded from the virus upon replication. *In vivo* experiments in mice with the nLuc construct revealed virus at the site of insertion (footpad)

within 6 hours post-infection with signal appearing in the inguinal, lumbar and cervical lymph nodes, abdominal region and snout by 24 h post-infection. The bioluminescence signal continued to expand through 48 h which suggested the presence of viral replication. Similar studies were performed with EEEV, Sindbis virus and Chikungunya virus with the two reporter constructs and similar results as to VEEV were noted. These data taken together indicate that bioreporters for use in assessing the location and natural history of virus infection can be created; however, the location and size of the insert are important considerations to insure retention of the reporter in the altered virus.

While optical imaging has demonstrated utility in tracking viral replication and distribution, its use is limited to mice and more specifically nude mice since hair attenuates the bioluminescent signal. Near infra-red fluorescent imaging is an alternative for improving signal and reducing background but limitations related to depth of penetration and the need for nude mice remains. Optical or fluorescent imaging for detection of viruses like VEEV that enter the brain may be problematic due to the attenuating characteristics of the skull. Nuclear imaging approaches such as positron emission tomography (PET) or single photon emission computed tomography (SPECT) are alternative bioreporter approaches which use short-lived radioisotopes and are amenable for use in rodents and NHP.

There are several reporter constructs reverse engineered into viruses utilizing enzymes and receptors which can be used to assess viral replication and distribution across rodent to non-rodent species. The most often used reporter is herpes simplex virus thymidine kinase (HSV-tk1, 1128 nucleotides) with either [$^{124}\text{I}/^{18}\text{F}$]-29-fluoro-29-deoxy-1- β -D-arabinofuranosyl-5-iodouracil ([$^{124}\text{I}/^{18}\text{F}$]-FIAU) or [^{18}F]-9-[4-fluoro-3-(hydroxymethyl)butyl]guanine ([^{18}F]-FHBG) as the enzyme substrate and PET as the imaging modality [14]. The viral thymidine kinase is translated

in the cell along with the viral RNA and phosphorylates the exogenously delivered radiolabeled [$^{124}\text{I}/^{18}\text{F}$]-FIAU or [^{18}F]-FHBG substrate, trapping it in cells, and thereby labeling cells which have been infected with the virus. Alternative reporter enzymes such as human mitochondrial thymidine kinase 2 (hmtk2; 1930 nucleotides) [15] and human deoxycytidine kinase (hdCK; 2470 nucleotides) [15] utilizing 2'-deoxy-2- ^{18}F -5-methyl-1- β -L-arabinofuranosyluracil ([^{18}F]-FMAU) and [^{18}F]-2'-deoxy-2'-fluoroarabinofuranosylcytosine as substrates, respectively, have been developed to avoid immunologic reactions in humans.

Several receptor based reporter systems such as the rat/human sodium iodide symporter (NIS) [16-18; 1929 nucleotides] and rat dopamine D2 receptor (D2R) [19; 2500 nucleotides] have been incorporated into viruses. The NIS reporter system is attractive because $^{123,124,131}\text{I}$, $^{99\text{m}}\text{TcO}_4^-$, and $\text{Na}^{18}\text{F}_4\text{B}$ can be used [19]. However, the size of NIS insert into a virus may present challenges with regards to it being removed from the virus through multiple replication cycles. Incorporation of the dopamine D2 receptor (D2R) into a virus and imaging receptor expression with [^{18}F]-fluoromethylspiperone has been utilized; however, the use of a mutant (D2R80A) proved more valuable because it completely uncoupled ligand binding with activation of G-protein linked signaling and adverse effects on the transduced cells [19]. Given the presence of D2R in the brain precludes the use of the D2R as a bioreporter for viruses known to get into the brain such as VEEV because of the high background signal from the constitutively expressed brain receptors.

While there are no reports of reengineered VEEV or Ebola virus to contain HSV-tk1 or NIS, one might model the VEEV insertion site for the optical reporters as a potential region for placement of the nuclear bioreporters. The advantage of nuclear imaging approaches, i.e., PET/SPECT, lies in the ability to image NHP, to assess brain uptake of the viruses and to track

virus replication in deep organs which are not easily discerned by optical imaging unless the organs are removed post-necropsy and evaluated *ex vivo*.

In addition to detecting viral replication and distribution, the biodistribution of the target of interest and therapeutic is equally important to show the presence of the target, to insure brain uptake for therapeutics against VEEV and to colocalize drug with site of the viral infection. Numerous PET tracers have been developed to brain receptors and such tools have been used in the development of therapies for CNS disorders [20, 21]. For example, radiolabeled ^{11}C and ^{18}F tracers to detect the amyloid or tau protein burden in Alzheimer's patients have been synthesized and used to correlate brain pathology with measures of cognitive decline [22] and to track the progression of disease in genetically engineered mice [23]. PET tracers specific for brain receptors, e.g., dopamine D2, serotonin, have been used to not only demonstrate the distribution of such receptors but also correlate brain receptor occupancy required for efficacy with plasma drug levels [24]. In addition to application in neuroscience, PET tracers have been utilized in oncology to demonstrate the presence of a target [25], generalized changes in tumor metabolism or proliferation [25] and both tumor uptake of the therapeutic and time course of drug retention [26]. In general, the types of approaches utilized in CNS and oncology can also be useful in the study of infectious diseases; however, unlike in these areas where the organ or tumor of interest is well defined virus distribution is more disseminated, potentially in low abundance early in the disease process and less well localized to specific organs.

By engineering reporters into viruses which are amenable to optical, PET and SPECT imaging and radiolabeling therapeutics, one can monitor virus and drug distribution, colocalize both agents and assess direct antiviral activity *in vivo* through reduction in reporter signal associated with reduced viral replication. Through monitoring the degree of viral replication and

tissue distribution, defined stages of infection can be delineated and the consequences of such infection can be evaluated using molecular imaging probes specific to cellular responses and/or metabolic changes. Characterization of viral infection and organ response in the presence and absence of therapeutics by molecular imaging paints a 'picture' of the infectious disease process that allows one to establish drug-target interactions, define drug mechanisms, assess secondary drug effects and dynamically monitor disease progression or regression.

Assessing the consequences of viral infection A major component of VEEV and Ebola virus infection is a robust inflammatory response followed by end organ damage and/or failure. Molecular imaging tools exist and in this section probes available to assess components of VEEV and Ebola virus infection such as inflammation and changes in metabolism, hypoxia, perfusion, vascular permeability and apoptosis will be reviewed.

Inflammation and PET/SPECT tracers for the assessment of macrophage accumulation and microglia/astrocyte activation in the brain have focused on monitoring the 18kDa translocator protein (TSPO) which is upregulated in inflammatory cells and activated microglia. The original TSPO tracer was [^{11}C](R)-PK11195 and a significant amount of preclinical and clinical work has been performed with this tracer. However, numerous novel radioligands such as [^{18}F]-DPA714, [^{11}C]-DPA713, [^{18}F]-PBR28, [^{18}F]-PBR111, [^{11}C]-DAA1106, [^{18}F]-DAA1106, [^{11}C]-SSR18075, [^{11}C]-CLINME, [^{123}I]-CLINDA and [^{11}C]-vinpocetine have been developed over the years [27]. TSPO radiotracers have proven to be selective markers of activated inflammatory cells in both the brain and peripheral organs. The TSPO radiotracer, [^{18}F]-DPA714, has been evaluated in animal models of epilepsy, stroke [28], quinolinic acid-induced striatal inflammation [29], inflammatory bowel disease [30] and rheumatoid arthritis [31] to assess the degree of inflammation and specificity of binding of the tracer to inflammatory

cells. In a model of lipopolysaccharide-induced lung inflammation, [^{18}F]-FEDAC, an additional radioligand for TSPO, identified the accumulation of neutrophils and macrophages in the lung and defined the time course of inflammation [32]. In all cases noted above, the specificity of the TSPO radiotracers was demonstrated by blocking binding with unlabeled tracer or another TSPO tracer and by demonstrating through immunohistochemistry that the areas of radiolabeling were both inflammatory cells and sites of TSPO expression. Thus, based on these data, one can conclude that the use of TSPO radiotracers is a viable method to track inflammatory cells and activated microglia in both brain and peripheral organs and as such can be a valuable tool for the assessment of neuroinflammation observed upon VEEV infection and tissue macrophage accumulation following Ebola infection.

The metabolic tracer, ^{18}F FDG, is an alternative PET tracer for use in assessing inflammation and consequences of infection. In areas of inflammation, macrophages are more metabolically active and higher uptake of ^{18}F FDG relative to non-inflamed tissues is noted. ^{18}F FDG has been used to assess inflammation associated with large vessel vasculitis, vascular graft infection, sarcoidosis and musculoskeletal infections [33]; however, the use of ^{18}F FDG cannot discern the difference between infection and aseptic inflammation. Direct *ex vivo* labeling of leukocytes with ^{18}F FDG and re-infusion has been used as an alternative means of tracking inflammation related to infection and the results are comparable to that noted for ^{111}In -oxine labeled leukocytes [34]. While ^{18}F FDG has utility in assessing inflammation associated with infection, it may have greater utility in evaluating the metabolic consequences of infection.

The sequelae following Ebola virus infection involves hypo-perfusion, hypoxia, coagulopathy, hyper-permeability and necrosis which may result in a phenotype of organ hypo-metabolism. With VEEV infection and involvement of the brain, metabolic alterations may be

associated with encephalitis progression. As has been described in the areas of oncology and neurology [35, 36], metabolic probes applied for these diseases may be utilized in the characterization of the consequences of Ebola virus and VEEV infection. ^{18}F FDG has routinely been used to assess tissue metabolism. With Alzheimer's disease and mild cognitive impairment reductions in glucose metabolism as assessed by PET have been noted [36]. The hypometabolism is believed to be associated with reduced neuronal activity and neuronal loss [37, 38]. With VEEV infection and altered activity of neurons, one could use ^{18}F FDG to demonstrate overall changes in brain metabolism and depending on the animal being evaluated, i.e., mouse vs non-human primate, one would be able to map regional differences in brain metabolic activity. Marked changes in glucose metabolism in other organs can also be detected under well controlled experimental conditions designed to eliminate spurious metabolic changes, e.g., reduce sensory stimulation and brain activation, or increased muscle metabolism due to chills. Alterations in liver or lymphatic system metabolism related to Ebola infection can be monitored post-infection. Unlike classical approaches to evaluating VEEV and Ebola viruses where such measures could only be performed at the end of the study, molecular imaging allows for the evaluation of changes in glucose metabolism at earlier time points and as such there is the potential to define markers of viral infection and/or drug treatment before overt clinical signs.

While ^{18}F FDG PET is a good marker of general organ metabolism, more selective tracers are available to evaluate specific metabolic pathways. A recently synthesized PET tracer, [^{18}F]-N-(methyl-(2-fluoroethyl)-1H-[1,2,3]triazole-4-yl)glucosamine ([^{18}F]NFTG), was developed for the measurement of glycogen synthase I levels which is part of the glycogen metabolism pathway [39,40]. With Ebola virus infection, one might propose that catabolic activities in liver and muscle can be evaluated dynamically using [^{18}F]NFTG since prior to infection and during

cellular quiescence glycogen synthase I levels are high and as a consequence [^{18}F]NFTG should also be high [40]. With infection, one would suspect a reduction in glycogen synthesis and reduced [^{18}F]NFTG uptake. Another tracer capable of monitoring the pentose phosphate pathway, [^{18}F]-2-deoxy-2-fluoroarabinose, can be used to monitor the synthesis of nucleotides to better understand mechanisms involved in virus replication, impact of infection on host based nucleotide production and the energy status of tissues since the pathway is involved in NADPH synthesis [41]. Effects of viral infection on protein synthesis can be evaluated using a variety of ^{11}C -labeled natural and unnatural amino acids [39]. Fatty acid metabolism and β -oxidation can be monitored utilizing [^{18}F]-fluoro-4-thia-oleate ([^{18}F]FTO)[42]. Taken independently or collectively, the molecular probes provide early measures of cellular necrosis, altered organ function, i.e., hyper- or hypo-metabolism, and defines where the virus may have the greatest impact. By utilizing the various metabolic PET tracers, one can develop an understanding of the consequences of infection on cellular glucose-glycogen metabolism, fatty acid metabolism and protein synthesis over the course of the disease evolution and potentially identify pathways for therapeutic intervention, pathways leading to end organ damage and differential effects of viruses (if they exist) across organs.

Coupled with the metabolic probes, PET/SPECT tracers exist to directly gauge the level of tissue hypoxia, vascular permeability and cellular apoptosis which are known to occur with Ebola and potentially VEEV infection. The most often used PET tracers for detection of hypoxia within viable tissue are [^{18}F]fluormisonidazole ([^{18}F]FMISO) (43) and [^{64}Cu]Cu-diacetyl-bis(N-methylthiosemicarbazone)([^{64}Cu]ATSM)(44). In viable cells that are hypoxic, the PET tracers are reduced to a reactive intermediate by cellular reductases which covalently bind to thiol groups of intracellular proteins and thereby accumulate in areas of hypoxia. While [^{18}F]FMISO

has been used clinically to assess hypoxia in tumors (43), it has also been shown to accumulate in ischemic areas associated with stroke in a rat model of middle cerebral artery occlusion (45). With viral infection and the associated hypercoagulopathy as noted for Ebola virus and altered flow and vascular permeability, one might suspect that brain and tissue hypoxia occurs and as such [^{18}F]FMISO or [^{64}Cu]ATSM which has an advantage of a longer half-life, i.e., 12.7 hours, can be used to monitor the consequences of vascular damage on tissue homeostasis.

Vascular damage is often associated with altered permeability and in the brain an altered blood brain barrier. With albumin being a major constituent of blood, radiolabeling of albumin with PET or SPECT isotopes may be a useful tool to evaluate vascular permeability. Kilbourn and colleagues in 1987 developed a method of labeling albumin and fibrinogen with ^{18}F (46) while others, more recently, devised strategies for labeling albumin *in vivo* (47). In normal animals, [^{18}F]albumin was retained in the circulation and served as a blood pool agent; however, with damage extravascular [^{18}F]albumin was noted and collocated with regions of inflammatory cell infiltrate (47). Thus, with Ebola virus and VEEV infection the accumulation of extravascular [^{18}F]albumin can be used to identify areas of enhanced vascular permeability and provide a tool to dynamically assess hemorrhage, microhemorrhage and general vascular dysfunction leading to leaky vessels in both the brain and peripheral organs.

Vascular damage, hypoxia and altered metabolic activity could lead to areas of necrosis and/or apoptosis. While areas of necrosis would be associated with areas devoid of [^{18}F]FDG and [^{18}F]FMISO activity and potentially high [^{18}F]albumin due to vascular damage, areas of apoptosis or early cell loss would be more difficult to detect. A novel PET tracer for detection of apoptosis, [^{18}F]5-fluoropentyl-2-methyl-malonic acid ([^{18}F]ML10), has been developed (48). It has been demonstrated in a mouse model of stroke that [^{18}F]ML10 is selective for cells

undergoing apoptosis and colocalizes with the histologic marker, TUNNEL. Application of [¹⁸F]ML10 PET imaging to the study of viral infection may define early cellular changes that could precede overt functional changes and identify regions especially in the brain that are more severely affected by viruses like VEEV.

In summary, numerous PET/SPECT approaches that have been developed for studies in oncology and neurology can be adapted to the evaluation of the consequences of viral infection. By combining measurements of tissue inflammation, metabolism, hypoxia, necrosis, apoptosis and vascular damage, one can track the consequences of infection on organ function, define the stages of tissue damage and identify appropriate therapeutic strategies for intervention. The dynamic nature of molecular imaging allows one not only assess the consequences of infection but also to evaluate mechanisms of infectious disease progression.

Conclusions Molecular imaging has great potential in advancing our understanding of infectious diseases. Classical imaging approaches that have been developed and applied to the study of oncology, neurology, cardiology and inflammation can be adapted to study viruses directly and to assess the consequences of BSL3/4 pathogens on tissues across the course of disease progression. By tracking viral replication and tissue distribution of virus, one can map the progression of the disease and correlate changes in tissue pathology using additional molecular imaging probes with tissue viral load. Unlike current methods which monitor plasma viral load as an index of infection, measuring tissue viral levels by imaging more directly assesses the degree of infection and when related to tissue levels of a PET radiolabeled drug, a relationship between plasma and tissue drug and virus levels can be established to better direct therapeutic intervention. Linking measures of tissue inflammation, metabolism, hypoxia, necrosis, apoptosis and vascular damage with tissue viral load over time can also provide

mechanistic information relating to viral stimulus and tissue response. At present, utilizing the available imaging probes and performing studies where samples from the same animals can be taken for proteomic and genomic analysis biomarkers of infection and disease progression can be developed and evaluated in relation to a specific stage of the disease. With the development of new molecular imaging probes, some of which may be virus specific, and application of additional imaging approaches like MRI, it is possible to characterize the disease more thoroughly and provide both structural and functional information to aid in therapeutic drug discovery efforts.

Acknowledgements

Funding for the preparation of the article was provided by Department of Defense Chemical Biological Defense Program through the Defense Threat Reduction Agency (DTRA) under USAMRIID project number 1323839.

Conflict of Interest

The author has no conflicts of interest.

Disclaimer

Opinions, interpretations, conclusions, and recommendations stated within the article are those of the author and are not necessarily endorsed by the U.S. Army.

References

1. Bocan TM, Panchal RG, Bavari S (2015) Applications of in vivo imaging in the evaluation of the pathophysiology of viral and bacterial infections and in development of countermeasures to BSL3/4 pathogens. *Mol Imaging Biol* 17(1):4-17. doi. 10.1007/s11307-014-0759-7
2. Bowen GS, Fashunell TR, Dean PB, Gregg MB (1976) Clinical aspects of human Venezuelan equine encephalitis in Texas. *Bull Pan Am Health Organ* 10:46-57
3. Steele KE, Twenhafel NA (2010) REVIEW PAPER: Pathology of animal models of alphavirus encephalitis. *Vet Pathol* 47(5):790-805. doi. 10.1177/0300985810372508
4. Bente D, Gren J, Strong JE, Feldmann H (2009) Disease modeling for Ebola and Marburg viruses. *Disease Models & Mechanisms* 2:12-17.
5. Geisbert TW, Hensley LE, Larsen T, Young HA, Reed DS, Geisbert JB, Scott DP, Kagan E, Jahrling PB, Davis JK (2003) Pathogenesis of Ebola hemorrhagic fever in *Cynomolgus* macaques. Evidence that dendritic cells are early and sustained targets of infection. *Am. J. Pathol.* 163:2347-2370.
6. Geisbert TW, Young HA, Jahrling PB, Davis KJ, Larsen T, Kagan E, Hensley LE (2003) Pathogenesis of Eblola hemorrhagic fever in primate models. Evidence that hemorrhage is not a direct effect of virus-induced cytolysis of endothelial cells. *Am. J. Pathol.* 163:2371-2382.
7. Milho R, Smith CM, Marques S, Alenquer M, May JS, Gillet L, Gaspar M, Efstathiou S, Simas JP, Stevenson PG (2009) *In vivo* imaging of murid herpesvirus-4 infection. *J Gen Virol* 90:21-32

8. Oliver SL, Zerboni L, Sommer M, Rajamani J, Arvin AM (2008) Development of recombinant varicella-zoster viruses expressing luciferase fusion proteins for live in vivo imaging in human skin and dorsal root ganglia xenografts. *J Virol Meth* 154:182-193
9. Patterson M, Poussard A, Taylor K, Seregin A, Smith J, Peng BH, Walker A, Linde J, Smith J, Salazar M, Paessler S (2011) Rapid, non-invasive imaging of alphaviral brain infection: Reducing animal numbers and morbidity to identify efficacy of potential vaccines and antivirals. *Vaccine* 29:9345-9351
10. Ziegler SA, Nuckois J, McGee CE, Huang YJ, Vanlandingham DL, Tesh RB, Higgs S (2011) *In vivo* imaging of Chikungunya virus in mice and aedes mosquitoes using *renilla* luciferase clone. *Vector Borne Zoonotic Disease* 11:1471-1477
11. Goff A, Twenhafel N, Garrison A, Mucker E, Lawler j, Paragas J (2007) In vivo imaging of cidofovir treatment of cowpox virus infection. *Virus Res* 128:88-98
12. Yamada S, Kosugi I, Katano H, Fukui Y, Kawasaki H, Arai Y, Kurane I, Inoue N (2010) In vivo imaging assay for the convenient evaluation of antiviral compounds against cytomegalovirus in mice. *Antiviral Res* 88:45-52
13. Sun C, Gardner CL, Watson AM, Ryman KD, Klimstra WB (2014) Stable, high-level expression of reporter proteins from improved alphavirus expression vectors to track replication and dissemination during encephalitic and arthritogenic disease. *J Virol* 88:2035-2046
14. Brader P, Serganova I, Blasberg RG (2013) Noninvasive molecular imaging using reporter genes. *J Nucl Med* 54:167-172
15. Ponomarev V, Doubrovin M, Shavrin A, Serganova I, Beresten T, Ageyeva L, Cai C, Balatoni J, Alauddin M, Gelovani J (2007) A human-derived reporter gene for

- noninvasive imaging in humans: mitochondrial thymidine kinase type 2. *J Nucl Med* 48:819–826
16. Haberkorn U (2001) Gene therapy with sodium/iodide symporter in hepatocarcinoma. *Exp Clin Endocrinol Diabetes* 109:60–62
17. Haddad D, Chen NG, Zhang Q, Chen CH, Ya Y, Gonzalez L, Carpenter SG, Carson J, Au J, Mittra A, Gonen M, Zanzonico PB, Fong Y, Szalay AA (2011) Insertion of the human sodium iodide symporter to facilitate deep tissue imaging does not alter oncolytic or replication capability of a novel vaccinia virus. *J Transl Med* 9:1-14
18. Pennheiter AR, Russell SJ, Carlson SK (2012) The sodium iodide symporter (NIS) as an imaging reporter for gene, viral, and cell-based therapies. *Current Gene Therapy* 12:33-47
19. Liang Q, Satyamurthy N, Barrio JR, Toyokuni T, Phelps MP, Gambhir SS, Herschman HR (2001) Noninvasive, quantitative imaging in living animals of a mutant dopamine D2 receptor reporter gene in which ligand binding is uncoupled from signal transduction. *Gene Ther* 8:1490–1498
20. Piel M, Vernaleken I, Rosch F (2014) Positron emission tomography in CNS drug discovery and drug monitoring. *J Med Chem* 57:9232-58
21. Hargreaves RJ, Rabiner EA (2014) Translational PET imaging research. *Neurobiol Dis* 61:32-8
22. Eisenmenger LB, Huo EJ, Hoffman JM, Minoshima S, Matesan MC, Lewis DH, Lopresti BJ, Mathis CA, Okonkwo DO, Mountz JM (2016) Advances in PET imaging of degenerative, cerebrovascular, and traumatic causes of dementia. *Semin Nucl Med* 46:57-87

23. Waldron AM, Wintmolders C, Bottelbergs A, Kelley JB, Schmidt ME, Stroobants S, Langlois X, Staelens S (2015) In vivo molecular neuroimaging of glucose utilization and its association with fibrillar amyloid- β load in aged APPPS1-21 mice *Alzheimer's Res Therap* 7:76
24. Sparshatt A, Taylor D, Patel MX, Kapur S (2010) A systematic review of aripiprazole-dose, plasma concentration, receptor occupancy, and response: implications for therapeutic drug monitoring. *J Clin Psychiatry* 71:1447-56
25. Chen K, Chen X (2011) Positron emission tomography imaging of cancer biology: Current status and future prospects. *Semin Oncol* 38:70-86
26. Ping LW, Meyer LA, Capretto DA, Sherman CD, Anderson CJ (2008) Receptor-binding, biodistribution, and metabolism studies of ^{64}Cu -DOTA-cetuximab, A PET-imaging agent for epidermal growth-factor-receptor-positive tumors. *Cancer Biother Radiopharm* 23:158-71
27. Jacobs AH, Tavitian B, INMiND consortium (2012) Noninvasive molecular imaging of neuroinflammation. *J Cereb Blood Flow Metab* 32:1392-1415
28. Harhausen D, Sudmann V, Khojasteh U, Muller J, Zille M, Graham K, Thiele A, Dyrks T, Dirnagl U, Wunder A (2013) Specific imaging of inflammation with the 18kDa translocator protein ligand DPA-714 in animal models of epilepsy and stroke. *PLoS ONE* 8(8):e69529
29. Lavisse S, Inoue K, Jan C, Peyronneau MA, Petit F, Goutal S, Dauguet J, Guillermier M, Dolle F, Rbah-Vidal L, Va Camp N, Aron-Badin R, Remy P, Hantraye P (2015) [^{18}F]DPA714 PET imaging of translocator protein TSPO (18 kDa) in the normal and

excitotoxically-lesioned nonhuman primate brain. *Eur J Nucl Med Mol Imaging* 42:478-494

30. Bernards N, Pottier G, Theze B, Dolle F, Boisgard R (2015) In vivo evaluation of inflammatory bowel disease with the aid of μ PET and the translocator protein 18kDa radioligand [^{18}F]DPA-714. *Mol Imaging Biol* 17:67-75
31. Pottier G, Bernards N, Dolle F, Boisgard R (2014) [^{18}F]DPA-714 as a biomarker for positron emission tomography imaging of rheumatoid arthritis in an animal model. *Arthritis Res Therap* 16:R69
32. Hatori A, Yui J, Yamasaki T, Xie L, Kumata K, Fujinaga M, Yoshia Y, Ogawa M, Nengaki N, Kawamura K, Fukumura T, Zhang M-R (2012) PET imaging of lung inflammation with [^{18}F]FEDAC, a radioligand for translocator protein (18kDa). *PLoS ONE* 7(9):e45065
33. Vaidyanathan S, Patel CN, Scarsbrook AF, Chowdhury FU (2015) FDG PET/CT in infection and inflammation – current and emerging clinical applications. *Clin Radiol* 70:787-200
34. Rini JN, Bhargava KK, Tronco GG, Singer C, Caprioli R, Marwin SE, Richardson HL, Nichols KJ, Pugliese PV, Palestro CJ (2006) PET with FDG-labeled leukocytes versus scintigraphy with ^{111}In -oxine-labeled leukocytes for detection of infection. *Radiol* 238:978-87
35. Lewsi DY, Soloviev D, Brindle KM (2015) Imaging tumor metabolism using positron emission tomography. *Cancer J* 21:129-136
36. Mosconi L (2005) Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. *Eur J Nucl Med Imaging* 32:486-510

37. Herholz K (2003) PET studies in dementia. *Ann Nucl Med* 17:79-89
38. Mielke R, Kessler J, Szelies B, Herholz K, Wienhard K, Heiss WD (1998) Normal and pathological aging – findings of positron-emission-tomography. *J Neural Transm* 105:821-37
39. Carroll L, Witney TH, Aboagye EO (2013) Design and synthesis of novel ¹⁸F-radiolabeled glucosamine derivatives for cancer imaging. *Med Chem Comm* 4:653-656
40. Witney TH, Corroll L, Alam IS, Chandrashekran A, Nguyen QD, Sala R, Harris R, DeBerardinis RJ, Agarwal R, Aboagye EO (2014) A novel radiotracer to image glycogen metabolism in tumors by positron emission tomography. *Cancer Res* 74:1319-28
41. Clark PM, Flores G, Evdokimov NM, McCracken MN, Chai T, Nair-Gill E, O'Mahoney F, Beaven SW, Faull KF, Phelps ME, Jung ME, Wittle ON (2014) Positron emission tomography probe demonstrates a striking concentration of ribose salvage in the liver. *Proc Natl Acad Sci USA* 111:E2866-74
42. DeGrado TR, Bhattacharyya F, Pandey MK, Belanger AP, Wang S (2010) Synthesis and preliminary evaluation of 19-18F-fluor-4-thia-oleate as a PET probe of fatty acid oxidation. *J Nucl Med* 51:1310-17
43. Mees G, Sierckx R, Vangestel C, Van de Wiele C (2009) Molecular imaging of hypozia with radiolabelled agents. *Eur J Nucl Med Mol Imaging* 36:1674-1686
44. Lewis JS, McCarthy DW, McCarthy TJ, Fujibayashi Y, Welch MJ (1999) Evaluation of [⁶⁴Cu]ATSM in vitro and in vivo in a hypoxic tumor model. *J Nucl Med* 40:177-183
45. Rojas S, Herance JR, Abad S, Jimenez C, Pareto D, Ruiz A, Torrent E, Figueiras FP, Popota F, Fernandez-Soriano FJ, Plana AM, Gispert JD (2011) Evaluation of hypoxic

tissue dynamics with ^{18}F -FMISO PET in a rat model of permanent cerebral ischemia.

Mol Imaging Biol 13:558-64

46. Kilbourn MR, Dence CS, Welch MJ, Mathias CJ (1987) Fluorine-18 labeling of proteins. *J Nucl Med* 28:462-470
47. Niu G, Lang L, Kiesewetter DO, Ma Y, Sun Z, Guo N, Guo J, Wu C, Chen X (2014) In vivo labeling of serum albumin for PET. *J Nucl Med* 55:1150-1156
48. Reshef A, Shirvan A, Waterhouse RN, Grimberg H, Levin G, Cohen A, Ulysse LG, Friedman G, Antoni G, Ziv H (2008) Molecular imaging of neurovascular cell death in experimental cerebral stroke by PET. *J Nucl Med* 49:1520-1528