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Stem Cell

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Coronavirus and Stem Cell Research Literatures

Mark Herbert

World Development Institute
39-06 Main Street, Flushing, Queens, New York 11354, USA, ma708090@gmail.com

Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus is mainly spread during close contact and via respiratory droplets that are produced when a person talks, coughs, or sneezes. Respiratory droplets may be produced during breathing, however, current research indicates that the virus is not considered airborne. People may also contract COVID-19 by touching a contaminated surface (Fomite) and then inadvertently transfer the pathogen to a mucous membrane (such as the eyes, nose, or mouth). It is most contagious when people are symptomatic, although spread may be possible before symptoms appear. The virus can live on surfaces up to 72 hours. Time from exposure to onset of symptoms is generally between two and fourteen days, with an average of five days. The standard method of diagnosis is by reverse transcription polymerase chain reaction (rRT-PCR) from a nasopharyngeal swab. The infection can also be diagnosed from a combination of symptoms, risk factors and a chest CT scan showing features of pneumonia. This article introduces recent research reports as references in the related studies.
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Key words: stem cell; coronavirus; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus is mainly spread during close contact and via respiratory droplets that are produced when a person talks, coughs, or sneezes. Respiratory droplets may be produced during breathing, however, current research indicates that the virus is not considered airborne. People may also contract COVID-19 by touching a contaminated surface (Fomite) and then inadvertently transfer the pathogen to a mucous membrane (such as the eyes, nose, or mouth). It is most contagious when people are symptomatic, although spread may be possible before symptoms appear. The virus can live on surfaces up to 72 hours. Time from exposure to onset of symptoms is generally between two and fourteen days, with an average of five days. The standard method of diagnosis is by reverse transcription polymerase chain reaction (rRT-PCR) from a nasopharyngeal swab. The infection can also be diagnosed from a combination of symptoms, risk factors and a chest CT scan showing features of pneumonia. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Anis, E. A., et al. (2017). "Transduction of hematopoietic stem cells to stimulate RNA interference against feline infectious peritonitis." J Feline Med Surg **19**(6): 680-686.

Objectives The goals of the study were: (1) to develop and evaluate non-replicating lentivirus vectors coding for feline coronavirus (FCoV)-specific micro (mi)RNA as a potential antiviral therapy for feline infectious peritonitis (FIP); (2) to assess the feasibility of transducing hematopoietic stem cells (HSCs) with ex vivo introduction of the miRNA-expressing lentivirus vector; and (3) to assess the ability of the expressed miRNA to inhibit FCoV replication in HSCs in vitro. Methods HSCs were obtained from feline bone marrow and replicated in vitro. Three lentiviruses were constructed, each expressing a different anti-FCoV miRNA. HSCs were stably transduced with the miRNA-expressing lentivirus vector that produced the most effective viral inhibition in a feline cell line. The effectiveness of the transduction and the expression of anti-FCoV miRNA were tested by infecting the HSCs with two different strains of FCoV. The inhibition of coronavirus replication was determined by relative quantification of the inhibition of intracellular viral genomic RNA synthesis using real-time, reverse-transcription PCR. The assessment of virus replication inhibition was determined via titration of extracellular virus using the TCID50 assay. Results Inhibition of FCoV was most significant in feline cells expressing miRNA-L2 that targeted the viral leader sequence, 48 h postinfection. miRNA-L2 expression in stably transduced HSCs resulted in 90% and 92% reductions in FIPV WSU 79-1146 genomic RNA synthesis and extracellular virus production, respectively, as well as 74% and 80% reduction in FECV WSU 79-1683 genomic RNA synthesis and extracellular virus production, respectively, as compared with an infected negative control sample producing non-targeting miRNA. Conclusions and relevance These preliminary results show that genetic modification of HSCs for constitutive production of anti-coronavirus miRNA will reduce FCoV replication.

Athmer, J., et al. (2017). "In Situ Tagged nsp15 Reveals Interactions with Coronavirus Replication/Transcription Complex-Associated Proteins." mBio **8**(1).

Coronavirus (CoV) replication and transcription are carried out in close proximity to restructured endoplasmic reticulum (ER) membranes in replication/transcription complexes (RTC). Many of the CoV nonstructural proteins (nsps) are required for RTC function; however, not all of their functions are known. nsp15 contains an endoribonuclease domain that is conserved in the CoV family. While the enzymatic activity and crystal structure of nsp15 are well defined, its role in replication remains elusive. nsp15 localizes to sites of RNA replication, but whether it acts independently or requires additional interactions for its function remains unknown. To begin to address these questions, we created an in situ tagged form of nsp15 using the prototypic CoV, mouse hepatitis virus (MHV). In MHV, nsp15 contains the genomic RNA packaging signal (P/S), a 95-bp RNA stem-loop structure that is not required for viral replication or nsp15 function. Utilizing this knowledge, we constructed an internal hemagglutinin (HA) tag that replaced the P/S. We found that nsp15-HA was localized to discrete perinuclear puncta and strongly colocalized with nsp8 and nsp12, both well-defined members of the RTC, but not the membrane (M) protein, involved in virus assembly. Finally, we found that nsp15 interacted with RTC-associated proteins nsp8 and nsp12 during infection, and this interaction was RNA independent. From this, we conclude that nsp15 localizes and interacts with CoV proteins in the RTC, suggesting it plays a direct or indirect role in virus replication. Furthermore, the use of in situ epitope tags could be used to determine novel nsp-nsp interactions in coronaviruses. IMPORTANCE: Despite structural and biochemical data demonstrating that the coronavirus nsp15 protein contains an endoribonuclease domain, its precise function during coronavirus infection remains unknown. In this work, we created a novel in situ tagged form of nsp15 to study interactions and localization during infection. This in situ tag was tolerated by MHV and did not affect viral replication. Utilizing this tag, we established that nsp15 localized to sites of replication but not sites of assembly throughout infection. Furthermore, we found that nsp15 interacted with the putative viral primase nsp8 and polymerase nsp12 during CoV infection. The strong association of nsp15 with replication complexes and interactions with replicative CoV enzymes suggest nsp15 is involved in CoV replication. These data and tools developed in this study help elucidate the function of nsp15 during infection and may be used to uncover other novel viral protein interactions.

Baglivo, M., et al. (2020). "Natural small molecules as inhibitors of coronavirus lipid-dependent attachment to host cells: a possible strategy for reducing SARS-COV-2 infectivity?" Acta Biomed **91**(1): 161-164.

BACKGROUND: Viral infectivity depends on interactions between components of the host cell plasma membrane and the virus envelope. Here we review strategies that could help stem the advance of the SARS-COV-2 epidemic. METHODS AND RESULTS: We focus on the role of lipid structures, such as lipid rafts and cholesterol, involved in the process, mediated by endocytosis, by which viruses attach to and infect cells. Previous studies have shown that many naturally derived substances, such as cyclodextrin and sterols, could reduce the infectivity

of many types of viruses, including the coronavirus family, through interference with lipid-dependent attachment to human host cells. CONCLUSIONS: Certain molecules prove able to reduce the infectivity of some coronaviruses, possibly by inhibiting viral lipid-dependent attachment to host cells. More research into these molecules and methods would be worthwhile as it could provide insights the mechanism of transmission of SARS-COV-2 and, into how they could become a basis for new antiviral strategies.

Baranov, P. V., et al. (2005). "Programmed ribosomal frameshifting in decoding the SARS-CoV genome." Virology **332**(2): 498-510.

Programmed ribosomal frameshifting is an essential mechanism used for the expression of orf1b in coronaviruses. Comparative analysis of the frameshift region reveals a universal shift site U_UUA_AAC, followed by a predicted downstream RNA structure in the form of either a pseudoknot or kissing stem loops. Frameshifting in SARS-CoV has been characterized in cultured mammalian cells using a dual luciferase reporter system and mass spectrometry. Mutagenic analysis of the SARS-CoV shift site and mass spectrometry of an affinity tagged frameshift product confirmed tandem tRNA slippage on the sequence U_UUA_AAC. Analysis of the downstream pseudoknot stimulator of frameshifting in SARS-CoV shows that a proposed RNA secondary structure in loop II and two unpaired nucleotides at the stem I-stem II junction in SARS-CoV are important for frameshift stimulation. These results demonstrate key sequences required for efficient frameshifting, and the utility of mass spectrometry to study ribosomal frameshifting.

Beaird, O. E., et al. (2016). "Current practices for treatment of respiratory syncytial virus and other non-influenza respiratory viruses in high-risk patient populations: a survey of institutions in the Midwestern Respiratory Virus Collaborative." Transpl Infect Dis **18**(2): 210-215.

BACKGROUND: The optimal treatment for respiratory syncytial virus (RSV) infection in adult immunocompromised patients is unknown. We assessed the management of RSV and other non-influenza respiratory viruses in Midwestern transplant centers. METHODS: A survey assessing strategies for RSV and other non-influenza respiratory viral infections was sent to 13 centers. RESULTS: Multiplex polymerase chain reaction assay was used for diagnosis in 11/12 centers. Eight of 12 centers used inhaled ribavirin (RBV) in some patient populations. Barriers included cost, safety, lack of evidence, and inconvenience. Six of 12 used intravenous immunoglobulin (IVIG), mostly in combination with RBV. Inhaled RBV was used more than oral, and in the post-stem cell transplant population, patients with lower respiratory tract infection (LRTI), graft-versus-host disease, and more recent transplantation were treated at higher rates. Ten centers had experience with lung transplant patients; all used either oral or inhaled RBV for LRTI, 6/10 treated upper respiratory tract infection (URTI). No center treated non-lung solid organ transplant (SOT) recipients with URTI; 7/11 would use oral or inhaled RBV in the same group with LRTI. Patients with hematologic malignancy without hematopoietic stem cell transplantation were treated with RBV at a similar frequency to non-lung SOT recipients. Three of 12 centers, in severe cases, treated parainfluenza and metapneumovirus, and 1/12 treated coronavirus. CONCLUSIONS: Treatment of RSV in immunocompromised patients varied greatly. While most centers treat LRTI, treatment of URTI was variable. No consensus was found regarding the use of oral versus inhaled RBV, or the use of IVIG. The presence of such heterogeneity demonstrates the need for further studies defining optimal treatment of RSV in immunocompromised hosts.

Beury, D., et al. (2020). "Use of whole-genome sequencing in the molecular investigation of care-associated HCoV-OC43 infections in a hematopoietic stem cell transplant unit." J Clin Virol **122**: 104206.

BACKGROUND: While respiratory viral infections are recognized as a frequent cause of illness in hematopoietic stem cell transplantation (HSCT) recipients, HCoV-OC43 infections have rarely been investigated as healthcare-associated infections in this population. OBJECTIVES: In this report, HCoV-OC43 isolates collected from HSCT patients were retrospectively characterized to identify potential clusters of infection that may stand for a hospital transmission. STUDY DESIGN: Whole-genome and S gene sequences were obtained from nasal swabs using next-generation sequencing and phylogenetic trees were constructed. Similar identity matrix and determination of the most common ancestor were used to compare clusters of patient's sequences. Amino acids substitutions were analysed. RESULTS: Genotypes B, E, F and G were identified. Two clusters of patients were defined from chronological data and phylogenetic trees. Analyses of amino acids substitutions of the S protein sequences identified substitutions specific for genotype F strains circulating among European people. CONCLUSIONS: HCoV-OC43 may be implicated in healthcare-associated infections.

Blau, D. M., et al. (2001). "Targeted disruption of the Ceacam1 (MHVR) gene leads to reduced

susceptibility of mice to mouse hepatitis virus infection." J Virol **75**(17): 8173-8186.

The CEACAM1 glycoproteins (formerly called biliary glycoproteins; BGP, C-CAM, CD66a, or MHVR) are members of the carcinoembryonic antigen family of cell adhesion molecules. In the mouse, splice variants of CEACAM1 have either two or four immunoglobulin (Ig) domains linked through a transmembrane domain to either a short or a long cytoplasmic tail. CEACAM1 has cell adhesion activity and acts as a signaling molecule, and long-tail isoforms inhibit the growth of colon and prostate tumor cells in rodents. CEACAM1 isoforms serve as receptors for several viral and bacterial pathogens, including the murine coronavirus mouse hepatitis virus (MHV) and Haemophilus influenzae, Neisseria gonorrhoeae, and Neisseria meningitidis in humans. To elucidate the mechanisms responsible for the many biological activities of CEACAM1, we modified the expression of the mouse Ceacam1 gene in vivo. Manipulation of the Ceacam1 gene in mouse embryonic stem cells that contained the Ceacam1a allele yielded a partial knockout. We obtained one line of mice in which the insert in the Ceacam1a gene had sustained a recombination event. This resulted in the markedly reduced expression of the two CEACAM1a isoforms with four Ig domains, whereas the expression of the two isoforms with two Ig domains was doubled relative to that in wild-type BALB/c (+/+) mice. Homozygous (p/p) Ceacam1a-targeted mice (Ceacam1aDelta4D) had no gross tissue abnormalities and were viable and fertile; however, they were more resistant to MHV A59 infection and death than normal (+/+) mice. Following intranasal inoculation with MHV A59, p/p mice developed markedly fewer and smaller lesions in the liver than +/+ or heterozygous (+/p) mice. The titers of virus produced in the livers were 50- to 100-fold lower in p/p mice than in +/p or +/+ mice. p/p mice survived a dose 100-fold higher than the lethal dose of virus for +/+ mice. +/p mice were intermediate between +/+ and p/p mice in susceptibility to liver damage, virus growth in liver, and susceptibility to killing by MHV. Ceacam1a-targeted mice provide a new model to study the effects of modulation of receptor expression on susceptibility to MHV infection in vivo.

Bredenbeek, P. J., et al. (1990). "The primary structure and expression of the second open reading frame of the polymerase gene of the coronavirus MHV-A59; a highly conserved polymerase is expressed by an efficient ribosomal frameshifting mechanism." Nucleic Acids Res **18**(7): 1825-1832.

Sequence analysis of a substantial part of the polymerase gene of the murine coronavirus MHV-A59 revealed the 3' end of an open reading frame (ORF1a) overlapping with a large ORF (ORF1b; 2733 amino acids) which covers the 3' half of the polymerase gene. The expression of ORF1b occurs by a ribosomal frameshifting mechanism since the ORF1a/ORF1b overlapping nucleotide sequence is capable of inducing ribosomal frameshifting in vitro as well as in vivo. A stem-loop structure and a pseudoknot are predicted in the nucleotide sequence involved in ribosomal frameshifting. Comparison of the predicted amino acid sequence of MHV ORF1b with the amino acid sequence deduced from the corresponding gene of the avian coronavirus IBV demonstrated that in contrast to the other viral genes this ORF is extremely conserved. Detailed analysis of the predicted amino acid sequence revealed sequence elements which are conserved in many DNA and RNA polymerases.

Cabeca, T. K., et al. (2013). "Human coronavirus ocurrence in different populations of Sao Paulo: A comprehensive nine-year study using a pancoronavirus RT-PCR assay." Braz J Microbiol **44**(1): 335-339.

Human coronaviruses (HCoVs) are considered one of the most common respiratory viruses associated with respiratory tract illnesses. An emergent human coronavirus was identified as the causal agent of an epidemic of severe acute respiratory syndrome (SARS) during 2002-2003. The severity of the disease combined with its rapid spread requires the continuous surveillance of coronaviruses in worldwide populations. Epidemiological and clinical data of HCoVs infectious in the Brazilian population are scarce and restricted to one or two groups of patients. Our study aimed to investigate retrospectively the presence of HCoVs in different populations of Sao Paulo presenting acute respiratory tract infections (ARIs) during the years of 2001-2010. A pancoronavirus RT-PCR was performed in this study. Coronaviruses were detected in 126 (11.5%) of 1,087 specimens. Peaks detection frequency was observed during 2002-2004 and 2008-2009, with the highest detection in 2008. The prevalence of HCoVs was higher among children with heart diseases (24.6%), patients under stem cell transplantation program (24.3%) and renal transplanted patients (20.2%). Coryza, cough and fever were the most common symptoms at presentation of positive cases and wheezing, a lower respiratory tract infection symptom was reported by 12% of the total, and 27% of high at-risk patients. HCoVs may have an important role among patients with underlying conditions and transplanted ones.

Campbell, A. P., et al. (2010). "Respiratory virus pneumonia after hematopoietic cell transplantation (HCT): associations between viral load in bronchoalveolar lavage samples, viral RNA detection

in serum samples, and clinical outcomes of HCT." J Infect Dis **201**(9): 1404-1413.

BACKGROUND: Few data exist on respiratory virus quantitation in lower respiratory samples and detection in serum from hematopoietic cell transplant (HCT) recipients with respiratory virus-associated pneumonia. METHODS: We retrospectively identified HCT recipients with respiratory syncytial virus (RSV), parainfluenza virus, influenza virus, metapneumovirus (MPV), and coronavirus (CoV) detected in bronchoalveolar lavage (BAL) samples, and we tested stored BAL and/or serum samples by quantitative polymerase chain reaction. RESULTS: In 85 BAL samples from 82 patients, median viral loads were as follows: for RSV (n = 35), 2.6 x 10(6) copies/mL; for parainfluenza virus (n = 35), 4.9 x 10(7) copies/mL; for influenza virus (n = 9), 6.8 x 10(5) copies/mL; for MPV (n = 7), 3.9 x 10(7) copies/mL; and for CoV (n = 4), 1.8 x 10(5) copies/mL. Quantitative viral load was not associated with mechanical ventilation or death. Viral RNA was detected in serum samples from 6 of 66 patients: 4 of 41 with RSV pneumonia, 1 with influenza B, and 1 with MPV/influenza A virus/CoV coinfection (influenza A virus and MPV RNA detected). RSV detection in serum was associated with high viral load in BAL samples (p =.05), and viral RNA detection in serum was significantly associated with death (adjusted rate ratio, 1.8; (p =.02). CONCLUSION: Quantitative polymerase chain reaction detects high viral loads in BAL samples from HCT recipients with respiratory virus pneumonia. Viral RNA is also detectable in the serum of patients with RSV, influenza, and MPV pneumonia and may correlate with the severity of disease.

Campbell, A. P., et al. (2015). "Clinical outcomes associated with respiratory virus detection before allogeneic hematopoietic stem cell transplant." Clin Infect Dis **61**(2): 192-202.

BACKGROUND: The management of respiratory virus infections prior to hematopoietic cell transplant (HCT) is difficult. We examined whether respiratory virus detection before HCT influenced the requirement for bronchoscopy, hospitalization, and overall survival following HCT. METHODS: Pre-HCT and weekly post-HCT nasal washes were collected through day 100 from patients with and without symptoms. Samples were tested by multiplex polymerase chain reaction for respiratory syncytial virus, parainfluenza viruses 1-4, influenza A and B, human metapneumovirus, adenovirus, and human rhinoviruses, coronaviruses, and bocavirus. RESULTS: Of 458 patients, 116 (25%) had respiratory viruses detected pre-HCT. Overall, patients with viruses detected pre-HCT had fewer days alive and out of the hospital and lower survival at day 100 (adjusted hazard ratio [aHR], 2.4; 95% confidence interval [CI], 1.3-4.5; P =.007) than patients with negative samples; this risk was also present with rhinovirus alone (aHR for mortality, 2.6; 95% CI, 1.2-5.5; P =.01). No difference in bronchoscopy incidence was seen in patients with and without respiratory viruses (aHR, 1.3; 95% CI,.8-2.0; P =.32). In symptomatic patients, those with respiratory viruses detected had increased overall mortality compared with patients without viruses detected (unadjusted HR, 3.5; 95% CI, 1.0-12.1; P =.05); among asymptomatic patients, detection of respiratory viruses was not associated with increased mortality. CONCLUSIONS: These data support routine testing for respiratory viruses among symptomatic patients before HCT, and delay of transplant with virus detection when feasible, even for detection of rhinovirus alone. Further study is needed to address whether asymptomatic patients should undergo screening for respiratory virus detection before HCT.

Chai, Q., et al. (2013). "Maturation of lymph node fibroblastic reticular cells from myofibroblastic precursors is critical for antiviral immunity." Immunity **38**(5): 1013-1024.

The stromal scaffold of the lymph node (LN) paracortex is built by fibroblastic reticular cells (FRCs). Conditional ablation of lymphotoxin-beta receptor (LTbetaR) expression in LN FRCs and their mesenchymal progenitors in developing LNs revealed that LTbetaR-signaling in these cells was not essential for the formation of LNs. Although T cell zone reticular cells had lost podoplanin expression, they still formed a functional conduit system and showed enhanced expression of myofibroblastic markers. However, essential immune functions of FRCs, including homeostatic chemokine and interleukin-7 expression, were impaired. These changes in T cell zone reticular cell function were associated with increased susceptibility to viral infection. Thus, myofibroblasic FRC precursors are able to generate the basic T cell zone infrastructure, whereas LTbetaR-dependent maturation of FRCs guarantees full immunocompetence and hence optimal LN function during infection.

Chang, R. Y., et al. (1994). "A cis-acting function for the coronavirus leader in defective interfering RNA replication." J Virol **68**(12): 8223-8231.

To test the hypothesis that the 65-nucleotide (nt) leader on subgenomic mRNAs suffices as a 5'-terminal cis-acting signal for RNA replication, a corollary to the notion that coronavirus mRNAs behave as replicons, synthetic RNA transcripts of a cloned, reporter-containing N mRNA (mRNA 7) of the bovine coronavirus with a precise 5' terminus and a 3' poly (A)

of 68 nt were tested for replication after being transfected into helper virus-infected cells. No replication was observed, but synthetic transcripts of a cloned reporter-containing defective interfering (DI) RNA differing from the N mRNA construct by 433 nt of continuous 5'-proximal genomic sequence between the leader and the N open reading frame did replicate and become packaged, indicating the insufficiency of the leader alone as a 5' signal for replication of transfected RNA molecules. The leader was shown to be a necessary part of the cis-acting signal for DI RNA replication, however, since removal of terminal bases that destroyed a predicted intraleader stem-loop also destroyed replicating ability. Surprisingly, when the same stem-loop was disrupted by base substitutions, replication appeared only minimally impaired and the leader was found to have rapidly reverted to wild type during DI RNA replication, a phenomenon reminiscent of high-frequency leader switching in the mouse hepatitis coronavirus. These results suggest that once a minimal structural requirement for leader is fulfilled for initiation of DI RNA replication, the wild-type leader is strongly preferred for subsequent replication. They also demonstrate that, in contrast to reported natural mouse hepatitis coronavirus DI RNAs, the DI RNA of the bovine coronavirus does not require sequence elements originating from discontinuous downstream regions within the polymerase gene for replication or for packaging.

Chen, J., et al. (2003). "An alternate pathway for recruiting template RNA to the brome mosaic virus RNA replication complex." J Virol **77**(4): 2568-2577.

The multidomain RNA replication protein 1a of brome mosaic virus (BMV), a positive-strand RNA virus in the alphavirus-like superfamily, plays key roles in assembly and function of the viral RNA replication complex. 1a, which encodes RNA capping and helicase-like domains, localizes to endoplasmic reticulum membranes, recruits BMV 2a polymerase and viral RNA templates, and forms membrane-bound, capsid-like spherules in which RNA replication occurs. cis-acting signals necessary and sufficient for RNA recruitment by 1a have been mapped in BMV genomic RNA2 and RNA3. Both signals comprise an extended stem-loop whose apex matches the conserved sequence and structure of the TPsiC stem-loop in tRNAs (box B). Mutations show that this box B motif is crucial to 1a responsiveness of wild-type RNA2 and RNA3. We report here that, unexpectedly, some chimeric mRNAs expressing the 2a polymerase open reading frame from RNA2 were recruited by 1a to the replication complex and served as templates for negative-strand RNA synthesis, despite lacking the normally essential, box B-containing 5' signal. Further studies showed that this template recruitment required high-efficiency translation of the RNA templates. Moreover, multiple small frameshifting insertion or deletion mutations throughout the N-terminal region of the open reading frame inhibited this template recruitment, while an in-frame insertion did not. Providing 2a in trans did not restore template recruitment of RNAs with frameshift mutations. Only those deletions in the N-terminal region of 2a that abolished 2a interaction with 1a abolished template recruitment of the RNA. These and other results indicate that this alternate pathway for 1a-dependent RNA recruitment involves 1a interaction with the translating mRNA via the 1a-interactive N-terminal region of the nascent 2a polypeptide. Interaction with nascent 2a also may be involved in 1a recruitment of 2a polymerase to membranes.

Chen, Y., et al. (2007). "A novel subset of putative stem/progenitor CD34+Oct-4+ cells is the major target for SARS coronavirus in human lung." J Exp Med **204**(11): 2529-2536.

Identification of the nature of severe acute respiratory syndrome (SARS)-infected cells is crucial toward understanding the pathogenesis. Using multicolor colocalization techniques, we previously reported that SARS (+) cells in the lung of fatally infected patients expressed the only known functional receptor, angiotensin-converting enzyme 2, and also a binding receptor, liver/lymph node-specific ICAM-3-grabbing non-integrin (CD209L). In this study, we show that SARS-infected cells also express the stem/progenitor cell markers CD34 and Oct-4, and do not express cytokeratin or surfactant. These putative lung stem/progenitor cells can also be identified in some non-SARS individuals and can be infected by SARS-coronavirus ex vivo. Infection of these cells may contribute to the loss of lung repair capacity that leads to respiratory failure as clinically observed.

Choi, J. H., et al. (2013). "Respiratory viral infections after hematopoietic stem cell transplantation in children." J Korean Med Sci **28**(1): 36-41.

This study was performed to characterize respiratory viral infections in pediatric patients undergoing hematopoietic stem cell transplantation (HSCT). Study samples included 402 respiratory specimens obtained from 358 clinical episodes that occurred in the 116 children of the 175 consecutive HSCT cohort at Seoul National University Children's Hospital, Korea from 2007 to 2010. Multiplex reverse-transcription polymerase chain reactions were performed for rhinovirus, respiratory syncytial virus (RSV), parainfluenza viruses (PIVs), adenovirus, human coronavirus (hCoV), influenza viruses and human metapneumovirus. Viruses were identified in 89 clinical episodes that occurred in 58 patients.

Among the 89 clinical episodes, frequently detected viruses were rhinovirus in 25 (28.1%), RSV in 23 (25.8%), PIV-3 in 16 (18.0%), adenovirus in 12 (13.5%), and hCoV in 10 (11.2%). Lower respiratory tract infections were diagnosed in 34 (38.2%). Neutropenia was present in 24 (27.0%) episodes and lymphopenia was in 31 (34.8%) episodes. Sixty-three percent of the clinical episodes were hospital-acquired. Three patients died of respiratory failure caused by respiratory viral infections. Respiratory viral infections in pediatric patients who have undergone HSCT are common and are frequently acquired during hospitalization. Continuous monitoring is required to determine the role of respiratory viruses in immunocompromised children and the importance of preventive strategies.

Curry, S. M., et al. (2018). "Porcine epidemic diarrhea virus reduces feed efficiency in nursery pigs." J Anim Sci **96**(1): 85-97.

Porcine epidemic diarrhea virus (PEDV) infects enterocytes and in nursery pigs, results in diarrhea, anorexia, and reduced performance. Therefore, the objective of this study was to determine how PEDV infection influenced growth performance and repartitioning of amino acids and energy in nursery pigs. A total of 32 barrows and gilts, approximately 1 wk post-wean (BW = 8.46 +/- 0.50 kg), and naive for PEDV were obtained, weighed, and allotted based on sex and BW to one of two treatments: 1) Control, PEDV naive and 2) PEDV-inoculated (PEDV) with eight pens of two pigs each per treatment. On day post-inoculation (dpi) 0, PEDV pigs were inoculated via intragastric gavage with PEDV isolate (USA/Iowa/18984/2013). Pig and feeder weights were recorded at dpi -7, 0, 5, and 20 in order to calculate ADG, ADFI, and G:F. Eight pigs per treatment were euthanized on dpi 5 and 20, and tissues and blood were collected. At dpi 5, all PEDV pigs were PCR positive for PEDV in feces. Overall, PEDV pigs tended ($P < 0.10$) to increase ADFI, which resulted in reduced ($P < 0.05$) feed efficiency. At dpi 5, PEDV pigs had reduced ($P < 0.05$) villus height and increased ($P < 0.05$) stem cell proliferation in the jejunum compared with Control pigs. Pigs inoculated with PEDV had increased ($P < 0.05$) serum haptoglobin and increased insulin-to-glucose ratios compared with Control pigs at dpi 5. Markers of muscle proteolysis were not different ($P > 0.05$) between treatments within dpi; however, at dpi 5, 20S proteasome activity was increased ($P < 0.05$) in longissimus dorsi of PEDV pigs compared with Control pigs. Liver and jejunum gluconeogenic enzyme activities were not different ($P > 0.05$) between treatments within dpi. Overall, PEDV-inoculated pigs did recover the absorptive capacity that was reduced during PEDV infection by increasing proliferation of intestinal stem cells. However, the energy and nutrients needed to recover the epithelium may be originating from available luminal nutrients instead of muscle proteolysis and gluconeogenesis. This study provides insight into the effects of an enteric coronavirus on postabsorptive metabolism in nursery pigs.

Curry, S. M., et al. (2017). "Effects of porcine epidemic diarrhea virus infection on nursery pig intestinal function and barrier integrity." Vet Microbiol **211**: 58-66.

The pig intestinal epithelium can be compromised by pathogens leading to reduced integrity and function. Porcine epidemic diarrhea virus (PEDV), recently detected in North America, exemplifies intestinal epithelial insult. Although several studies have investigated the molecular aspects and host immune response to PEDV, there are little data on the impact of PEDV on pig intestinal physiology. The objective of this study was to investigate the longitudinal impact of PEDV on nursery pig intestinal function and integrity. Fifty recently-weaned, 5-week-old barrows and gilts (BW=9.92+/-0.49kg) were sorted based on body weight (BW) and sex into two treatments: 1) Control or 2) PEDV inoculated. At 2, 5, 7, and 14days post inoculation (dpi), 4 pigs per treatment were euthanized and jejunum sections collected. PEDV antigen was detected in inoculated pigs by immunohistochemistry in 50% (2/4) at dpi 2, 100% (4/4) at dpi 5, and none at later time points. PEDV-infected pigs had reduced ($P<0.05$) villus height and decreased transepithelial resistance compared with controls. Total acidic mucins, particularly sialomucin, were reduced in PEDV pigs at dpi 2 and then increased compared with controls at dpi 7 and 14. In addition, PEDV pigs had increased stem cell proliferation ($P<0.05$) and a numerical increase in DNA fragmentation compared with controls through dpi 7 which coincided with an observed return of digestive function to that of controls. Collectively, these data reveal that PEDV infection results in time-dependent changes not only in intestinal morphology but also barrier integrity and function.

Dube, M., et al. (2018). "Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43." J Virol **92**(17).

Human coronaviruses (HCoVs) are recognized respiratory pathogens for which accumulating evidence indicates that in vulnerable patients the infection can cause more severe pathologies. HCoVs are not always confined to the upper respiratory tract and can invade the central nervous system (CNS) under still unclear circumstances. HCoV-induced neuropathologies in humans are difficult to diagnose

early enough to allow therapeutic interventions. Making use of our already described animal model of HCoV neuropathogenesis, we describe the route of neuropropagation from the nasal cavity to the olfactory bulb and piriform cortex and then the brain stem. We identified neuron-to-neuron propagation as one underlying mode of virus spreading in cell culture. Our data demonstrate that both passive diffusion of released viral particles and axonal transport are valid propagation strategies used by the virus. We describe for the first time the presence along axons of viral platforms whose static dynamism is reminiscent of viral assembly sites. We further reveal that HCoV OC43 modes of propagation can be modulated by selected HCoV OC43 proteins and axonal transport. Our work, therefore, identifies processes that may govern the severity and nature of HCoV OC43 neuropathogenesis and will make possible the development of therapeutic strategies to prevent occurrences.IMPORTANCE Coronaviruses may invade the CNS, disseminate, and participate in the induction of neurological diseases. Their neuropathogenicity is being increasingly recognized in humans, and the presence and persistence of human coronaviruses (HCoV) in human brains have been proposed to cause long-term sequelae. Using our mouse model relying on natural susceptibility to HCoV OC43 and neuronal cell cultures, we have defined the most relevant path taken by HCoV OC43 to access and spread to and within the CNS toward the brain stem and spinal cord and studied in cell culture the underlying modes of intercellular propagation to better understand its neuropathogenesis. Our data suggest that axonal transport governs HCoV OC43 egress in the CNS, leading to the exacerbation of neuropathogenesis. Exploiting knowledge on neuroinvasion and dissemination will enhance our ability to control viral infection within the CNS, as it will shed light on underlying mechanisms of neuropathogenesis and uncover potential druggable molecular virus-host interfaces.

Eichenberger, E. M., et al. (2019). "Incidence, significance, and persistence of human coronavirus infection in hematopoietic stem cell transplant recipients." Bone Marrow Transplant **54**(7): 1058-1066.

Hematopoietic stem cell transplant (HSCT) recipients are at increased risk of respiratory viral infections and their associated complications. Unlike other respiratory viruses, little is known about the clinical significance of human coronavirus infection (HCoV) in this population. We retrospectively identified all HSCT recipients who were transplanted between May 2013 and June 2017 at our institution and characterized the cumulative incidence of post-transplant HCoV infection. Of 678 patients who underwent HSCT during the study period, 112 (17%) developed HCoV infection, making HCoV the fourth most common respiratory viral infection. Thirty-four (30%) HCoV-infected patients progressed to proven or probable lower respiratory tract infection (LRTI). Age >/=50, graft-versus-host disease, corticosteroids, hypoalbuminemia, and inpatient status at the time of infection were independently associated with progression to LRTI. Twenty-seven (59%) patients who underwent repeat NP swab had persistent viral shedding for >/=21 days, with a median duration of 4 weeks of viral shedding. We conclude that HCoV is common and clinically significant in HSCT recipients, with nearly one-third of patients progressing to proven or probable LRTI. Evaluating for LRTI risk factors found in this study may identify patients who require closer surveillance and aggressive supportive care when infected with HCoV.

Fischer, S. A. (2008). "Emerging viruses in transplantation: there is more to infection after transplant than CMV and EBV." Transplantation **86**(10): 1327-1339.

Transplant physicians and surgeons are familiar with the risks, clinical behavior, and management of cytomegalovirus in transplant recipients. Donor-transmitted viral infections are uncommon but in recent years have brought to light the clinical manifestations of rabies, West Nile virus, and lymphocytic choriomeningitis virus in the early posttransplant period. Later posttransplant, infection with viruses circulating in the community can occur with a number of pathogens, including some vaccine-preventable illnesses such as measles and mumps. Recent advances in molecular microbiology have made it possible to diagnose a growing number of community-acquired viral pathogens infecting transplant recipients. This article reviews some of the emerging and reemerging viral pathogens infecting solid organ and hematopoietic stem-cell recipients, including adenovirus, bocavirus, coronavirus, human herpesvirus-6, lymphocytic choriomeningitis virus, measles, mumps, metapneumovirus, parainfluenza, rotavirus, respiratory syncytial virus, and West Nile virus.

Folz, R. J. and M. A. Elkordy (1999). "Coronavirus pneumonia following autologous bone marrow transplantation for breast cancer." Chest **115**(3): 901-905.

Infectious bronchitis virus, otherwise known as coronavirus, can cause mild upper respiratory tract illnesses in children and adults. Rarely has coronavirus been linked, either by serology or nasal wash, to pneumonia. We report a case of a young woman who,

following treatment for stage IIIA breast cancer using a high-dose chemotherapy regimen followed by autologous bone marrow and stem cell transplantation, developed respiratory failure and was found to have coronavirus pneumonia as diagnosed by electron microscopy from BAL fluid. We propose that coronavirus should be considered in the differential diagnosis of acute respiratory failure in cancer patients who have undergone high-dose chemotherapy and autologous hematopoietic support.

Fontana, L. and L. Strasfeld (2019). "Respiratory Virus Infections of the Stem Cell Transplant Recipient and the Hematologic Malignancy Patient." Infect Dis Clin North Am **33**(2): 523-544.

Respiratory virus infections in hematologic stem cell transplant recipients and patients with hematologic malignancies are increasingly recognized as a cause of significant morbidity and mortality. The often overlapping clinical presentation makes molecular diagnostic strategies imperative for rapid diagnosis and to inform understanding of the changing epidemiology of each of the respiratory viruses. Most respiratory virus infections are managed with supportive therapy, although there is effective antiviral therapy for influenza. The primary focus should remain on primary prevention infection control procedures and isolation precautions, avoidance of ill contacts, and vaccination for influenza.

Goebel, S. J., et al. (2007). "A hypervariable region within the 3' cis-acting element of the murine coronavirus genome is nonessential for RNA synthesis but affects pathogenesis." J Virol **81**(3): 1274-1287.

The 3' cis-acting element for mouse hepatitis virus (MHV) RNA synthesis resides entirely within the 301-nucleotide 3' untranslated region (3' UTR) of the viral genome and consists of three regions. Encompassing the upstream end of the 3' UTR are a bulged stem-loop and an overlapping RNA pseudoknot, both of which are essential to MHV and common to all group 2 coronaviruses. At the downstream end of the genome is the minimal signal for initiation of negative-strand RNA synthesis. Between these two ends is a hypervariable region (HVR) that is only poorly conserved between MHV and other group 2 coronaviruses. Paradoxically, buried within the HVR is an octanucleotide motif (oct), 5'-GGAAGAGC-3', which is almost universally conserved in coronaviruses and is therefore assumed to have a critical biological function. We conducted an extensive mutational analysis of the HVR. Surprisingly, this region tolerated numerous deletions, rearrangements, and point mutations. Most striking, a mutant deleted of the entire HVR was only minimally impaired in tissue culture relative to the wild type. By contrast, the HVR deletion mutant was highly attenuated in mice, causing no signs of clinical disease and minimal weight loss compared to wild-type virus. Correspondingly, replication of the HVR deletion mutant in the brains of mice was greatly reduced compared to that of the wild type. Our results show that neither the HVR nor oct is essential for the basic mechanism of MHV RNA synthesis in tissue culture. However, the HVR appears to play a significant role in viral pathogenesis.

Gross, A. E. and M. L. Bryson (2015). "Oral Ribavirin for the Treatment of Noninfluenza Respiratory Viral Infections: A Systematic Review." Ann Pharmacother **49**(10): 1125-1135.

OBJECTIVE: To review clinical outcomes data for patients treated with oral ribavirin for noninfluenza respiratory viral infections (NIRVIs). DATA SOURCES: MEDLINE, EMBASE, and PubMed Central (1972 to June 1, 2015) were queried with the following search term combinations: "Oral" AND "ribavirin" AND ("respiratory syncytial virus" OR "metapneumovirus" OR "parainfluenza" OR "coronavirus" OR "rhinovirus" OR "enterovirus" OR "adenovirus"). STUDY SELECTION AND DATA EXTRACTION: Included studies must have characterized the clinical outcomes of a cohort of patients treated with oral ribavirin for symptomatic NIRVIs. Case reports and series with <5 cases, conference abstracts, and articles written in languages other than English were excluded. DATA SYNTHESIS: Of the 1256 unique reports, 15 met inclusion criteria: 12 retrospective, 3 prospective, and 3 comparative with untreated control groups. All studies except for 2 Middle East respiratory syndrome coronavirus (MERS-CoV) studies were in immunocompromised patients (9 malignancy/stem cell transplant, 4 lung transplant). The mortality rate ranged from 0% to 31% in malignancy/stem cell transplant recipients treated with oral ribavirin, and 1/108 (0.9%) ribavirin-treated lung transplant recipients died at 30 days. Three studies (one each for malignancy, lung transplant, and MERS-CoV) suggested a clinical outcomes benefit with oral ribavirin compared with supportive care alone; however, the nonrandomized design precludes efficacy determination. Hemolysis was the most common adverse reaction, occurring in 14% (54/375) of patients. Ribavirin was discontinued in 4% of patients secondary to adverse reactions. CONCLUSIONS: Oral ribavirin should be considered for the treatment of NIRVI in immunocompromised adults (malignancy/stem cell transplant or lung transplant) or adults with MERS-CoV.

Guan, B. J., et al. (2012). "Genetic evidence of a long-range RNA-RNA interaction between the genomic 5' untranslated region and the nonstructural protein 1 coding region in murine and bovine coronaviruses." J Virol **86**(8): 4631-4643.

Higher-order RNA structures in the 5' untranslated regions (UTRs) of the mouse hepatitis coronavirus (MHV) and bovine coronavirus (BCoV), separate species in the betacoronavirus genus, appear to be largely conserved despite an approximately 36% nucleotide sequence divergence. In a previous study, each of three 5'-end-proximal cis-acting stem-loop domains in the BCoV genome, I/II, III, and IV, yielded near-wild-type (wt) MHV phenotypes when used by reverse genetics to replace its counterpart in the MHV genome. Replacement with the BCoV 32-nucleotide (nt) inter-stem-loop fourth domain between stem-loops III and IV, however, required blind cell passaging for virus recovery. Here, we describe suppressor mutations within the transplanted BCoV 32-nt domain that along with appearance of potential base pairings identify an RNA-RNA interaction between this domain and a 32-nt region approximately 200 nt downstream within the nonstructural protein 1 (Nsp1)-coding region. Mfold and phylogenetic covariation patterns among similarly grouped betacoronaviruses support this interaction, as does cotransplantation of the BCoV 5' UTR and its downstream base-pairing domain. Interestingly, cotransplantation of the BCoV 5' UTR and BCoV Nsp1 coding region directly yielded an MHV wt-like phenotype, which demonstrates a cognate interaction between these two BCoV regions, which in the MHV genome act in a fully interspecies-compliant manner. Surprisingly, the 30-nt inter-stem-loop domain in the MHV genome can be deleted and viral progeny, although debilitated, are still produced. These results together identify a previously undescribed long-range RNA-RNA interaction between the 5' UTR and Nsp1 coding region in MHV-like and BCoV-like betacoronaviruses that is cis acting for viral fitness but is not absolutely required for viral replication in cell culture.

Guan, B. J., et al. (2011). "An optimal cis-replication stem-loop IV in the 5' untranslated region of the mouse coronavirus genome extends 16 nucleotides into open reading frame 1." J Virol **85**(11): 5593-5605.

The 288-nucleotide (nt) 3' untranslated region (UTR) in the genome of the bovine coronavirus (BCoV) and 339-nt 3' UTR in the severe acute respiratory syndrome (SARS) coronavirus (SCoV) can each replace the 301-nt 3' UTR in the mouse hepatitis coronavirus (MHV) for virus replication, thus demonstrating common 3' cis-replication signals. Here, we show that replacing the 209-nt MHV 5' UTR with the approximately 63%-sequence-identical 210-nt BCoV 5' UTR by reverse genetics does not yield viable virus, suggesting 5' end signals are more stringent or possibly are not strictly 5' UTR confined. To identify potential smaller, 5'-common signals, each of three stem-loop (SL) signaling domains and one inter-stem-loop domain from the BCoV 5' UTR was tested by replacing its counterpart in the MHV genome. The SLI/II domain (nucleotides 1 to 84) and SLIII domain (nucleotides 85 to 141) each immediately enabled near-wild-type (wt) MHV-like progeny, thus behaving similarly to comparable 5'-proximal regions of the SCoV 5' UTR as shown by others. The inter-stem-loop domain (nt 142 to 173 between SLs III and IV) enabled small plaques only after genetic adaptation. The SLIV domain (nt 174 to 210) required a 16-nt extension into BCoV open reading frame 1 (ORF1) for apparent stabilization of a longer BCoV SLIV (nt 174 to 226) and optimal virus replication. Surprisingly, pleiomorphic SLIV structures, including a terminal loop deletion, were found among debilitated progeny from intra-SLIV chimeras. The results show the inter-stem-loop domain to be a potential novel species-specific cis-replication element and that cis-acting SLIV in the viral genome extends into ORF1 in a manner that stabilizes its lower stem and is thus not 5' UTR confined.

Gustin, K. M., et al. (2009). "Bovine coronavirus nonstructural protein 1 (p28) is an RNA binding protein that binds terminal genomic cis-replication elements." J Virol **83**(12): 6087-6097.

Nonstructural protein 1 (nsp1), a 28-kDa protein in the bovine coronavirus (BCoV) and closely related mouse hepatitis coronavirus, is the first protein cleaved from the open reading frame 1 (ORF 1) polyprotein product of genome translation. Recently, a 30-nucleotide (nt) cis-replication stem-loop VI (SLVI) has been mapped at nt 101 to 130 within a 288-nt 5'-terminal segment of the 738-nt nsp1 cistron in a BCoV defective interfering (DI) RNA. Since a similar nsp1 coding region appears in all characterized groups 1 and 2 coronavirus DI RNAs and must be translated in cis for BCoV DI RNA replication, we hypothesized that nsp1 might regulate ORF 1 expression by binding this intra-nsp1 cistronic element. Here, we (i) establish by mutation analysis that the 72-nt intracistronic SLV immediately upstream of SLVI is also a DI RNA cis-replication signal, (ii) show by gel shift and UV-cross-linking analyses that cellular proteins of approximately 60 and 100 kDa, but not viral proteins, bind SLV and SLVI, (SLV-VI) and (iii) demonstrate by gel shift analysis that nsp1 purified from Escherichia coli does not bind SLV-VI but does bind three 5' untranslated region (UTR)- and one 3' UTR-located cis-replication SLs. Notably, nsp1 specifically binds SLIII and its

flanking sequences in the 5' UTR with approximately 2.5 muM affinity. Additionally, under conditions enabling expression of nsp1 from DI RNA-encoded subgenomic mRNA, DI RNA levels were greatly reduced, but there was only a slight transient reduction in viral RNA levels. These results together indicate that nsp1 is an RNA-binding protein that may function to regulate viral genome translation or replication but not by binding SLV-VI within its own coding region.

Hakki, M., et al. (2015). "The clinical impact of coronavirus infection in patients with hematologic malignancies and hematopoietic stem cell transplant recipients." J Clin Virol **68**: 1-5.

BACKGROUND: Compared to other respiratory viruses, relatively little is known about the clinical impact of coronavirus (CoV) infection after hematopoietic stem cell transplant (HSCT) or in patients with hematologic malignancies. OBJECTIVES: To characterize the role of CoV in respiratory tract infections among HSCT and hematologic malignancy patients. STUDY DESIGN: We conducted a retrospective review of all cases of CoV infection documented by polymerase chain reaction, (PCR)-based testing on nasopharyngeal and bronchoalveolar lavage fluid samples between June 2010 and 2013. Cases of CoV infection occurring in HSCT and hematologic malignancy patients were identified and the clinical characteristics of these cases were compared to other respiratory viruses. RESULTS: CoV was identified in 2.6% (n=43) of all samples analyzed (n=1661) and in 6.8% of all samples testing positive for a respiratory virus (n=631). 33 of 38 (86.8%) of patients in whom CoV was identified were HSCT and hematologic malignancy patients. Among these patients, CoV was detected in 9.7% of unique infection episodes, with only rhinovirus/enterovirus (RhV/EnV) infection being more common. Group I CoV subtypes accounted for 76.3% of cases, and 57% of infections were diagnosed between December and March. CoV infection was associated with upper respiratory tract symptoms in most patients, similar to other respiratory viruses. Possible and proven lower respiratory tract disease was less common compared to other respiratory viruses except RhV/EnV. CONCLUSIONS: CoV is frequently detected in HSCT and hematologic malignancy patients in whom suspicion for a respiratory viral infection exists, but is less likely to progress to lower respiratory tract disease than most other respiratory viruses.

Hirsch, H. H., et al. (2013). "Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus." Clin Infect Dis **56**(2): 258-266.

Community-acquired respiratory virus (CARV) infections have been recognized as a significant cause of morbidity and mortality in patients with leukemia and those undergoing hematopoietic stem cell transplantation (HSCT). Progression to lower respiratory tract infection with clinical and radiological signs of pneumonia and respiratory failure appears to depend on the intrinsic virulence of the specific CARV as well as factors specific to the patient, the underlying disease, and its treatment. To better define the current state of knowledge of CARVs in leukemia and HSCT patients, and to improve CARV diagnosis and management, a working group of the Fourth European Conference on Infections in Leukaemia (ECIL-4) 2011 reviewed the literature on CARVs, graded the available quality of evidence, and made recommendations according to the Infectious Diseases Society of America grading system. Owing to differences in screening, clinical presentation, and therapy for influenza and adenovirus, ECIL-4 recommendations are summarized for CARVs other than influenza and adenovirus.

Hsin, W. C., et al. (2018). "Nucleocapsid protein-dependent assembly of the RNA packaging signal of Middle East respiratory syndrome coronavirus." J Biomed Sci **25**(1): 47.

BACKGROUND: Middle East respiratory syndrome coronavirus (MERS-CoV) consists of a positive-sense, single-stranded RNA genome and four structural proteins: the spike, envelope, membrane, and nucleocapsid protein. The assembly of the viral genome into virus particles involves viral structural proteins and is believed to be mediated through recognition of specific sequences and RNA structures of the viral genome. METHODS AND RESULTS: A culture system for the production of MERS coronavirus-like particles (MERS VLPs) was determined and established by electron microscopy and the detection of coexpressed viral structural proteins. Using the VLP system, a 258-nucleotide RNA fragment, which spans nucleotides 19,712 to 19,969 of the MERS-CoV genome (designated PS258(19712-19969)ME), was identified to function as a packaging signal. Assembly of the RNA packaging signal into MERS VLPs is dependent on the viral nucleocapsid protein. In addition, a 45-nucleotide stable stem-loop substructure of the PS258(19712-19969)ME interacted with both the N-terminal domain and the C-terminal domain of the viral nucleocapsid protein. Furthermore, a functional SARS-CoV RNA packaging signal failed to assemble into the MERS VLPs, which indicated virus-specific assembly of the RNA genome. CONCLUSIONS: A MERS-oV RNA

packaging signal was identified by the detection of GFP expression following an incubation of MERS VLPs carrying the heterologous mRNA GFP-PS258(19712-19969)ME with virus permissive Huh7 cells. The MERS VLP system could help us in understanding virus infection and morphogenesis.

Hutspardol, S., et al. (2015). "Significant Transplantation-Related Mortality from Respiratory Virus Infections within the First One Hundred Days in Children after Hematopoietic Stem Cell Transplantation." Biol Blood Marrow Transplant **21**(10): 1802-1807.

Respiratory viral infections (RVI) are important in hematopoietic stem cell transplantations (HSCT) and knowledge regarding incidence, morbidity, mortality, and long-term pulmonary complications is limited. We report a study to evaluate incidence and outcomes, both short and long-term, of RVI in children receiving HSCT. Between January 2000 and December 2012, 844 patients underwent hematopoietic stem cell transplantation (HSCT) at the Hospital for Sick Children: 491 were allogeneic and 353 were autologous. When screening for causes of death in the first year after HSCT in the 844 patients, we found that RVI as a cause of death was only evident in the first 100 days after HSCT. Fifty-four (6.5%) patients were found to have an RVI within the first 100 days after HSCT (allogeneic = 32, autologous = 22). Upper and lower respiratory tract infections were documented in 31 (57%) and 23 (43%) patients, respectively. Viruses were parainfluenza (35%), respiratory syncytial virus (28%), influenza (22%), adenovirus (7%), human metapneumovirus (4%), coronavirus (2%), and rhinovirus (2%). Three patients relapsed with their primary disease before day 100 and were excluded. The overall mortality for the remaining 51 patients was 10% (allogeneic = 4, autologous = 1). All 5 deaths were directly attributable to RVI and all 5 deaths occurred in patients with a lower respiratory tract infection. The remaining patients were followed for a median of 4.3 years (range, 1.4 to 11.8) and no chronic pulmonary complications were observed. A clear seasonal pattern for contracting RVI was evident with 65% of total RVI occurring between October and March (35 of 427 versus 19 of 417, P =.03). Given the significant mortality from RVI and the challenges in preventing them, choosing the time to start HSCT, whenever possible, may help prevent RVI and improve outcomes.

Ireland, D. D., et al. (2009). "RNase L mediated protection from virus induced demyelination." PLoS Pathog **5**(10): e1000602.

IFN-alpha/beta plays a critical role in limiting viral spread, restricting viral tropism and protecting mice from neurotropic coronavirus infection. However, the IFN-alpha/beta dependent mechanisms underlying innate anti-viral functions within the CNS are poorly understood. The role of RNase L in viral encephalomyelitis was explored based on its functions in inhibiting translation, inducing apoptosis, and propagating the IFN-alpha/beta pathway through RNA degradation intermediates. Infection of RNase L deficient (RL (-/-)) mice with a sub-lethal, demyelinating mouse hepatitis virus variant revealed that the majority of mice succumbed to infection by day 12 p.i. However, RNase L deficiency did not affect overall control of infectious virus, or diminish IFN-alpha/beta expression in the CNS. Furthermore, increased morbidity and mortality could not be attributed to altered proinflammatory signals or composition of cells infiltrating the CNS. The unique phenotype of infected RL (-/-) mice was rather manifested in earlier onset and increased severity of demyelination and axonal damage in brain stem and spinal cord without evidence for enhanced neuronal infection. Increased tissue damage coincided with sustained brain stem infection, foci of microglia infection in grey matter, and increased apoptotic cells. These data demonstrate a novel protective role for RNase L in viral induced CNS encephalomyelitis, which is not reflected in overall viral control or propagation of IFN-alpha/beta mediated signals. Protective function is rather associated with cell type specific and regional restriction of viral replication in grey matter and ameliorated neurodegeneration and demyelination.

Ison, M. G., et al. (2003). "Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia." Clin Infect Dis **36**(9): 1139-1143.

Little is known about the impact of human rhinovirus (HRV) and coronavirus infections in hematopoietic stem cell transplant (HSCT) recipients. We tested bronchoalveolar lavage (BAL) samples obtained from HSCT recipients with acute pulmonary infiltrates for HRV (n=122) and coronavirus (n=46) by reverse-transcriptase polymerase chain reaction. HRV RNA was detected in 6 (8%) of 77 patients, and coronavirus RNA was detected in 0 of 46 of BAL samples from HSCT recipients. The fatality rate in HRV-infected patients was high (83%), but all patients had significant coinfections, and the overall mortality rate was not different from that of patients who were negative for HRV in BAL samples. These results suggest that HRV may be a cause of lower respiratory tract infections in HSCT recipients and that its detection in BAL samples is associated with frequent copathogens. Whether the poor prognosis is due to HRV or the copathogen is not clear.

Jolicoeur, P. and L. Lamontagne (1995). "Impairment of bone marrow pre-B and B cells in MHV3 chronically-infected mice." Adv Exp Med Biol **380**: 193-195.

Mouse hepatitis virus type 3 (MHV3) appears to be an excellent model for the study of the relationship between viral-induced immunodeficiency and the development of chronic disease. Animal surviving acute hepatitis develop a chronic disease characterized by viral persistency in various organs, by a humoral immunodeficiency, and eventually die within the next three months postinfection. To verify if B cell immunodeficiency occurs during the chronic disease, percentage and absolute number of bone marrow B lineage cell subpopulations were recorded at various times postinfection (p.i.) in pathogenic L2-MHV3-infected (C57BL/6 x A/J) F1 mice. Absolute numbers of B (cmu+smu+) cells decreased as early as three days p.i. up to 15 days p.i., and then gradually returned toward normal values in L2-MHV3-infected mice during the chronic disease. In contrast, pre-B (cmu+smu-) cells were less significantly decrease during the chronic disease. In addition, abnormally enlarged cells (> 13 microns) were detected either in bone marrow pre-B or B cells from L2-MHV3-infected mice.

Jung, K., et al. (2015). "Comparative pathogenesis of US porcine epidemic diarrhea virus (PEDV) strain PC21A in conventional 9-day-old nursing piglets vs. 26-day-old weaned pigs." Vet Microbiol **178**(1-2): 31-40.

Our study demonstrated potential mechanisms by which porcine epidemic diarrhea virus (PEDV) infection induces greater disease severity of nursing vs. weaned conventional pigs. Twenty-six-day-old weaned [PEDV-inoculated (n=11); mock (n=9)] and 9-day-old nursing pigs [PEDV-inoculated (n=9); mock (n=11)] were inoculated orally [8.9 log10 genomic equivalents (GE)/pig] with PC21A strain or mock (MEM). Pigs were monitored for clinical signs and PEDV RNA titers in feces and serum. For pathology and immunofluorescence staining for Ki67 (marker for crypt proliferation) and LGR5 (marker for crypt stem cell), 3-4 pigs were euthanized at postinoculation days (PIDs) 1, 3 and 5. Severe watery diarrhea and atrophic enteritis with moderate to high PEDV RNA titers in feces (7.5-12.2 log10 GE/ml) and low viral RNA titers in serum (5.6-8.6 log10 GE/ml) were observed in all inoculated nursing piglets at PIDs 1-5. In contrast, weaned pigs did not show evidence of PEDV infection at PID 1. Pigs exhibited high fecal shedding titers at PIDs 2-5 and mild to severe atrophic enteritis at PIDs 3-5, indicating a longer incubation for PEDV infection. While uninoculated or inoculated 27-31-day-old pigs showed large numbers of Ki67- or LGR5-positive cells in the intestinal crypts, there was a lack of LGR5-positive cells and low proliferation of crypts in jejunum of uninoculated 10-14-day-old piglets, possibly causing a slower turnover of enterocytes; however, the number of LGR5-positive cells and proliferation of intestinal crypts increased remarkably at 3-5 days after inoculation. Biologic mediators that promote crypt stem cell regeneration would be targets to improve the intestinal epithelium renewal during PEDV infection.

Kang, H., et al. (2006). "Putative cis-acting stem-loops in the 5' untranslated region of the severe acute respiratory syndrome coronavirus can substitute for their mouse hepatitis virus counterparts." J Virol **80**(21): 10600-10614.

Consensus covariation-based secondary structural models for the 5' 140 nucleotides of the 5' untranslated regions (5'UTRs) from mouse hepatitis virus (MHV) and severe acute respiratory syndrome coronavirus (SCoV) were developed and predicted three major helical stem-loop structures, designated stem-loop 1 (SL1), SL2, and SL4. The SCoV 5'UTR was predicted to contain a fourth stem-loop, named SL3, in which the leader transcriptional regulatory sequence (TRS) is folded into a hairpin loop. cDNAs corresponding to MHV/SCoV chimeric genomes were constructed by replacing the complete MHV 5'UTR with the corresponding SCoV sequence and by separately replacing MHV 5'UTR putative SL1, putative SL2, TRS, and putative SL4 with the corresponding SCoV sequences. Chimeric genomes were transcribed in vitro, and viruses were recovered after electroporation into permissive cells. Genomes in which the MHV 5'UTR SL1, SL2, and SL4 were individually replaced by their SCoV counterparts were viable. Chimeras containing the complete SCoV 5'UTR or the predicted SCoV SL3 were not viable. A chimera containing the SCoV 5'UTR in which the SCoV TRS was replaced with the MHV TRS was also not viable. The chimera containing the entire SCoV 5'UTR failed to direct the synthesis of any virus-specific RNA. Replacing the SCoV TRS with the MHV TRS in the MHV/5'UTR SCoV chimera permitted the synthesis of minus-sense genome-sized RNA but did not support the production of positive- or minus-sense subgenomic RNA7. A similar phenotype was obtained with the MHV/SCoV SL3 chimera. These results suggest a role for the TRS in the replication of minus-sense genomic RNA in addition to its known function in subgenomic RNA synthesis.

Kim, S. H., et al. (2017). "Atypical presentations of MERS-CoV infection in immunocompromised hosts." J Infect Chemother **23**(11): 769-773.

During the 2015 Korean MERS outbreak, we experienced atypical presentations of MERS-CoV infections in three immunocompromised hosts that warranted exceptional management. Case 1 showed delayed symptom development after a four-day asymptomatic period, Case 2 experienced a 20-day incubation period, and Case 3 exhibited persistent viral shedding without clinical deterioration. Recognizing these exceptions is extremely important in the management of MERS-CoV-exposed or -infected patients and for control of potential MERS outbreaks.

Kim, Y. N. and S. Makino (1995). "Characterization of a murine coronavirus defective interfering RNA internal cis-acting replication signal." J Virol **69**(8): 4963-4971.

The mouse hepatitis virus (MHV) sequences required for replication of the JHM strain of MHV defective interfering (DI) RNA consist of three discontinuous genomic regions: about 0.47 kb from both terminal sequences and a 0.13-kb internal region present at about 0.9 kb from the 5' end of the DI genome. In this study, we investigated the role of the internal 0.13-kb region in MHV RNA replication. Overall sequences of the 0.13-kb regions from various MHV strains were similar to each other, with nucleotide substitutions in some strains; MHV-A59 was exceptional, with three nucleotide deletions. Computer-based secondary-structure analysis of the 0.13-kb region in the positive strand revealed that most of the MHV strains formed the same or a similar main stem-loop structure, whereas only MHV-A59 formed a smaller main stem-loop structure. The RNA secondary structures in the negative strands were much less uniform among the MHV strains. A series of DI RNAs that contained MHV-JHM-derived 5'- and 3'-terminal sequences plus internal 0.13-kb regions derived from various MHV strains were constructed. Most of these DI RNAs replicated in MHV-infected cells, except that MRP-A59, with a 0.13-kb region derived from MHV-A59, failed to replicate. Interestingly, replication of MRP-A59 was temperature dependent; it occurred at 39.5 degrees C but not at 37 or 35 degrees C, whereas a DI RNA with an MHV-JHM-derived 0.13-kb region replicated at all three temperatures. At 37 degrees C, synthesis of MRP-A59 negative-strand RNA was detected in MHV-infected and MRP-A59 RNA-transfected cells. Another DI RNA with the internal 0.13-kb region deleted also synthesized negative-strand RNA in MHV-infected cells. MRP-A59-transfected cells were shifted from 39.5 to 37 degrees C at 5.5 h postinfection, a time when most MHV negative-strand RNAs have already accumulated; after the shift, MRP-A59 positive-strand RNA synthesis ceased. The minimum sequence required for maintenance of the positive-strand major stem-loop structure and biological function of the MHV-JHM 0.13-kb region was about 57 nucleotides. Function was lost in the 50-nucleotide sequence that formed a positive-strand stem-loop structure identical to that of MHV-A59. These studies suggested that the RNA structure made by the internal sequence was important for positive-strand MHV RNA synthesis.

Ko, J. H., et al. (2018). "Host susceptibility to MERS-CoV infection, a retrospective cohort study of the 2015 Korean MERS outbreak." J Infect Chemother **24**(2): 150-152.

To evaluate host susceptibility factors to Middle East respiratory syndrome coronavirus (MERS-CoV) infection, we conducted a retrospective cohort study from the single largest exposure event of the 2015 Korean MERS outbreak. A total of 175 patients were closely exposed to a super-spreader, 26 of which were infected (14.9%). In a multivariate analysis, history of autologous stem cell transplantation (HR, 31.151; 95% CI, 5.447-178.145; $P < 0.001$) and tachypnea at ED (HR, 4.392; 95% CI, 1.402-13.761; $P = 0.011$) were significantly associated with MERS-CoV infection.

Kyuwa, S., et al. (2000). "Replication of enterotropic and polytropic murine coronaviruses in cultured cell lines of mouse origin." Exp Anim **49**(4): 251-257.

To understand the virus-cell interactions that occur during murine coronavirus infection, six murine cell lines (A3-1M, B16, CMT-93, DBT, IC-21 and J774A.1) were inoculated with eight murine coronaviruses, including prototype strains of both polytropic and enterotropic biotypes, and new isolates. All virus strains produced a cytopathic effect (CPE) with cell-to-cell fusion in B16, DBT, IC-21 and J774A.1 cells. The CPE was induced most rapidly in IC-21 cells and was visible microscopically in all cell lines tested. In contrast, the coronaviruses produced little CPE in A3-1M and CMT-93 cells. Although most virus-infected cells, except KQ3E-infected A3-1M, CMT-93 and J774A.1 cells, produced progeny viruses in the supernatants when assayed by plaque formation on DBT cells, the kinetics of viral replication were dependent on both the cell line and virus strain; replication of prototype strains was higher than that of new isolates. There was no significant difference in replication of enterotropic and polytropic strains. B16 cells supported the highest level of viral replication. To determine the sensitivity of the cell lines to murine coronaviruses, the 50% tissue culture infectious dose of the coronaviruses was determined with B16, DBT, IC-21 and J774A.1 cells, and compared to that with DBT cells. The results indicate that IC-21 cells were the most sensitive to murine coronaviruses. These data suggest that B16 and IC-21

cells are suitable for large-scale preparation and isolation of murine coronaviruses, respectively.

Lee, K. H., et al. (2019). "Characteristics of community-acquired respiratory viruses infections except seasonal influenza in transplant recipients and non-transplant critically ill patients." J Microbiol Immunol Infect.

BACKGROUND/PURPOSE: Transplant recipients are vulnerable to life-threatening community-acquired respiratory viruses (CA-RVs) infection (CA-RVI). Even if non-transplant critically ill patients in intensive care unit (ICU) have serious CA-RVI, comparison between these groups remains unclear. We aimed to evaluate clinical characteristics and mortality of CA-RVI except seasonal influenza A/B in transplant recipients and non-transplant critically ill patients in ICU. METHODS: We collected 37,777 CA-RVs multiplex real-time reverse transcription-polymerase chain reaction test results of individuals aged >/=18 years from November 2012 to November 2017. The CA-RVs tests included adenovirus, coronavirus 229E/NL63/OC43, human bocavirus, human metapneumovirus, parainfluenza virus 1/2/3, rhinovirus, and respiratory syncytial virus A/B. RESULTS: We found 286 CA-RVI cases, including 85 solid organ transplantation recipients (G1), 61 hematopoietic stem cell transplantation recipients (G2), and 140 non-transplant critically ill patients in ICU (G3), excluding those with repeated isolation within 30 days. Adenovirus positive rate and infection cases were most prominent in G2 ($p < 0.001$). The median time interval between transplantation and CA-RVI was 30 and 20 months in G1 and G2, respectively. All-cause in-hospital mortality was significantly higher in G3 than in G1 or G2 (51.4% vs. 28.2% or 39.3%, $p = 0.002$, respectively). The mechanical ventilation (MV) was the independent risk factor associated with all-cause in-hospital mortality in all three groups (hazard ratio, 3.37, 95% confidence interval, 2.04-5.56, $p < 0.001$). CONCLUSIONS: This study highlights the importance of CA-RVs diagnosis in transplant recipients even in long-term posttransplant period, and in non-transplant critically ill patients in ICU with MV.

Li, K., et al. (2016). "Middle East Respiratory Syndrome Coronavirus Causes Multiple Organ Damage and Lethal Disease in Mice Transgenic for Human Dipeptidyl Peptidase 4." J Infect Dis **213**(5): 712-722.

Middle East respiratory syndrome coronavirus (MERS-CoV) causes life-threatening disease. Dipeptidyl peptidase 4 (DPP4) is the receptor for cell binding and entry. There is a need for small-animal models of MERS, but mice are not susceptible to MERS because murine dpp4 does not serve as a receptor. We developed transgenic mice expressing human DPP4 (hDPP4) under the control of the surfactant protein C promoter or cytokeratin 18 promoter that are susceptible to infection with MERS-CoV. Notably, mice expressing hDPP4 with the cytokeratin 18 promoter developed progressive, uniformly fatal disease following intranasal inoculation. High virus titers were present in lung and brain tissues 2 and 6 days after infection, respectively. MERS-CoV-infected lungs revealed mononuclear cell infiltration, alveolar edema, and microvascular thrombosis, with airways generally unaffected. Brain disease was observed, with the greatest involvement noted in the thalamus and brain stem. Animals immunized with a vaccine candidate were uniformly protected from lethal infection. These new mouse models of MERS-CoV should be useful for investigation of early disease mechanisms and therapeutic interventions.

Li, L., et al. (2019). "Porcine Intestinal Enteroids: a New Model for Studying Enteric Coronavirus Porcine Epidemic Diarrhea Virus Infection and the Host Innate Response." J Virol **93**(5).

Porcine epidemic diarrhea virus (PEDV), a member of the group of alphacoronaviruses, is the pathogen of a highly contagious gastrointestinal swine disease. The elucidation of the events associated with the intestinal epithelial response to PEDV infection has been limited by the absence of good in vitro porcine intestinal models that recapitulate the multicellular complexity of the gastrointestinal tract. Here, we generated swine enteroids from the intestinal crypt stem cells of the duodenum, jejunum, or ileum and found that the generated enteroids are able to satisfactorily recapitulate the complicated intestinal epithelium in vivo and are susceptible to infection by PEDV. PEDV infected multiple types of cells, including enterocytes, stem cells, and goblet cells, and exhibited segmental infection discrepancies compared with ileal enteroids and colonoids, and this finding was verified in vivo Moreover, the clinical isolate PEDV-JMS propagated better in ileal enteroids than the cell-adapted isolate PEDV-CV777, and PEDV infection suppressed interferon (IFN) production early during the infection course. IFN lambda elicited a potent antiviral response and inhibited PEDV in enteroids more efficiently than IFN alpha (IFN-alpha). Therefore, swine enteroids provide a novel in vitro model for exploring the pathogenesis of PEDV and for the in vitro study of the interplay between a host and a variety of swine enteric viruses.IMPORTANCE PEDV is a highly contagious enteric coronavirus that causes significant economic losses, and the lack of a good in vitro model system is a major roadblock to an

in-depth understanding of PEDV pathogenesis. Here, we generated a porcine intestinal enteroid model for PEDV infection. Utilizing porcine intestinal enteroids, we demonstrated that PEDV infects multiple lineages of the intestinal epithelium and preferably infects ileal enteroids over colonoids and that enteroids prefer to respond to IFN lambda 1 over IFN-alpha. These events recapitulate the events that occur in vivo This study constitutes the first use of a primary intestinal enteroid model to investigate the susceptibility of porcine enteroids to PEDV and to determine the antiviral response following infection. Our study provides important insights into the events associated with PEDV infection of the porcine intestine and provides a valuable in vitro model for studying not only PEDV but also other swine enteric viruses.

Li, L., et al. (2008). "Structural lability in stem-loop 1 drives a 5' UTR-3' UTR interaction in coronavirus replication." J Mol Biol **377**(3): 790-803.

The leader RNA of the 5' untranslated region (UTR) of coronaviral genomes contains two stem-loop structures denoted SL1 and SL2. Herein, we show that SL1 is functionally and structurally bipartite. While the upper region of SL1 is required to be paired, we observe strong genetic selection against viruses that contain a deletion of A35, an extrahelical nucleotide that destabilizes SL1, in favor of genomes that contain a diverse panel of destabilizing second-site mutations, due to introduction of a noncanonical base pair near A35. Viruses containing destabilizing SL1-DeltaA35 mutations also contain one of two specific mutations in the 3' UTR. Thermal denaturation and imino proton solvent exchange experiments reveal that the lower half of SL1 is unstable and that second-site SL1-DeltaA35 substitutions are characterized by one or more features of the wild-type SL1. We propose a "dynamic SL1" model, in which the base of SL1 has an optimized lability required to mediate a physical interaction between the 5' UTR and the 3' UTR that stimulates subgenomic RNA synthesis. Although not conserved at the nucleotide sequence level, these general structural characteristics of SL1 appear to be conserved in other coronaviral genomes.

Ling, T. Y., et al. (2006). "Identification of pulmonary Oct-4+ stem/progenitor cells and demonstration of their susceptibility to SARS coronavirus (SARS-CoV) infection in vitro." Proc Natl Acad Sci U S A **103**(25): 9530-9535.

In this study, we report a serum-free culture system for primary neonatal pulmonary cells that can support the growth of octamer-binding transcription factor 4+ (Oct-4+) epithelial colonies with a surrounding mesenchymal stroma. In addition to Oct-4, these cells also express other stem cell markers such as stage-specific embryonic antigen 1 (SSEA-1), stem cell antigen 1 (Sca-1), and Clara cell secretion protein (CCSP) but not c-Kit, CD34, and p63, indicating that they represent a subpopulation of Clara cells that have been implicated as lung stem/progenitor cells in lung injury models. These colony cells can be kept for weeks in primary cultures and undergo terminal differentiation to alveolar type-2- and type-1-like pneumocytes sequentially when removed from the stroma. In addition, we have demonstrated the presence of Oct-4+ long-term BrdU label-retaining cells at the bronchoalveolar junction of neonatal lung, providing a link between the Oct-4+ cells in vivo and in vitro and strengthening their identity as putative neonatal lung stem/progenitor cells. Lastly, these Oct-4+ epithelial colony cells, which also express angiotensin-converting enzyme 2, are the target cells for severe acute respiratory syndrome coronavirus infection in primary cultures and support active virus replication leading to their own destruction. These observations imply the possible involvement of lung stem/progenitor cells, in addition to pneumocytes, in severe acute respiratory syndrome coronavirus infection, accounting for the continued deterioration of lung tissues and apparent loss of capacity for lung repair.

Liu, P., et al. (2013). "Functional analysis of the stem loop S3 and S4 structures in the coronavirus 3'UTR." Virology **443**(1): 40-47.

We designed a series of mutations to separately destabilize two helical stems (designated S3 and S4) predicted by a covariation-based model of the coronavirus 3'UTR (Zust et al., 2008). Mouse hepatitis virus genomes containing three or four nucleotide mutations that destabilize either S3 or S4 were viable, whereas genomes carrying these mutations in both S3 and S4 were not viable. A genome carrying these mutations in S3 and S4 plus compensatory mutations restoring base-pairing yielded a virus with wild type phenotype. Larger mutations which completely disrupt S3 or S4 generated various phenotypes. Mutations opening up S3 were lethal. Disruptions of S4 generated both viable and lethal mutants. Genomes carrying the original mutations in S3 or S4 plus compensatory mutations restoring base pairing were viable and had robust growth phenotypes. These results support the Zust model for the coronavirus 3'UTR and suggest that the S3 stem is required for virus viability.

Madhugiri, R., et al. (2018). "Structural and functional conservation of cis-acting RNA elements in coronavirus 5'-terminal genome regions." Virology **517**: 44-55.

Structure predictions suggest a partial conservation of RNA structure elements in coronavirus terminal genome regions. Here, we determined the structures of stem-loops (SL) 1 and 2 of two alphacoronaviruses, human coronavirus (HCoV) 229E and NL63, by RNA structure probing and studied the functional relevance of these putative cis-acting elements. HCoV-229E SL1 and SL2 mutants generated by reverse genetics were used to study the effects on viral replication of single-nucleotide substitutions predicted to destabilize the SL1 and SL2 structures. The data provide conclusive evidence for the critical role of SL1 and SL2 in HCoV-229E replication and, in some cases, revealed parallels with previously characterized betacoronavirus SL1 and SL2 elements. Also, we were able to rescue viable HCoV-229E mutants carrying replacements of SL2 with equivalent betacoronavirus structural elements. The data obtained in this study reveal a remarkable degree of structural and functional conservation of 5'-terminal RNA structural elements across coronavirus genus boundaries.

Mallick, B., et al. (2009). "MicroRNome analysis unravels the molecular basis of SARS infection in bronchoalveolar stem cells." PLoS One **4**(11): e7837.

Severe acute respiratory syndrome (SARS), caused by the coronavirus SARS-CoV, is an acute infectious disease with significant mortality. A typical clinical feature associated with SARS is pulmonary fibrosis and associated lung failure. In the aftermath of the SARS epidemic, although significant progress towards understanding the underlying molecular mechanism of the infection has been made, a large gap still remains in our knowledge regarding how SARS-CoV interacts with the host cell at the onset of infection. The rapidly changing viral genome adds another variable to this equation. We have focused on a novel concept of microRNA (miRNA)-mediated host-virus interactions in bronchoalveolar stem cells (BASCs) at the onset of infection by correlating the "BASC-microRNome" with their targets within BASCs and viral genome. This work encompasses miRNA array data analysis, target prediction, and miRNA-mRNA enrichment analysis and develops a complex interaction map among disease-related factors, miRNAs, and BASCs in SARS pathway, which will provide some clues for diagnostic markers to view an overall interplay leading to disease progression. Our observation reveals the BASCs (Sca-1+ CD34+ CD45-Pecam-), a subset of Oct-4+ ACE2+ epithelial colony cells at the broncho-alveolar duct junction, to be the prime target cells of SARS-CoV infection. Upregulated BASC miRNAs-17*, -574-5p, and -214 are co-opted by SARS-CoV to suppress its own replication and evade immune elimination until successful transmission takes place. Viral Nucleocapsid and Spike protein targets seem to co-opt downregulated miR-223 and miR-98 respectively within BASCs to control the various stages of BASC differentiation, activation of inflammatory chemokines, and downregulation of ACE2. All these effectively accounts for a successful viral transmission and replication within BASCs causing continued deterioration of lung tissues and apparent loss of capacity for lung repair. Overall, this investigation reveals another mode of exploitation of cellular miRNA machinery by virus to their own advantage.

Mangale, V., et al. (2017). "Neural precursor cells derived from induced pluripotent stem cells exhibit reduced susceptibility to infection with a neurotropic coronavirus." Virology **511**: 49-55.

The present study examines the susceptibility of mouse induced pluripotent stem cell-derived neural precursor cells (iPSC-NPCs) to infection with the neurotropic JHM strain of mouse hepatitis virus (JHMV). Similar to NPCs derived from striatum of day 1 postnatal GFP-transgenic mice (GFP-NPCs), iPSC-derived NPCs (iPSC-NPCs) are able to differentiate into terminal neural cell types and express MHC class I and II in response to IFN-gamma treatment. However, in contrast to postnatally-derived NPCs, iPSC-NPCs express low levels of carcinoembryonic antigen-cell adhesion molecule 1a (CEACAM1a), the surface receptor for JHMV, and are less susceptible to infection and virus-induced cytopathic effects. The relevance of this in terms of therapeutic application of NPCs resistant to viral infection is discussed.

Mikulska, M., et al. (2014). "Epidemiology of viral respiratory tract infections in an outpatient haematology facility." Ann Hematol **93**(4): 669-676.

Viral respiratory tract infections (VRTI) are an important cause of morbidity and mortality in haematology patients, particularly after haematopoietic stem cell transplantation (HSCT). The incidence, clinical presentation and outcome of symptomatic and asymptomatic VRTI in HSCT outpatient unit were prospectively evaluated during a single influenza season (January-March 2011). Pharyngeal swabs were performed at the first visit and if new symptoms were present. Molecular multiplex assay for 12 respiratory viruses was performed by the regional reference laboratory. Among 264 swabs from 193 outpatients, 58 (22 %) resulted positive for 61 viruses (influenza, n = 20; respiratory syncytial virus [RSV], n = 21; rhinovirus, n = 12; coronavirus, n = 4; adenovirus, n = 3; parainfluenza, n = 1). VRTI were detected more frequently in the presence of symptoms than in asymptomatic patients: 49 out of 162 (30 %) vs. 9 out

of 102 (9 %), p < 0.001. Influenza-like illness syndrome (ILI) was significantly associated with a VRTI if compared to other presentations (42 %), while the European Centre for Disease Prevention and Control definition was not (30 %). Positive predictive value (PPV) of ILI for influenza was 17 %. Influenza and RSV peak periods were contemporary. Influenza prophylaxis was given to 25 patients following exposure. Low rate of progression from upper to lower respiratory tract infection (approximately 5 % for influenza and RSV), no nosocomial epidemics and no VRTI-related deaths were observed. VRTI are very frequent in high-risk haematology outpatients, but symptoms are aspecific and PPV of ILI is low. Symptoms of influenza and RSV overlap. Thus, microbiological diagnosis and contact preventive measures are crucial. Rather than universal influenza prophylaxis, prompt diagnosis and treatment of only documented infections could be pursued.

Milano, F., et al. (2010). "Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients." Blood **115**(10): 2088-2094.

Little is known about clinical and virologic manifestations of rhinovirus (HRV) and coronavirus (HCoV) infections after hematopoietic cell transplantation (HCT). We performed surveillance for 1 year and describe the natural history of these infections during the first 100 days after allogeneic HCT, when symptom surveys and upper respiratory samples were collected weekly. Samples were tested using RT-PCR for HRVs and HCoVs (OC43, 229E, HKU1, and NL63). Among 215 patients, 64 (30%) patients had 67 infections. Day 100 cumulative incidence estimate was 22.3% for HRV and 11.1% for HCoV. Median duration of viral shedding was 3 weeks; prolonged shedding of at least 3 months occurred in 6 of 45 patients with HRV and 3 of 22 with HCoV. Six patients with HRV and 9 with HCoV were asymptomatic. HRV infection was associated with rhinorrhea, congestion, postnasal drip, sputum, and cough; HCoV infection was not associated with respiratory symptoms or hepatic dysfunction. Lower respiratory infection developed in 2 patients with HRV before day 100, and 1 each with HRV and HCoV after day 100. HRV and HCoV infections are common in the first 100 days after HCT, viral shedding lasts more than 3 weeks in half, and lower respiratory infection is rare.

Molenkamp, R. and W. J. Spaan (1997). "Identification of a specific interaction between the coronavirus mouse hepatitis virus A59 nucleocapsid protein and packaging signal." Virology **239**(1): 78-86.

The coronavirus mouse hepatitis virus (MHV) is an enveloped positive stranded RNA virus. In infected cells MHV produces a 3' coterminal nested set of subgenomic messenger RNAs. Only the genomic RNA, however, is encapsidated by the nucleocapsid protein and incorporated in infectious MHV virions. It is believed that an RNA packaging signal (Ps), present only in the genomic RNA, is responsible for this selectivity. Earlier studies mapped this signal to a 69-nt stem-loop structure positioned in the 3' end of ORF1b. The selective encapsidation mechanism probably initiates by specific interaction of the packaging signal with the nucleocapsid protein. In this study we demonstrate the in vitro interaction of the MHV-A59 nucleocapsid protein with the packaging signal of MHV using gel retardation and UV cross-linking assays. This interaction was observed not only with the nucleocapsid protein from infected cells but also with that from purified virions and from cells expressing a recombinant nucleocapsid protein. The specificity of the interaction was demonstrated by competition experiments with nonlabeled Ps containing RNAs, tRNA, and total cytoplasmic RNA. The results indicated that no virus specific modification of the N-protein or the presence of other viral proteins are required for this in vitro intervention. The assays described in this report provide us with a powerful tool for studying encapsidation (initiation) in more detail.

Morales, L., et al. (2013). "Transmissible gastroenteritis coronavirus genome packaging signal is located at the 5' end of the genome and promotes viral RNA incorporation into virions in a replication-independent process." J Virol **87**(21): 11579-11590.

Preferential RNA packaging in coronaviruses involves the recognition of viral genomic RNA, a crucial process for viral particle morphogenesis mediated by RNA-specific sequences, known as packaging signals. An essential packaging signal component of transmissible gastroenteritis coronavirus (TGEV) has been further delimited to the first 598 nucleotides (nt) from the 5' end of its RNA genome, by using recombinant viruses transcribing subgenomic mRNA that included potential packaging signals. The integrity of the entire sequence domain was necessary because deletion of any of the five structural motifs defined within this region abrogated specific packaging of this viral RNA. One of these RNA motifs was the stem-loop SL5, a highly conserved motif in coronaviruses located at nucleotide positions 106 to 136. Partial deletion or point mutations within this motif also abrogated packaging. Using TGEV-derived defective minigenomes replicated in trans by a helper virus, we have shown that TGEV RNA packaging is a replication-independent process. Furthermore, the last

494 nt of the genomic 3' end were not essential for packaging, although this region increased packaging efficiency. TGEV RNA sequences identified as necessary for viral genome packaging were not sufficient to direct packaging of a heterologous sequence derived from the green fluorescent protein gene. These results indicated that TGEV genome packaging is a complex process involving many factors in addition to the identified RNA packaging signal. The identification of well-defined RNA motifs within the TGEV RNA genome that are essential for packaging will be useful for designing packaging-deficient biosafe coronavirus-derived vectors and providing new targets for antiviral therapies.

Ogimi, C., et al. (2017). "Prolonged Shedding of Human Coronavirus in Hematopoietic Cell Transplant Recipients: Risk Factors and Viral Genome Evolution." J Infect Dis **216**(2): 203-209.

Background: Recent data suggest that human coronavirus (HCoV) pneumonia is associated with significant mortality in hematopoietic cell transplant (HCT) recipients. Investigation of risk factors for prolonged shedding and intrahost genome evolution may provide critical information for development of novel therapeutics. Methods: We retrospectively reviewed HCT recipients with HCoV detected in nasal samples by polymerase chain reaction (PCR). HCoV strains were identified using strain-specific PCR. Shedding duration was defined as time between first positive and first negative sample. Logistic regression analyses were performed to evaluate factors for prolonged shedding (>/=21 days). Metagenomic next-generation sequencing (mNGS) was conducted when >/=4 samples with cycle threshold values of <28 were available. Results: Seventeen of 44 patients had prolonged shedding. Among 31 available samples, 35% were OC43, 32% were NL63, 19% were HKU1, and 13% were 229E; median shedding duration was similar between strains (P =.79). Bivariable logistic regression analyses suggested that high viral load, receipt of high-dose steroids, and myeloablative conditioning were associated with prolonged shedding. mNGS among 5 subjects showed single-nucleotide polymorphisms from OC43 and NL63 starting 1 month following onset of shedding. Conclusions: High viral load, high-dose steroids, and myeloablative conditioning were associated with prolonged shedding of HCoV in HCT recipients. Genome changes were consistent with the expected molecular clock of HCoV.

Ogimi, C., et al. (2017). "Clinical Significance of Human Coronavirus in Bronchoalveolar Lavage Samples From Hematopoietic Cell Transplant Recipients and Patients With Hematologic Malignancies." Clin Infect Dis **64**(11): 1532-1539.

Background.: The possible role of human coronavirus (HCoV) in lower respiratory tract disease (LRTD) in hematopoietic cell transplant (HCT) recipients and patients with hematologic malignancies (HM) has not been well studied. Methods.: We conducted a retrospective review of HCT/HM patients with HCoV detected in bronchoalveolar lavage (BAL). HCoV strains were identified in BAL samples using strain-specific polymerase chain reaction. Mortality rates were compared among HCT recipients with LRTD caused by HCoV, respiratory syncytial virus (RSV), influenza virus, or parainfluenza virus (PIV) by multivariable Cox regression analysis. Results.: We identified 35 patients (37 episodes) with HCoV LRTD. Among 23 available BAL samples, 48% were strain OC43, 22% were NL63, 17% were 229E, and 13% were HKU1. Overall, 21 patients (60%) required oxygen therapy at diagnosis and 19 (54%) died within 90 days of diagnosis. Respiratory copathogens were detected in 21 episodes (57%), including viruses (n = 12), fungi (n = 10), and bacteria (n = 8). Mortality rates were not different between patients with and without copathogens (P =.65). In multivariable models, mortality associated with HCoV LRTD was similar to that seen with RSV, influenza, and PIV LRTD in HCT recipients (adjusted hazard ratio, 1.34 [95% confidence interval,.66-2.71], P =.41 vs RSV, adjusted for cell source, cytopenia, copathogens, oxygen use, and steroid use). Conclusions.: HCoV LRTD in patients with HCT or HM is associated with high rates of oxygen use and mortality. Mortality associated with HCoV LRTD in HCT recipients appears to be similar to that seen with RSV, influenza virus, and PIV.

Okumura, A., et al. (1996). "Maintenance of pluripotency in mouse embryonic stem cells persistently infected with murine coronavirus." J Virol **70**(6): 4146-4149.

A persistently coronavirus-infected embryonic stem (ES) cell line A3/MHV was established by infecting an ES cell line, A3-1, with mouse hepatitis virus type-2. Although almost all A3/MHV cells were found infected, both A3/MHV and A3-1 cells expressed comparable levels of cell surface differentiation markers. In addition, A3/MHV cells retained the ability to form embryoid bodies. These results suggest that persistent coronavirus infection does not affect the differentiation of ES cells.

Pinana, J. L., et al. (2018). "Epidemiologic and Clinical Characteristics of Coronavirus and Bocavirus Respiratory Infections after Allogeneic Stem Cell Transplantation: A Prospective Single-Center Study." Biol Blood Marrow Transplant **24**(3): 563-570.

Epidemiologic data about coronaviruses (CoVs) and human bocavirus (HBoV) in the setting of

allogeneic hematopoietic stem cell transplantation (allo-HSCT) are scarce. We conducted a prospective longitudinal study on respiratory viral infections (RVIs) in allo-HSCT recipients with respiratory symptoms from December 2013 until June 2016. Respiratory virus in upper and/or lower respiratory tract (URT and LRT) specimens were tested using Luminex xTAG RVP Fast v1 assay. Seventy-nine consecutive allo-HSCT recipients developed a total of 192 virologically documented RVI episodes over 30 months. The median follow-up after RVI was 388 days (range, 5 to 923). CoV or HBoV was detected in 27 of 192 episodes (14%); 18 of 79 recipients (23%) developed a total of 21 CoV RVI episodes, whereas 6 recipients (8%) had 1 HBoV RVI episode each. Fourteen CoV RVI episodes were limited to the URT, whereas 7 affected the LRT. Co-pathogens were detected in 8 (38%) CoV cases. Type OC43 CoV was the dominant type (48%) followed by NL63 (24%), KHU1 (19%), and 229E (9%); the CoV hospitalization rate was 19%, whereas mortality was 5% (1 patient without any other microbiologic documentation). Among the 6 recipients with HBoV (3%), only 1 had LRT involvement and no one died from respiratory failure. In 5 cases (83%) HBoV was detected along with other viral co-pathogens. CoV RVIs are common after allo-HSCT, and in a significant proportion of cases CoV progressed to LRT and showed moderate to severe clinical features. In contrast, HBoV RVIs were rare and mostly presented in the context of co-infections.

Plaisted, W. C., et al. (2014). "T cell mediated suppression of neurotropic coronavirus replication in neural precursor cells." Virology **449**: 235-243.

Neural precursor cells (NPCs) are the subject of intense investigation for their potential to treat neurodegenerative disorders, yet the consequences of neuroinvasive virus infection of NPCs remain unclear. This study demonstrates that NPCs support replication following infection by the neurotropic JHM strain of mouse hepatitis virus (JHMV). JHMV infection leads to increased cell death and dampens IFN-gamma-induced MHC class II expression. Importantly, cytokines secreted by CD4+ T cells inhibit JHMV replication in NPCs, and CD8+ T cells specifically target viral peptide-pulsed NPCs for lysis. Furthermore, treatment with IFN-gamma inhibits JHMV replication in a dose-dependent manner. Together, these findings suggest that T cells play a critical role in controlling replication of a neurotropic virus in NPCs, a finding which has important implications when considering immune modulation for NPC-based therapies for treatment of human neurologic diseases.

Plaisted, W. C., et al. (2016). "Remyelination Is Correlated with Regulatory T Cell Induction Following Human Embryoid Body-Derived Neural Precursor Cell Transplantation in a Viral Model of Multiple Sclerosis." PLoS One **11**(6): e0157620.

We have recently described sustained clinical recovery associated with dampened neuroinflammation and remyelination following transplantation of neural precursor cells (NPCs) derived from human embryonic stem cells (hESCs) in a viral model of the human demyelinating disease multiple sclerosis. The hNPCs used in that study were derived by a novel direct differentiation method (direct differentiation, DD-NPCs) that resulted in a unique gene expression pattern when compared to hNPCs derived by conventional methods. Since the therapeutic potential of human NPCs may differ greatly depending on the method of derivation and culture, we wanted to determine whether NPCs differentiated using conventional methods would be similarly effective in improving clinical outcome under neuroinflammatory demyelinating conditions. For the current study, we utilized hNPCs differentiated from a human induced pluripotent cell line via an embryoid body intermediate stage (EB-NPCs). Intraspinal transplantation of EB-NPCs into mice infected with the neurotropic JHM strain of mouse hepatitis virus (JHMV) resulted in decreased accumulation of CD4+ T cells in the central nervous system that was concomitant with reduced demyelination at the site of injection. Dampened neuroinflammation and remyelination was correlated with a transient increase in CD4+FOXP3+ regulatory T cells (Tregs) concentrated within the peripheral lymphatics. However, compared to our earlier study, pathological improvements were modest and did not result in significant clinical recovery. We conclude that the genetic signature of NPCs is critical to their effectiveness in this model of viral-induced neurologic disease. These comparisons will be useful for understanding what factors are critical for the sustained clinical improvement.

Prentice, E., et al. (2004). "Coronavirus replication complex formation utilizes components of cellular autophagy." J Biol Chem **279**(11): 10136-10141.

The coronavirus mouse hepatitis virus (MHV) performs RNA replication on double membrane vesicles (DMVs) in the cytoplasm of the host cell. However, the mechanism by which these DMVs form has not been determined. Using genetic, biochemical, and cell imaging approaches, the role of autophagy in DMV formation and MHV replication was investigated. The results demonstrated that replication complexes co-localize with the autophagy proteins, microtubule-associated protein light-chain 3 and Apg12. MHV infection induces autophagy by a

mechanism that is resistant to 3-methyladenine inhibition. MHV replication is impaired in autophagy knockout, APG5-/-, embryonic stem cell lines, but wild-type levels of MHV replication are restored by expression of Apg5 in the APG5-/-cells. In MHV-infected APG5-/-cells, DMVs were not detected; rather, the rough endoplasmic reticulum was dramatically swollen. The results of this study suggest that autophagy is required for formation of double membrane-bound MHV replication complexes and that DMV formation significantly enhances the efficiency of replication. Furthermore, the rough endoplasmic reticulum is implicated as the possible source of membranes for replication complexes.

Raman, S. and D. A. Brian (2005). "Stem-loop IV in the 5' untranslated region is a cis-acting element in bovine coronavirus defective interfering RNA replication." J Virol **79**(19): 12434-12446.

The 210-nucleotide (nt) 5' untranslated region (UTR) in the positive-strand bovine coronavirus (BCoV) genome is predicted to contain four higher-order structures identified as stem-loops I to IV, which may function as cis-acting elements in genomic RNA replication. Here, we describe evidence that stem-loop IV, a bulged stem-loop mapping at nt 186 through 215, (i) is phylogenetically conserved among group 2 coronaviruses and may have a homolog in groups 1 and 3, (ii) exists as a higher-order structure on the basis of enzyme probing, (iii) is required as a higher-order element for replication of a BCoV defective interfering (DI) RNA in the positive but not the negative strand, and (iv) as a higher-order structure in wild-type (wt) and mutant molecules that replicate, specifically binds six cellular proteins in the molecular mass range of 25 to 58 kDa as determined by electrophoretic mobility shift and UV cross-linking assays; binding to viral proteins was not detected. Interestingly, the predicted stem-loop IV homolog in the severe acute respiratory syndrome (SARS) coronavirus appears to be group 1-like in that it is in part duplicated with a group 1-like conserved loop sequence and is not group 2-like, as would be expected by the SARS coronavirus group 2-like 3' UTR structure. These results together indicate that stem-loop IV in the BCoV 5' UTR is a cis-acting element for DI RNA replication and that it might function through interactions with cellular proteins. It is postulated that stem-loop IV functions similarly in the virus genome.

Sato, K., et al. (2018). "Experimental Adaptive Evolution of Simian Immunodeficiency Virus SIVcpz to Pandemic Human Immunodeficiency Virus Type 1 by Using a Humanized Mouse Model." J Virol **92**(4).

Human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, originated from simian immunodeficiency virus from chimpanzees (SIVcpz), the precursor of the human virus, approximately 100 years ago. This indicates that HIV-1 has emerged through the cross-species transmission of SIVcpz from chimpanzees to humans. However, it remains unclear how SIVcpz has evolved into pandemic HIV-1 in humans. To address this question, we inoculated three SIVcpz strains (MB897, EK505, and MT145), four pandemic HIV-1 strains (NL4-3, NLCSFV3, JRCSF, and AD8), and two nonpandemic HIV-1 strains (YBF30 and DJO0131). Humanized mice infected with SIVcpz strain MB897, a virus phylogenetically similar to pandemic HIV-1, exhibited a peak viral load comparable to that of mice infected with pandemic HIV-1, while peak viral loads of mice infected with SIVcpz strain EK505 or MT145 as well as nonpandemic HIV-1 strains were significantly lower. These results suggest that SIVcpz strain MB897 is preadapted to humans, unlike the other SIVcpz strains. Moreover, viral RNA sequencing of MB897-infected humanized mice identified a nonsynonymous mutation in env, a G413R substitution in gp120. The infectivity of the gp120 G413R mutant of MB897 was significantly higher than that of parental MB897. Furthermore, we demonstrated that the gp120 G413R mutant of MB897 augments the capacity for viral replication in both in vitro cell cultures and humanized mice. Taken together, this is the first experimental investigation to use an animal model to demonstrate a gain-of-function evolution of SIVcpz into pandemic HIV-1.IMPORTANCE From the mid-20th century, humans have been exposed to the menace of infectious viral diseases, such as severe acute respiratory syndrome coronavirus, Ebola virus, and Zika virus. These outbreaks of emerging/reemerging viruses can be triggered by cross-species viral transmission from wild animals to humans, or zoonoses. HIV-1, the causative agent of AIDS, emerged by the cross-species transmission of SIVcpz, the HIV-1 precursor in chimpanzees, around 100 years ago. However, the process by which SIVcpz evolved to become HIV-1 in humans remains unclear. Here, by using a hematopoietic stem cell-transplanted humanized-mouse model, we experimentally recapitulate the evolutionary process of SIVcpz to become HIV-1. We provide evidence suggesting that a strain of SIVcpz, MB897, preadapted to infect humans over other SIVcpz strains. We further demonstrate a gain-of-function evolution of SIVcpz in infected humanized mice. Our study reveals that pandemic HIV-1 has emerged through at least two steps: preadaptation and subsequent gain-of-function mutations.

Shahani, L., et al. (2017). "Antiviral therapy for respiratory viral infections in immunocompromised patients." Expert Rev Anti Infect Ther **15**(4): 401-415.

INTRODUCTION: Respiratory viruses (influenza, parainfluenza, respiratory syncytial virus, coronavirus, human metapneumovirus, and rhinovirus) represent the most common causes of respiratory viral infections in immunocompromised patients. Also, these infections may be more severe in immunocompromised patients than in the general population. Early diagnosis and treatment of viral infections continue to be of paramount importance in immunocompromised patients; because once viral replication and invasive infections are evident, prognosis can be grave. Areas covered: The purpose of this review is to provide an overview of the main antiviral agents used for the treatment of respiratory viral infections in immunocompromised patients and review of the new agents in the pipeline. Expert commentary: Over the past decade, important diagnostic advances, specifically, the use of rapid molecular testing has helped close the gap between clinical scenarios and pathogen identification and enhanced early diagnosis of viral infections and understanding of the role of prolonged shedding and viral loads. Advancements in novel antiviral therapeutics with high resistance thresholds and effective immunization for preventable infections in immunocompromised patients are needed.

Splichal, I., et al. (1994). "Ontogeny of interferon alpha secreting cells in the porcine fetal hematopoietic organs." Immunol Lett **43**(3): 203-208.

We examined the ontogeny of IFN-alpha Secreting Cells (IFN-alpha SC) in different hematopoietic organs and blood of porcine fetuses at different stages of gestation. Cells were induced to produce IFN-alpha by incubation with the coronavirus TGEV and IFN-alpha SC were detected by ELISPOT. A striking finding was that IFN-alpha SC could be detected in the fetal liver as early as at 26 days of gestation, i.e., during the first quarter of gestation, a period at which T-cell markers could not be detected by flow cytometry. In addition, IFN-alpha SC could be detected in the cord blood, the spleen and the bone marrow of fetuses at later stages of gestation. These data indicate that IFN-alpha SC appear very early during the ontogeny of the immune system, long before the development of the specific immune system, and may therefore represent an early antiviral defence mechanism. IFN-alpha SC were found to be associated with hematopoietic organs, which argues for their hematopoietic lineage.

Srinivasan, A., et al. (2013). "Detection of respiratory viruses in asymptomatic children undergoing allogeneic hematopoietic cell transplantation." Pediatr Blood Cancer **60**(1): 149-151.

Detection of respiratory viruses by molecular methods, in children without respiratory symptoms undergoing hematopoietic cell transplantation (HCT), has not been well described. A prospective study of 33 asymptomatic children detected respiratory viruses in 8 of 33 (24%) patients before HCT. Human rhinovirus (HRV) was detected in five patients, and human adenovirus (hADV) in three patients. Two additional patients shed HRV, and one shed human coronavirus (hCoV), post-HCT. Two patients had co-infections. Of the 11 asymptomatic patients where respiratory virus was detected, 3 (27%) later developed an upper respiratory tract infection, from the same virus.

Srinivasan, A., et al. (2013). "Prospective detection of respiratory pathogens in symptomatic children with cancer." Pediatr Infect Dis J **32**(3): e99-e104.

BACKGROUND:: The data on human rhinovirus, coronavirus, bocavirus, metapneumovirus, Chlamydophila pneumoniae, Mycoplasma pneumoniae and Bordetella pertussis infections in children with cancer is limited. METHODS:: We sought to determine prospectively the prevalence of respiratory pathogens in these children, using multiplexed-polymerase chain reaction. RESULTS:: We enrolled 253 children with upper or lower respiratory tract infection (LRTI) during a 1-year period. A respiratory virus was detected in 193 (76%) patients; 156 (81%) patients had upper respiratory tract infection. Human rhinovirus was the most common virus detected in 97 (62%) and 24 (65%) patients with upper respiratory tract infection and LRTI, respectively. Leukemia or lymphoma was the most common underlying diagnosis in 95 (49%) patients followed by solid tumor 47 (24%), posthematopoietic stem cell transplant 28 (15%) and brain tumor in 23 (12%) patients. By multiple logistic regression analysis, human bocavirus was the most commonly detected respiratory virus in patients with LRTI (P = 0.008; odds ratio, 4.52; 95% confidence interval: 1.48-13.79). Coinfection with >1 virus was present in 47 (24%) patients, and did not increase the risk for LRTI. Two (0.7%) patients succumbed to LRTI from parainfluenza virus-3 and respiratory syncytial virus/human rhinovirus infection, respectively. C. pneumoniae and M. pneumoniae were detected in 4 and 3 patients, respectively. CONCLUSIONS:: Human rhinovirus was the most common virus detected in children with cancer and posthematopoietic stem cell transplant hospitalized with an acute respiratory illness, and was not associated with increased morbidity. Prospective studies with viral load determination and asymptomatic controls are

needed to study the association of these emerging respiratory viruses with LRTI in children with cancer and posthematopoietic stem cell transplant.

Su, Y. P., et al. (2014). "Dependence of coronavirus RNA replication on an NH2-terminal partial nonstructural protein 1 in cis." J Virol **88**(16): 8868-8882.

UNLABELLED: Genomes of positive (+)-strand RNA viruses use cis-acting signals to direct both translation and replication. Here we examine two 5'-proximal cis-replication signals of different character in a defective interfering (DI) RNA of the bovine coronavirus (BCoV) that map within a 322-nucleotide (nt) sequence (136 nt from the genomic 5' untranslated region and 186 nt from the nonstructural protein 1 [nsp1]-coding region) not found in the otherwise-identical nonreplicating subgenomic mRNA7 (sgmRNA7). The natural DI RNA is structurally a fusion of the two ends of the BCoV genome that results in a single open reading frame between a partial nsp1-coding region and the entire N gene. (i) In the first examination, mutation analyses of a recently discovered long-range RNA-RNA base-paired structure between the 5' untranslated region and the partial nsp1-coding region showed that it, possibly in concert with adjacent stem-loops, is a cis-acting replication signal in the (+) strand. We postulate that the higher-order structure promotes (+)-strand synthesis. (ii) In the second examination, analyses of multiple frame shifts, truncations, and point mutations within the partial nsp1-coding region showed that synthesis of a PEFP core amino acid sequence within a group A lineage betacoronavirus-conserved NH2-proximal WAPEFPWM domain is required in cis for DI RNA replication. We postulate that the nascent protein, as part of an RNA-associated translating complex, acts to direct the DI RNA to a critical site, enabling RNA replication. We suggest that these results have implications for viral genome replication and explain, in part, why coronavirus sgmRNAs fail to replicate. IMPORTANCE: cis-Acting RNA and protein structures that regulate (+)-strand RNA virus genome synthesis are potential sites for blocking virus replication. Here we describe two: a previously suspected 5'-proximal long-range higher-order RNA structure and a novel nascent NH2-terminal protein component of nsp1 that are common among betacoronaviruses of group A lineage.

Tan, Y. W., et al. (2012). "Binding of the 5'-untranslated region of coronavirus RNA to zinc finger CCHC-type and RNA-binding motif 1 enhances viral replication and transcription." Nucleic Acids Res **40**(11): 5065-5077.

Coronaviruses RNA synthesis occurs in the cytoplasm and is regulated by host cell proteins. In a screen based on a yeast three-hybrid system using the 5'-untranslated region (5'-UTR) of SARS coronavirus (SARS-CoV) RNA as bait against a human cDNA library derived from HeLa cells, we found a positive candidate cellular protein, zinc finger CCHC-type and RNA-binding motif 1 (MADP1), to be able to interact with this region of the SARS-CoV genome. This interaction was subsequently confirmed in coronavirus infectious bronchitis virus (IBV). The specificity of the interaction between MADP1 and the 5'-UTR of IBV was investigated and confirmed by using an RNA pull-down assay. The RNA-binding domain was mapped to the N-terminal region of MADP1 and the protein binding sequence to stem-loop I of IBV 5'-UTR. MADP1 was found to be translocated to the cytoplasm and partially co-localized with the viral replicase/transcriptase complexes (RTCs) in IBV-infected cells, deviating from its usual nuclear localization in a normal cell using indirect immunofluorescence. Using small interfering RNA (siRNA) against MADP1, defective viral RNA synthesis was observed in the knockdown cells, therefore indicating the importance of the protein in coronaviral RNA synthesis.

Tanaka, T., et al. (2012). "Severe acute respiratory syndrome coronavirus nsp1 facilitates efficient propagation in cells through a specific translational shutoff of host mRNA." J Virol **86**(20): 11128-11137.

Severe acute respiratory syndrome (SARS) coronavirus (SCoV) is an enveloped virus containing a single-stranded, positive-sense RNA genome. Nine mRNAs carrying a set of common 5' and 3' untranslated regions (UTR) are synthesized from the incoming viral genomic RNA in cells infected with SCoV. A nonstructural SCoV nsp1 protein causes a severe translational shutoff by binding to the 40S ribosomal subunits. The nsp1-40S ribosome complex further induces an endonucleolytic cleavage near the 5'UTR of host mRNA. However, the mechanism by which SCoV viral proteins are efficiently produced in infected cells in which host protein synthesis is impaired by nsp1 is unknown. In this study, we investigated the role of the viral UTRs in evasion of the nsp1-mediated shutoff. Luciferase activities were significantly suppressed in cells expressing nsp1 together with the mRNA carrying a luciferase gene, while nsp1 failed to suppress luciferase activities of the mRNA flanked by the 5'UTR of SCoV. An RNA-protein binding assay and RNA decay assay revealed that nsp1 bound to stem-loop 1 (SL1) in the 5'UTR of SCoV RNA and that the specific interaction with nsp1 stabilized the mRNA carrying SL1. Furthermore,

experiments using an SCoV replicon system showed that the specific interaction enhanced the SCoV replication. The specific interaction of nsp1 with SL1 is an important strategy to facilitate efficient viral gene expression in infected cells, in which nsp1 suppresses host gene expression. Our data indicate a novel mechanism of viral gene expression control by nsp1 and give new insight into understanding the pathogenesis of SARS.

Tirotta, E., et al. (2010). "Cell replacement therapies to promote remyelination in a viral model of demyelination." J Neuroimmunol **224**(1-2): 101-107.

Persistent infection of the central nervous system (CNS) of mice with the neuroadapted JHM strain of mouse hepatitis (MHV) is characterized by ongoing demyelination mediated by inflammatory T cells and macrophages that is similar both clinically and histologically with the human demyelinating disease multiple sclerosis (MS). Although extensive demyelination occurs in mice persistently infected with MHV there is only limited remyelination. Therefore, the MHV model of demyelination is a relevant model for studying disease and evaluating therapeutic approaches to protect cells of the oligodendrocyte lineage and promote remyelination. This concept is further highlighted as the etiology of MS remains enigmatic, but viruses have long been considered as potential triggering agents in initiating and/or maintaining MS symptoms. As such, understanding mechanisms associated with promoting repair within the CNS in the context of a persistent viral infection is critical given the possible viral etiology of MS. This review focuses on recent studies using either mouse neural stem cells (NSCs) or human oligodendrocyte progenitor cells (OPCs) derived from human embryonic stem cell (hESC) to promote remyelination in mice persistently infected with MHV. In addition, the potential role for chemokines in positional migration of transplanted cells is addressed.

Uhlenhaut, C., et al. (2012). "Use of a novel virus detection assay to identify coronavirus HKU1 in the lungs of a hematopoietic stem cell transplant recipient with fatal pneumonia." Transpl Infect Dis **14**(1): 79-85.

A 38-year-old female patient with systemic lupus erythematosus presented with pulmonary infiltrates and hypoxemia for several months following immunodepleting autologous hematopoietic stem cell transplantation. She was treated for influenza, which was isolated repeatedly from oropharynx and bronchoalveolar lavage (BAL) fluids, and later empirically for lupus pneumonitis, but died 6 months after transplant. Autopsy findings failed to show influenza in the lungs or lupus pneumonitis. A novel generic polymerase chain reaction (PCR)-based assay using degenerate primers identified human coronavirus (CoV) HKU1 RNA in BAL fluid at autopsy. CoV was confirmed by virus-specific PCRs of lung tissue at autopsy. Electron microscopy showed viral particles consistent with CoV HKU1 in lung tissue both at autopsy and from a previous biopsy. Although human CoV HKU1 infection is not usually severe, in highly immunocompromised patients, it can be associated with fatal pneumonia.

Verinaud, L., et al. (1999). "Lymphoid organ alterations enhanced by sub-lethal doses of coronaviruses in experimentally induced Trypanosoma cruzi infection in mice." Lab Anim Sci **49**(1): 35-41.

The effect of sub-lethal doses of coronaviruses on the course of disease in CBA mice experimentally infected with a mildly pathogenic strain of Trypanosoma cruzi was investigated. Mice were inoculated with either T. cruzi, 0.1 median lethal dose (LD50) of coronavirus (mouse hepatitis virus [MHV-3] or virus X), or both pathogens. Levels of parasitemia, mortality, and the extent of pathologic alterations in lymphoid organs were determined. Mice inoculated with T. cruzi had mild alterations in their lymphoid organs and survived infection. In contrast, mice inoculated with both pathogens died, and had significantly higher levels of parasitemia and profound alterations in lymphoid organs. These results indicate that the pathologic profile of T. cruzi infection can be profoundly altered by subclinical infection with coronaviruses.

Wang, L., et al. (2017). "Respiratory virus infection after allogeneic hematopoietic stem cell transplant in a tropical center: Predictive value of the immunodeficiency scoring index." Transpl Infect Dis **19**(3).

BACKGROUND: Respiratory virus infection (RVI) is a prevalent infection in patients after allogeneic hematopoietic stem cell transplant (allo-HSCT) and can result in significant morbidity and mortality. Ability to assess the potential severity of RVI is important in the management of such patients. METHODS: We reviewed the cases of RVI in allo-HSCT recipients and explored the predictive value of the immunodeficiency scoring index (ISI) established for respiratory syncytial virus (RSV) and its applicability for RVI caused by other respiratory viruses. RESULTS: RVI occurred year-round in our tropical transplant center, with peaks in the middle and end of the year. Ninety-five of the 195 recipients developed a total of 191 episodes of RVI, giving a cumulative incidence of 28% by 6 months and 52% by 24 months for the first episode of RVI. RSV, influenza, rhinovirus, and parainfluenza were the most common viruses. Pneumonia occurred in 63.64%, 42.31%, and

32.42% of adenovirus, influenza, and RSV RVI episodes, respectively, but was also non-negligible in the more benign viruses, such as coronavirus (31.58%) and rhinovirus (23.68%). Nineteen of the 63 episodes of viral pneumonia required mechanical ventilation and 14 deaths occurred within 6 weeks of the RVI. Receiver operating characteristic analysis showed that an ISI of >/=8 predicted pneumonia with a positive predictive value of >80% for RVI caused by RSV, influenza, adenovirus, and parainfluenza, while it was not predictive for coronavirus and rhinovirus. CONCLUSIONS: The ISI is a useful aid for decision-making during clinic consultation for patients presenting with symptoms suggestive of an RVI.

Weigt, S. S., et al. (2011). "Respiratory viral infections in hematopoietic stem cell and solid organ transplant recipients." Semin Respir Crit Care Med **32**(4): 471-493.

Respiratory viral infections (RVIs) are common causes of mild illness in immunocompetent children and adults with rare occurrences of significant morbidity or mortality. Complications are more common in the very young, very old, and those with underlying lung diseases. However, RVIs are increasingly recognized as a cause of morbidity and mortality in recipients of hematopoietic stem cell transplants (HSCT) and solid organ transplants (SOTs). Diagnostic techniques for respiratory syncytial virus (RSV), parainfluenza, influenza, and adenovirus have been clinically available for decades, and these infections are known to cause serious disease in transplant recipients. Modern molecular technology has now made it possible to detect other RVIs including human metapneumovirus, coronavirus, and bocavirus, and the role of these viruses in causing serious disease in transplant recipients is still being worked out. This article reviews the current information regarding epidemiology, pathogenesis, clinical presentation, diagnosis, and treatment of these infections, as well as the aspects of clinical significance of RVIs unique to HSCT or SOT.

Weinger, J. G., et al. (2012). "MHC mismatch results in neural progenitor cell rejection following spinal cord transplantation in a model of viral-induced demyelination." Stem Cells **30**(11): 2584-2595.

Transplantation of syngeneic neural progenitor cells (NPCs) into mice persistently infected with the JHM strain of mouse hepatitis virus (JHMV) results in enhanced differentiation into oligodendrocyte progenitor cells that is associated with remyelination, axonal sparing, and clinical improvement. Whether allogeneic NPCs are tolerated or induce immune-mediated rejection is controversial and poorly defined under neuroinflammatory demyelinating conditions. We have used the JHMV-induced demyelination model to evaluate the antigenicity of transplanted allogeneic NPCs within the central nervous system (CNS) of mice with established immune-mediated demyelination. Cultured NPCs constitutively expressed the costimulatory molecules CD80/CD86, and IFN-gamma treatment induced expression of MHC class I and II antigens. Injection of allogeneic C57BL/6 NPCs (H-2b background) led to a delayed type hypersensitivity response in BALB/c (H-2d background) mice associated with T-cell proliferation and IFN-gamma secretion following coculture with allogeneic NPCs. Transplantation of MHC-mismatched NPCs into JHMV-infected mice resulted in increased transcripts encoding the T-cell chemoattractant chemokines CXCL9 and CXCL10 that correlated with increased T-cell infiltration that was associated with NPC rejection. Treatment of MHC-mismatched mice with T-cell subset-specific depleting antibodies increased survival of allogeneic NPCs without affecting commitment to an oligodendrocyte lineage. Collectively, these results show that allogeneic NPCs are antigenic, and T-cells contribute to rejection following transplantation into an inflamed CNS suggesting that immunomodulatory treatments may be necessary to prolong survival of allogeneic cells.

Wen, C. C., et al. (2011). "Traditional Chinese medicine herbal extracts of Cibotium barometz, Gentiana scabra, Dioscorea batatas, Cassia tora, and Taxillus chinensis inhibit SARS-CoV replication." J Tradit Complement Med **1**(1): 41-50.

Development of anti-severe acute respiratory syndrome associated coronavirus (SARS-CoV) agents is pivotal to prevent the reemergence of the life-threatening disease, SARS. In this study, more than 200 extracts from Chinese medicinal herbs were evaluated for anti-SARS-CoV activities using a cell-based assay that measured SARS-CoV-induced cytopathogenic effect (CPE) in vitro on Vero E6 cells. Six herbal extracts, one each from Gentianae Radix (long dan; the dried rhizome of Gentiana scabra), Dioscoreae Rhizoma (shan yao; the tuber of Dioscorea batatas), Cassiae Semen (jue ming zi; the dried seed of Cassia tora) and Loranthi Ramus (sang ji sheng; the dried stem, with leaf of Taxillus chinensis) (designated as GSH, DBM, CTH and TCH, respectively), and two from Rhizoma Cibotii (gou ji; the dried rhizome of Cibotium barometz) (designated as CBE and CBM), were found to be potent inhibitors of SARS-CoV at concentrations between 25 and 200 mug/ml. The concentrations of the six extracts needed to inhibit 50% of Vero E6 cell proliferation (CC50) and 50% of viral replication (EC50) were determined. The resulting selective index values (SI = CC50/EC50)

of the most effective extracts CBE, GSH, DBM, CTH and TCH were > 59.4, > 57.5, > 62.1, > 59.4, and > 92.9, respectively. Among these extracts, CBM and DBM also showed significant inhibition of SARS-CoV 3CL protease activity with IC50 values of 39 mug/ml and 44 mug/ml, respectively. Our findings suggest that these six herbal extracts may have potential as candidates for future development of anti-SARS therapeutics.AbbreviationsSARS,severe acute respiratory syndromeCoV,coronavirusCPE,cytopathogenic effectTCM,traditional Chinese medicine.

Weng, J. R., et al. (2019). "Antiviral activity of Sambucus FormosanaNakai ethanol extract and related phenolic acid constituents against human coronavirus NL63." Virus Res **273**: 197767.

Human coronavirus NL63 (HCoV-NL63), one of the main circulating HCoVs worldwide, causes respiratory tract illnesses like runny nose, cough, bronchiolitis and pneumonia. Recently, a severe respiratory illness outbreak of HCoV-NL63has been reported in a long-term care facility. Sambucus FormosanaNakai, a species of elderberry, is a traditional medicinal herb with anti-inflammatory and antiviral potential. The study investigated the antiviral activity of Sambucus FormosanaNakai stem ethanol extract and some phenolic acid constituents against HCoV-NL63. The extract was less cytotoxic and concentration-dependently increased anti-HCoV-NL63 activities, including cytopathicity, sub-G1 fraction, virus yield (IC50=1.17mug/ml), plaque formation (IC50=4.67mug/ml) and virus attachment (IC50=15.75mug/ml). Among the phenolic acid constituents in Sambucus FormosanaNakai extract, caffeic acid, chlorogenic acid and gallic acid sustained the anti-HCoV-NL63 activity that was ranked in the following order of virus yield reduction: caffeic acid (IC50=3.54muM)>chlorogenic acid (IC50=43.45muM)>coumaric acid (IC50=71.48muM). Caffeic acid significantly inhibited the replication of HCoV-NL63 in a cell-type independent manner, and specifically blocked virus attachment (IC50=8.1muM). Therefore, the results revealed that Sambucus Formosana Nakai stem ethanol extract displayed the strong anti-HCoV-NL63 potential; caffeic acid could be the vital component with anti-HCoV-NL63 activity. The finding could be helpful for developing antivirals against HCoV-NL63.

Whitman, L. M., et al. (2012). "Olig1 function is required for remyelination potential of transplanted neural progenitor cells in a model of viral-induced demyelination." Exp Neurol **235**(1): 380-387.

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) resulting in cumulative neurologic deficits associated with progressive myelin loss. We have previously shown that transplantation of neural progenitor cells (NPCs) into mice persistently infected with the JHM strain of mouse hepatitis virus (JHMV) results in enhanced differentiation into oligodendrocyte progenitor cells (OPCs) that is associated with remyelination and axonal sparing. The current study examines the contributions of the transcription factor Olig1 on NPC differentiation and remyelination. Under defined conditions, NPCs preferentially differentiate into oligodendroglia whereas NPCs isolated from Olig1-deficient (Olig1-/-) mice exhibit enhanced differentiation into astrocytes. Transplantation of Olig1-/- and Olig1+/+ NPCs into JHMV-infected mice resulted in similar cell survival, proliferation, and selective migration to areas of demyelination. However, only recipients of wild type NPCs exhibited extensive remyelination compared to mice receiving Olig1-/- NPCs. In vivo characterization of NPCs revealed that Olig1+/+ NPCs preferentially differentiated into NG2-positive OPCs and formed processes expressing myelin basic protein that encircled axons. In contrast, the majority of transplanted Olig1-/- NPCs differentiated into GFAP-positive cells consistent with the astrocyte lineage. These results indicate that exogenous NPCs contribute to improved clinical and histological outcome and this is associated with remyelination by this donor population. Further, these findings reveal that Olig1function is required for the remyelination potential of NPCs after transplant, through specification and/or maintenance of oligodendroglial identity.

Wolfromm, A., et al. (2014). "Viral respiratory infections diagnosed by multiplex PCR after allogeneic hematopoietic stem cell transplantation: long-term incidence and outcome." Biol Blood Marrow Transplant **20**(8): 1238-1241.

Viral respiratory infections (VRIs) are frequent after hematopoietic stem cell transplantation and constitute a potential cause of mortality. We analyzed the incidence, risk factors, and prognosis of VRIs in a cohort of transplanted patients. More frequent viruses were human coronavirus and human rhinovirus followed by flu-like viruses and adenovirus. Risk factors for death were lymphocytopenia and high steroid dosage.

Wu, H. Y., et al. (2014). "Reselection of a genomic upstream open reading frame in mouse hepatitis coronavirus 5'-untranslated-region mutants." J Virol **88**(2): 846-858.

An AUG-initiated upstream open reading frame (uORF) encoding a potential polypeptide of 3 to 13

amino acids (aa) is found within the 5' untranslated region (UTR) of >75% of coronavirus genomes based on 38 reference strains. Potential CUG-initiated uORFs are also found in many strains. The AUG-initiated uORF is presumably translated following genomic 5'-end cap-dependent ribosomal scanning, but its function is unknown. Here, in a reverse-genetics study with mouse hepatitis coronavirus, the following were observed. (i) When the uORF AUG-initiating codon was replaced with a UAG stop codon along with a U112A mutation to maintain a uORF-harboring stem-loop 4 structure, an unimpaired virus with wild-type (WT) growth kinetics was recovered. However, reversion was found at all mutated sites within five virus passages. (ii) When the uORF was fused with genomic (main) ORF1 by converting three in-frame stop codons to nonstop codons, a uORF-ORF1 fusion protein was made, and virus replicated at WT levels. However, a frameshifting G insertion at virus passage 7 established a slightly 5'-extended original uORF. (iii) When uAUG-eliminating deletions of 20, 30, or 51 nucleotides (nt) were made within stem-loop 4, viable but debilitated virus was recovered. However, a C80U mutation in the first mutant and an A77G mutation in the second appeared by passage 10, which generated alternate uORFs that correlated with restored WT growth kinetics. In vitro, the uORF-disrupting nondeletion mutants showed enhanced translation of the downstream ORF1 compared with the WT. These results together suggest that the uORF represses ORF1 translation yet plays a beneficial but nonessential role in coronavirus replication in cell culture.

Yang, M., et al. (2003). "The effect of SARS coronavirus on blood system: its clinical findings and the pathophysiologic hypothesis." Zhongguo Shi Yan Xue Ye Xue Za Zhi **11**(3): 217-221.

Severe acute respiratory syndrome (SARS) has recently recognized as a new human infectious disease. A novel coronavirus was identified as the causative agent of SARS. This report summarizes the hematological findings in SARS patients and proposes a hypothesis for the pathophysiology of SARS coronavirus related abnormal hematopoiesis. Hematological changes in patients with SARS were common and included lymphopenia (68% - 90% of adults; 100% of children, n = 10), thrombocytopenia (20% - 45% of adults, 50% of children), and leukopenia (20% - 34% of adults, 70% of children). The possible mechanisms of this coronavirus on blood system may include (1) directly infect blood cells and bone marrow stromal cells via CD13 or CD66a; and/or (2) induce auto-antibodies and immune complexes to damage these cells. In addition, lung damage in SARS patients may also play a role on inducing thrombocytopenia by (1) increasing the consumption of platelets/megakaryocytes; and/or (2) reducing the production of platelets in the lungs. Since the most common hematological changes in SARS patients were lymphopenia and immunodeficiency. We postulate that hematopoietic growth factors such as G-CSF, by mobilizing endogenous blood stem cells and endogenous cytokines, could become a hematological treatment for SARS patients, which may enhance the immune system against these virus.

Yang, M., et al. (2004). "Hematological findings in SARS patients and possible mechanisms (review)." Int J Mol Med **14**(2): 311-315.

Severe acute respiratory syndrome (SARS) is a new human infectious disease. The causative agent of SARS is a novel coronavirus (SARS-CoV). This report summarizes the hematological findings in SARS patients and proposes the possible mechanisms of SARS-CoV related abnormal hematopoiesis. Hematological changes in patients with SARS are common and include lymphopenia, thrombocytopenia and occasionally leukopenia. A significant decrease was also observed in peripheral CD4+ and CD8+ T lymphocyte subsets and it was related to onset of SARS. A number of potential mechanisms may be involved. The development of auto-immune antibodies or immune complexes triggered by viral infection may play a major role in inducing lymphopenia and thrombocytopenia. Moreover, SARS-CoV may also directly infect hematopoietic stem/progenitor cells via CD13 or CD66a inducing their growth inhibition and apoptosis. The receptor for group I and III CoV is aminopeptidase N (CD13). CD13 has been identified in human bone marrow CD34+ cells, platelets, megakaryocytes, myeloid cells, and erythroid cells, but not in lymphocytes. The common receptor for group II CoV is CEACAM1a (CD66a). CD66a is an adhesion molecule expressed on bone marrow CD34+ cells, platelets, granulocytes and activated lymphocytes. In addition, glucocorticoids could induce lymphopenia and the use of steroids may account for the decrease of lymphocytes in some SARS patients. The increased consumption of platelets and/or the decreased production of platelets in the damaged lungs are a potential alternative but often overlooked mechanism that can contribute to thrombocytopenia in severe critical pulmonary conditions.

Yu, W. and J. L. Leibowitz (1995). "Specific binding of host cellular proteins to multiple sites within the 3' end of mouse hepatitis virus genomic RNA." J Virol **69**(4): 2016-2023.

The initial step in mouse hepatitis virus (MHV) RNA replication is the synthesis of negative-strand RNA from a positive-strand genomic RNA template. Our approach to begin studying MHV RNA

replication is to identify the cis-acting signals for RNA synthesis and the proteins which recognize these signals at the 3' end of genomic RNA of MHV. To determine whether host cellular and/or viral proteins interact with the 3' end of the coronavirus genome, an RNase T1 protection/gel mobility shift electrophoresis assay was used to examine cytoplasmic extracts from mock- and MHV-JHM-infected 17Cl-1 murine cells for the ability to form complexes with defined regions of the genomic RNA. We demonstrated the specific binding of host cell proteins to multiple sites within the 3' end of MHV-JHM genomic RNA. By using a set of RNA probes with deletions at either the 5' or 3' end or both ends, two distinct binding sites were located. The first protein-binding element was mapped in the 3'-most 42 nucleotides of the genomic RNA [3' (+42) RNA], and the second element was mapped within an 86-nucleotide sequence encompassing nucleotides 171 to 85 from the 3' end of the genome (171-85 RNA). A single potential stem-loop structure is predicted for the 3' (+)42 RNA, and two stem-loop structures are predicted for the 171-85 RNA. Proteins interacting with these two elements were identified by UV-induced covalent cross-linking to labeled RNAs followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. The RNA-protein complex formed with the 3'-most 42 nucleotides contains approximately five host polypeptides, a highly labeled protein of 120 kDa and four minor species with sizes of 103, 81, 70, and 55 kDa. The second protein-binding element, contained within a probe representing nucleotides 487 to 85 from the 3' end of the genome, also appears to bind five host polypeptides, 142, 120, 100, 55, and 33 kDa in size, with the 120-kDa protein being the most abundant. The RNA-protein complexes observed with MHV-infected cells in both RNase protection/gel mobility shift and UV cross-linking assays were identical to those observed with uninfected cells. The possible involvement of the interaction of host proteins with the viral genome during MHV replication is discussed.

Zhang, J. J., et al. (2006). "Promoter activity of SARS coronavirus 5' UTR sequence in eukaryotic cells." Sichuan Da Xue Xue Bao Yi Xue Ban **37**(1): 5-9.

OBJECTIVES: To investigate 5'UTR sequence in different SARS-CoV isolates, to identify the secondary structure, and to test the promoter activity of the cDNA sequence corresponding to SARS-CoV 5'UTR in eukaryotic cells. METHODS: 101 SARS-CoV 5'UTR were aligned. One typical sequence containing full 264 nt was then subjected to be predicted its secondary structure. The pGL3-5'UTR and pGL3-a-5'UTR were constructed by substitution of SV40 promoter with SARS-CoV 5'UTR cDNA or its antisense sequence. Then the recombinant plasmids were transfected into HepG2 cells and the luciferase activities were detected. A set of deletion mutant plasmids, of which pGL3-5' UTR-1, pGL3-5' UTR-2, pGL3-5'UTR-3 and pGL3-5'UTR-4 are with 3, 2, 1, and 0 residual stem-loops of 3' termini respectively,were constructed from pGL3-5'UTR and were transfected into HepG2 cells to express reporter gene luc+, with pGL3-5'UTR containing full sequence as control. The luciferase activities expressed by the plasmids were measured. And then the total RNA of the transfected cells was extracted. Subsequently, by 5' Rapid Amplication of cDNA Ends (5'RACE), the PCR product was sequenced. The luciferase expressed by pGL3-5'UTR in various cells, the lung carcinoma cell line A549, hepatoma cell line HepG2, kidney cell Vero E6, cervical cancer cell line HeLa and human umbilical vein endothelial cell line ECV304 were measured and compared with each other. RESULTS: The full sequence of the SARS-CoV 5' UTR is a 264nt, and 18 deletion mutants were found. Totally, 5 site substitutions were found in 101 5'UTR sequences. The SARS-CoV 5'UTR RNA folded to form a stable secondary structure containing four stem-loop domains. The biggest and most complex one is the stem-loop II appearing a pseudoknot. Comparing with pGL3-a-5'UTR, pGL3-5'UTR expressed luciferase obviously. Both pGL3-5'UTR containing full sequence and pGL3-5'UTR-1 containing three stem-loops of 3' termini expressed the luciferase well. However, when lost stem-loop I and II, the pGL3-5'UTR-2, pGL3-5'UTR-3 and pGL3-5'UTR-4 almost didn't express luciferase. The 56th nucleotide of SARS-CoV 5'UTR was found to be the initiation site for transcription. Transfected with expression luciferase plasmid pGL3-5' UTR in which SARS-CoV 5' UTR acts as the promoter, the luciferase could express in five cell lines in different degrees. Ranked by the luciferase activity from the highest to the lowest, the order is A549, HepG2, ECV304, HeLa and Vero E6. CONCLUSIONS: A: The 5'UTR sequences of different SARS-CoV isolates are relatively conserved, and a full sequence would form a secondary structure containing four stem-loop domains. B: The cDNA sequence corresponding to SARS-CoV 5'UTR possessed a promoter activity in eukaryotic cells. C: The promoter domain of the SARS-CoV 5'UTR contains both stem-loop I and II. D: The 56th nucleotide and its down stream TRS of SARS-CoV 5'UTR plays a key role in regulating transcription. E: Cells sourced from various tissues can provide efficient accessory factors for SARS-CoV 5'UTR sequence that acts as a promoter, and the lung-sourced cells may be the most suitable.

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References

1. Anis, E. A., et al. (2017). "Transduction of hematopoietic stem cells to stimulate RNA interference against feline infectious peritonitis." J Feline Med Surg 19(6): 680-686.
2. Athmer, J., et al. (2017). "In Situ Tagged nsp15 Reveals Interactions with Coronavirus Replication/Transcription Complex-Associated Proteins." mBio 8(1).
3. Baglivo, M., et al. (2020). "Natural small molecules as inhibitors of coronavirus lipid-dependent attachment to host cells: a possible strategy for reducing SARS-COV-2 infectivity?" Acta Biomed 91(1): 161-164.
4. Baidu. http://www.baidu.com. 2020.
5. Baranov, P. V., et al. (2005). "Programmed ribosomal frameshifting in decoding the SARS-CoV genome." Virology 332(2): 498-510.
6. Beaird, O. E., et al. (2016). "Current practices for treatment of respiratory syncytial virus and other non-influenza respiratory viruses in high-risk patient populations: a survey of institutions in the Midwestern Respiratory Virus Collaborative." Transpl Infect Dis 18(2): 210-215.
7. Beury, D., et al. (2020). "Use of whole-genome sequencing in the molecular investigation of care-associated HCoV-OC43 infections in a hematopoietic stem cell transplant unit." J Clin Virol 122: 104206.
8. Blau, D. M., et al. (2001). "Targeted disruption of the Ceacam1 (MHVR) gene leads to reduced susceptibility of mice to mouse hepatitis virus infection." J Virol 75(17): 8173-8186.
9. Bredenbeek, P. J., et al. (1990). "The primary structure and expression of the second open reading frame of the polymerase gene of the coronavirus MHV-A59; a highly conserved polymerase is expressed by an efficient ribosomal frameshifting mechanism." Nucleic Acids Res 18(7): 1825-1832.
10. Cabeca, T. K., et al. (2013). "Human coronavirus ocurrence in different populations of Sao Paulo: A comprehensive nine-year study using a pancoronavirus RT-PCR assay." Braz J Microbiol 44(1): 335-339.
11. Campbell, A. P., et al. (2010). "Respiratory virus pneumonia after hematopoietic cell transplantation (HCT): associations between viral load in bronchoalveolar lavage samples, viral RNA detection in serum samples, and clinical outcomes of HCT." J Infect Dis 201(9): 1404-1413.
12. Campbell, A. P., et al. (2015). "Clinical outcomes associated with respiratory virus detection before allogeneic hematopoietic stem cell transplant." Clin Infect Dis 61(2): 192-202.
13. Cancer Biology. http://www.cancerbio.net. 2020.
14. Chai, Q., et al. (2013). "Maturation of lymph node fibroblastic reticular cells from myofibroblastic precursors is critical for antiviral immunity." Immunity 38(5): 1013-1024.
15. Chang, R. Y., et al. (1994). "A cis-acting function for the coronavirus leader in defective interfering RNA replication." J Virol 68(12): 8223-8231.
16. Chen, J., et al. (2003). "An alternate pathway for recruiting template RNA to the brome mosaic virus RNA replication complex." J Virol 77(4): 2568-2577.
17. Chen, Y., et al. (2007). "A novel subset of putative stem/progenitor CD34+Oct-4+ cells is the major target for SARS coronavirus in human lung." J Exp Med 204(11): 2529-2536.
18. Choi, J. H., et al. (2013). "Respiratory viral infections after hematopoietic stem cell transplantation in children." J Korean Med Sci 28(1): 36-41.
19. Curry, S. M., et al. (2017). "Effects of porcine epidemic diarrhea virus infection on nursery pig intestinal function and barrier integrity." Vet Microbiol 211: 58-66.
20. Curry, S. M., et al. (2018). "Porcine epidemic diarrhea virus reduces feed efficiency in nursery pigs." J Anim Sci 96(1): 85-97.
21. Dube, M., et al. (2018). "Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43." J Virol 92(17).
22. Eichenberger, E. M., et al. (2019). "Incidence, significance, and persistence of human coronavirus infection in hematopoietic stem cell transplant recipients." Bone Marrow Transplant 54(7): 1058-1066.
23. Fischer, S. A. (2008). "Emerging viruses in transplantation: there is more to infection after transplant than CMV and EBV." Transplantation 86(10): 1327-1339.
24. Folz, R. J. and M. A. Elkordy (1999). "Coronavirus pneumonia following autologous bone marrow transplantation for breast cancer." Chest 115(3): 901-905.
25. Fontana, L. and L. Strasfeld (2019). "Respiratory Virus Infections of the Stem Cell Transplant Recipient and the Hematologic Malignancy Patient." Infect Dis Clin North Am 33(2): 523-544.
26. Goebel, S. J., et al. (2007). "A hypervariable region within the 3' cis-acting element of the murine coronavirus genome is nonessential for

RNA synthesis but affects pathogenesis." J Virol 81(3): 1274-1287.
27. Google. http://www.google.com. 2020.
28. Gross, A. E. and M. L. Bryson (2015). "Oral Ribavirin for the Treatment of Noninfluenza Respiratory Viral Infections: A Systematic Review." Ann Pharmacother 49(10): 1125-1135.
29. Guan, B. J., et al. (2011). "An optimal cis-replication stem-loop IV in the 5' untranslated region of the mouse coronavirus genome extends 16 nucleotides into open reading frame 1." J Virol 85(11): 5593-5605.
30. Guan, B. J., et al. (2012). "Genetic evidence of a long-range RNA-RNA interaction between the genomic 5' untranslated region and the nonstructural protein 1 coding region in murine and bovine coronaviruses." J Virol 86(8): 4631-4643.
31. Gustin, K. M., et al. (2009). "Bovine coronavirus nonstructural protein 1 (p28) is an RNA binding protein that binds terminal genomic cis-replication elements." J Virol 83(12): 6087-6097.
32. Hakki, M., et al. (2015). "The clinical impact of coronavirus infection in patients with hematologic malignancies and hematopoietic stem cell transplant recipients." J Clin Virol 68: 1-5.
33. Hirsch, H. H., et al. (2013). "Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus." Clin Infect Dis 56(2): 258-266.
34. Hsin, W. C., et al. (2018). "Nucleocapsid protein-dependent assembly of the RNA packaging signal of Middle East respiratory syndrome coronavirus." J Biomed Sci 25(1): 47.
35. Hutspardol, S., et al. (2015). "Significant Transplantation-Related Mortality from Respiratory Virus Infections within the First One Hundred Days in Children after Hematopoietic Stem Cell Transplantation." Biol Blood Marrow Transplant 21(10): 1802-1807.
36. Ireland, D. D., et al. (2009). "RNase L mediated protection from virus induced demyelination." PLoS Pathog 5(10): e1000602.
37. Ison, M. G., et al. (2003). "Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia." Clin Infect Dis 36(9): 1139-1143.
38. Jolicoeur, P. and L. Lamontagne (1995). "Impairment of bone marrow pre-B and B cells in MHV3 chronically-infected mice." Adv Exp Med Biol 380: 193-195.
39. Journal of American Science. http://www.jofamericanscience.org. 2020.
40. Jung, K., et al. (2015). "Comparative pathogenesis of US porcine epidemic diarrhea virus (PEDV) strain PC21A in conventional 9-day-old nursing piglets vs. 26-day-old weaned pigs." Vet Microbiol 178(1-2): 31-40.
41. Kang, H., et al. (2006). "Putative cis-acting stem-loops in the 5' untranslated region of the severe acute respiratory syndrome coronavirus can substitute for their mouse hepatitis virus counterparts." J Virol 80(21): 10600-10614.
42. Kim, S. H., et al. (2017). "Atypical presentations of MERS-CoV infection in immunocompromised hosts." J Infect Chemother 23(11): 769-773.
43. Kim, Y. N. and S. Makino (1995). "Characterization of a murine coronavirus defective interfering RNA internal cis-acting replication signal." J Virol 69(8): 4963-4971.
44. Ko, J. H., et al. (2018). "Host susceptibility to MERS-CoV infection, a retrospective cohort study of the 2015 Korean MERS outbreak." J Infect Chemother 24(2): 150-152.
45. Kyuwa, S., et al. (2000). "Replication of enterotropic and polytropic murine coronaviruses in cultured cell lines of mouse origin." Exp Anim 49(4): 251-257.
46. Lee, K. H., et al. (2019). "Characteristics of community-acquired respiratory viruses infections except seasonal influenza in transplant recipients and non-transplant critically ill patients." J Microbiol Immunol Infect.
47. Li, K., et al. (2016). "Middle East Respiratory Syndrome Coronavirus Causes Multiple Organ Damage and Lethal Disease in Mice Transgenic for Human Dipeptidyl Peptidase 4." J Infect Dis 213(5): 712-722.
48. Li, L., et al. (2008). "Structural lability in stem-loop 1 drives a 5' UTR-3' UTR interaction in coronavirus replication." J Mol Biol 377(3): 790-803.
49. Li, L., et al. (2019). "Porcine Intestinal Enteroids: a New Model for Studying Enteric Coronavirus Porcine Epidemic Diarrhea Virus Infection and the Host Innate Response." J Virol 93(5).
50. Life Science Journal. http://www.lifesciencesite.com. 2020.
51. Ling, T. Y., et al. (2006). "Identification of pulmonary Oct-4+ stem/progenitor cells and demonstration of their susceptibility to SARS coronavirus (SARS-CoV) infection in vitro." Proc Natl Acad Sci U S A 103(25): 9530-9535.
52. Liu, P., et al. (2013). "Functional analysis of the stem loop S3 and S4 structures in the coronavirus 3'UTR." Virology 443(1): 40-47.
53. Ma H, Chen G. Stem cell. The Journal of American Science 2005;1(2):90-92. doi:10.7537/marsjas010205.14.

http://www.jofamericanscience.org/journals/am-sci/0102/14-mahongbao.pdf.
54. Ma H, Cherng S. Eternal Life and Stem Cell. Nature and Science. 2007;5(1):81-96. doi:10.7537/marsnsj050107.10. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
55. Ma H, Cherng S. Nature of Life. Life Science Journal 2005;2(1):7-15. doi:10.7537/marslsj020105.03. http://www.lifesciencesite.com/lsj/life0201/life-0201-03.pdf.
56. Ma H, Yang Y. Turritopsis nutricula. Nature and Science 2010;8(2):15-20. doi:10.7537/marsnsj080210.03. http://www.sciencepub.net/nature/ns0802/03_1279_hongbao_turritopsis_ns0802_15_20.pdf.
57. Ma H. The Nature of Time and Space. Nature and science 2003;1(1):1-11. doi:10.7537/marsnsj010103.01. http://www.sciencepub.net/nature/0101/01-ma.pdf.
58. Madhugiri, R., et al. (2018). "Structural and functional conservation of cis-acting RNA elements in coronavirus 5'-terminal genome regions." Virology 517: 44-55.
59. Mallick, B., et al. (2009). "MicroRNome analysis unravels the molecular basis of SARS infection in bronchoalveolar stem cells." PLoS One 4(11): e7837.
60. Mangale, V., et al. (2017). "Neural precursor cells derived from induced pluripotent stem cells exhibit reduced susceptibility to infection with a neurotropic coronavirus." Virology 511: 49-55.
61. Marsland Press. http://www.sciencepub.net. 2020.
62. Marsland Press. http://www.sciencepub.org. 2020.
63. Mikulska, M., et al. (2014). "Epidemiology of viral respiratory tract infections in an outpatient haematology facility." Ann Hematol 93(4): 669-676.
64. Milano, F., et al. (2010). "Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients." Blood 115(10): 2088-2094.
65. Molenkamp, R. and W. J. Spaan (1997). "Identification of a specific interaction between the coronavirus mouse hepatitis virus A59 nucleocapsid protein and packaging signal." Virology 239(1): 78-86.
66. Morales, L., et al. (2013). "Transmissible gastroenteritis coronavirus genome packaging signal is located at the 5' end of the genome and promotes viral RNA incorporation into virions in a replication-independent process." J Virol 87(21): 11579-11590.
67. National Center for Biotechnology Information, U.S. National Library of Medicine. http://www.ncbi.nlm.nih.gov/pubmed. 2020.
68. Nature and Science. http://www.sciencepub.net/nature. 2020.
69. Ogimi, C., et al. (2017). "Clinical Significance of Human Coronavirus in Bronchoalveolar Lavage Samples From Hematopoietic Cell Transplant Recipients and Patients With Hematologic Malignancies." Clin Infect Dis 64(11): 1532-1539.
70. Ogimi, C., et al. (2017). "Prolonged Shedding of Human Coronavirus in Hematopoietic Cell Transplant Recipients: Risk Factors and Viral Genome Evolution." J Infect Dis 216(2): 203-209.
71. Okumura, A., et al. (1996). "Maintenance of pluripotency in mouse embryonic stem cells persistently infected with murine coronavirus." J Virol 70(6): 4146-4149.
72. Pinana, J. L., et al. (2018). "Epidemiologic and Clinical Characteristics of Coronavirus and Bocavirus Respiratory Infections after Allogeneic Stem Cell Transplantation: A Prospective Single-Center Study." Biol Blood Marrow Transplant 24(3): 563-570.
73. Plaisted, W. C., et al. (2014). "T cell mediated suppression of neurotropic coronavirus replication in neural precursor cells." Virology 449: 235-243.
74. Plaisted, W. C., et al. (2016). "Remyelination Is Correlated with Regulatory T Cell Induction Following Human Embryoid Body-Derived Neural Precursor Cell Transplantation in a Viral Model of Multiple Sclerosis." PLoS One 11(6): e0157620.
75. Prentice, E., et al. (2004). "Coronavirus replication complex formation utilizes components of cellular autophagy." J Biol Chem 279(11): 10136-10141.
76. Raman, S. and D. A. Brian (2005). "Stem-loop IV in the 5' untranslated region is a cis-acting element in bovine coronavirus defective interfering RNA replication." J Virol 79(19): 12434-12446.
77. Sato, K., et al. (2018). "Experimental Adaptive Evolution of Simian Immunodeficiency Virus SIVcpz to Pandemic Human Immunodeficiency Virus Type 1 by Using a Humanized Mouse Model." J Virol 92(4).
78. Shahani, L., et al. (2017). "Antiviral therapy for respiratory viral infections in immunocompromised patients." Expert Rev Anti Infect Ther 15(4): 401-415.
79. Splichal, I., et al. (1994). "Ontogeny of interferon alpha secreting cells in the porcine fetal

hematopoietic organs." Immunol Lett 43(3): 203-208.
80. Srinivasan, A., et al. (2013). "Detection of respiratory viruses in asymptomatic children undergoing allogeneic hematopoietic cell transplantation." Pediatr Blood Cancer 60(1): 149-151.
81. Srinivasan, A., et al. (2013). "Prospective detection of respiratory pathogens in symptomatic children with cancer." Pediatr Infect Dis J 32(3): e99-e104.
82. Stem Cell. http://www.sciencepub.net/stem. 2020.
83. Su, Y. P., et al. (2014). "Dependence of coronavirus RNA replication on an NH2-terminal partial nonstructural protein 1 in cis." J Virol 88(16): 8868-8882.
84. Tan, Y. W., et al. (2012). "Binding of the 5'-untranslated region of coronavirus RNA to zinc finger CCHC-type and RNA-binding motif 1 enhances viral replication and transcription." Nucleic Acids Res 40(11): 5065-5077.
85. Tanaka, T., et al. (2012). "Severe acute respiratory syndrome coronavirus nsp1 facilitates efficient propagation in cells through a specific translational shutoff of host mRNA." J Virol 86(20): 11128-11137.
86. Tirotta, E., et al. (2010). "Cell replacement therapies to promote remyelination in a viral model of demyelination." J Neuroimmunol 224(1-2): 101-107.
87. Uhlenhaut, C., et al. (2012). "Use of a novel virus detection assay to identify coronavirus HKU1 in the lungs of a hematopoietic stem cell transplant recipient with fatal pneumonia." Transpl Infect Dis 14(1): 79-85.
88. Verinaud, L., et al. (1999). "Lymphoid organ alterations enhanced by sub-lethal doses of coronaviruses in experimentally induced Trypanosoma cruzi infection in mice." Lab Anim Sci 49(1): 35-41.
89. Wang, L., et al. (2017). "Respiratory virus infection after allogeneic hematopoietic stem cell transplant in a tropical center: Predictive value of the immunodeficiency scoring index." Transpl Infect Dis 19(3).
90. Weigt, S. S., et al. (2011). "Respiratory viral infections in hematopoietic stem cell and solid organ transplant recipients." Semin Respir Crit Care Med 32(4): 471-493.
91. Weinger, J. G., et al. (2012). "MHC mismatch results in neural progenitor cell rejection following spinal cord transplantation in a model of viral-induced demyelination." Stem Cells 30(11): 2584-2595.
92. Wen, C. C., et al. (2011). "Traditional Chinese medicine herbal extracts of Cibotium barometz, Gentiana scabra, Dioscorea batatas, Cassia tora, and Taxillus chinensis inhibit SARS-CoV replication." J Tradit Complement Med 1(1): 41-50.
93. Weng, J. R., et al. (2019). "Antiviral activity of Sambucus FormosanaNakai ethanol extract and related phenolic acid constituents against human coronavirus NL63." Virus Res 273: 197767.
94. Whitman, L. M., et al. (2012). "Olig1 function is required for remyelination potential of transplanted neural progenitor cells in a model of viral-induced demyelination." Exp Neurol 235(1): 380-387.
95. Wikipedia. The free encyclopedia. http://en.wikipedia.org. 2020.
96. Wolfromm, A., et al. (2014). "Viral respiratory infections diagnosed by multiplex PCR after allogeneic hematopoietic stem cell transplantation: long-term incidence and outcome." Biol Blood Marrow Transplant 20(8): 1238-1241.
97. Wu, H. Y., et al. (2014). "Reselection of a genomic upstream open reading frame in mouse hepatitis coronavirus 5'-untranslated-region mutants." J Virol 88(2): 846-858.
98. Yang, M., et al. (2003). "The effect of SARS coronavirus on blood system: its clinical findings and the pathophysiologic hypothesis." Zhongguo Shi Yan Xue Ye Xue Za Zhi 11(3): 217-221.
99. Yang, M., et al. (2004). "Hematological findings in SARS patients and possible mechanisms (review)." Int J Mol Med 14(2): 311-315.
100. Yu, W. and J. L. Leibowitz (1995). "Specific binding of host cellular proteins to multiple sites within the 3' end of mouse hepatitis virus genomic RNA." J Virol 69(4): 2016-2023.
101. Zhang, J. J., et al. (2006). "Promoter activity of SARS coronavirus 5' UTR sequence in eukaryotic cells." Sichuan Da Xue Xue Bao Yi Xue Ban 37(1): 5-9.