SUPPLEMENTAL HANDOUT:

To begin, note that 35 to 40 million people are living with HIV, (about 1 in 200 people). Population-wide infection levels are declining, but not rapidly. This is, in part, because about half of infected individuals are unaware that they are infected, and because about 60% of infected individuals do not receive anti-retroviral drug therapy. Additionally, infections continue to arise because there are no effective HIV vaccines in place. This places HIV virus / AIDS disease at #3 on the worldwide mortality and morbidity list. Without anti-HIV drug therapies, about 50% of HIV-infected individuals die within 9 years.

For these reasons, it is valuable to know about HIV replication and pathogenesis.

By the end of the lecture, you will be familiar with HIV biology, and with relationships between HIV biology, HIV transmission, HIV variability, and the course of HIV – induced disease. (HIV therapeutics will be presented in a separate lecture.)

Slide 3: Retroviruses are enveloped, often spherical particles. In the electron microscope (EM), HIV can be distinguished by a conical core, within the limiting membrane envelope. Note that the envelope is studded with projections. The projections are envelope glycoproteins, which for HIV are glycoprotein (gp) 120/41 trimers.

Slide 4: A schematic of the virion identifies its parts. Starting from a point furthest from the center of the particle, there are envelope projections. The gp120 is the oval part here. GP120 binds receptors, CD4 and chemokine coreceptors CCR5/CXCR4. GP120 is noncovalently connected to gp41, the transmembrane rods here. GP41 is needed for the virus to enter cells. The blue circle represents the membrane envelope of the virus. In the core, there are two identical RNAs (retroviruses are diploid) along with key enzyme activities: Reverse transcriptase (RT), RNase H, integrages and protease. RT converts the virion RNAs to DNA, RNase H degrades the RNA after it has been copied into DNA, integrase catalyzes incorporation of the DNA into the host cell chromosome, and protease operates during virus assembly to cleave virion proteins.

Slide 5: This slide depicts the retroviral replication cycle. We can take the replication cycle stepwise, starting with the virus entering the cell (boxed in red).

The virus binds to receptors (top left). The virion membrane fuses with either the plasma membrane or endosomal membranes (top left). The core enters the cytoplasm.

Slide 6: Virus entry in greater detail is understood from knowledge of the viral gp120/41. The key functions of gp120/41 are virus binding to cells, and virus membrane fusion with host cell membranes.
The gp120 first binds to CD4 (in green here). This binding causes the gp120/41 to change its shape and expose a site for binding of a coreceptor. The coreceptors (in blue here) are 7-transmembrane G protein coupled receptors (you will remember these from MCBG courses). The gp120 then binds to coreceptors. This triggers dramatic changes in gp41 conformation, forcing part of gp41 into the host cell membrane. The gp41 then collapses into a hairpin–like coiled coil and this then brings viral and host cell membranes together, which eventually forms a pore that the viral core can come through, to start the infection.

Of note, the intermediate conformations of gp120/41 are vaccine antibody and antiviral drug targets.

Some individuals are profoundly resistant to HIV infection because they do not make functional CCR5 coreceptors. This has several implications including the possibility of curing HIV disease by stem cell replacement therapy (a heroic but tenable strategy that has been successful in one instance).

**Slide 7:** Reverse transcription (RT) is the next step. RT ensues in the cytoplasm. A double strand DNA form of the virus is made. This is called the Pre Integration Complex (PIC). The PIC enters the nucleus.

**Slide 8:** Focusing on the RT step here, note that the virion RNAs are organized into the virion. The RNAs are 5’ capped and 3’ polyadenylated. There are two identical RNAs in each virus particle. The genes on these RNAs are called gag (encode group antigens), pol (encode the enzymes in the virion, RT, RNaseH, integrase, protease) and env (encode gp120/41). A host cell tRNA is hybridized near the 5’ ends.

One virion RNA is depicted at the top right of this slide. The ends are direct repeats, labeled as “R”. There are also unique (labeled as “U”) regions at 5’ and 3’ ends (U5 and U3). In reverse transcription, U3 and U5 get copied twice, and so the DNA form (the provirus) has two Long Terminal Repeats (LTRs), in arrangement of U3-R-U5, depicted at bottom. The LTRs are the promoters for the next transcription steps.

The reverse transcription process is oversimplified here. If you want a more detailed understanding of this process, see https://www.youtube.com/watch?v=eS1GODinO8w, and / or figure 2 in http://www.ncbi.nlm.nih.gov/books/NBK19424/

A key point is that numerous anti-HIV drugs target the RT step.
Slide 9: After reverse transcription, the next steps are translocation into the nucleus (this is assisted by host importins – recall your MCBG course). Then integrase action takes place. Integrase catalyzes the insertion of the DNA into the host chromosome.

Slide 10: Focusing on the integration step here, note that the integration process requires a break in host DNA and then the insertion of the provirus via ligation. Integration sites are somewhat random although transcriptionally active parts of the host genome are most often the substrates for integration. After integration, the provirus is permanently in the host cell, but the cell is not yet producing any progeny viruses. This is referred as viral latency.

The somewhat random integration of retroviruses makes the viruses mutagens. This is a potential problem for gene therapy, because it is known that some animal retroviruses are indeed mutagenic and oncogenic. There are consequences to retroviral DNA integration.

Slide 11: The integrated DNA form is called the “provirus”. The provirus is transcribed by host RNA polymerase II (pol II) to make viral RNAs. Viral RNAs transport to the cytoplasm and are translated. Viral translation products include Gag (group antigen) proteins and also enzymes (as stated above) and also the envelope glycoproteins. These proteins participate in virion morphogenesis. New virions are budded from cells.

Slide 12: After budding, there is a maturation phase in which gag proteins are cleaved by viral proteases to form the condensed core.

Slide 13: The final maturation step is important because it is the target of HIV protease inhibitors. Maturation takes place when virions (at left) have their gag proteins cleaved by the virion protease. The gag fragments rearrange to form a condensed core. Without this rearrangement, the future reverse transcription steps that we described earlier cannot take place. Protease inhibitors block the HIV protease and therefore noninfectious virus particles are produced.

Slide 14: To review, the retroviral (i.e., HIV) replication cycle can be divided into two parts. The first part includes all steps up to proviral integration. Stopping the process at this stage is common. Stopping the process at this stage generates viral latency. Gene therapy vectors are re-purposed retroviruses, including HIV-based re-purposed viruses, engineered to complete only the first steps up to integration. The second part includes all steps after proviral integration. These steps typically take place in metabolically active cells. These steps culminate in new virus formation and spreading of infection.

Moving on to HIV transmission:
**Slide 16:** In the 1980s and thereafter, HIV has been extensively evaluated, and we know now that HIV is somewhat typical for a zoonotic virus, that is, a virus that transfers from animals to humans. There is strong phylogenetic evidence for at least three transfers of chimpanzee HIV to humans. This transfer from chimpanzees was more than 50 years ago, as there are historical samples, saved by clinicians, dating back to at least 1959. Retrospective lab tests (PCR and others) document HIV in humans for more than 50 years. The disease of AIDS, however, was not documented until the 1980s, and at first, it was rare opportunistic infections that hinted at some underlying cause. *This history offers a lesson about the value of observation and communication in clinical medicine.*

**Slide 17:** Perhaps the time span between first human infections and HIV epidemic is because the transmissibility of the virus is low. Probability of HIV transmission per coital act is about 1 in 1,000; probably increases about 2 or 3-fold if there is evidence of genital ulcer disease. The virus is not efficiently transmitted.

**Slide 18:** One can contrast a retrovirus like HIV with a paramyxovirus like measles. Measles has remarkably effective transmissibility; > 90% transfer efficiency to nearby infected individuals. HIV by contrast is not very contagious, not spread by respiratory, alimentary or vector routes. Consistent with these routes, recovery of HIV is not in feces or urine or tears or sweat or saliva. HIV is in plasma, semen, very low levels in vaginal cervical fluid.

**Slide 19:** Also consistent with low transmissibility, HIV infectivity is labile. The virus is inactivated by air drying, heating, bleach, alcohol, pH extremes. In large part this is because HIV is an enveloped virus, and membrane enveloped viruses are generally less stable than nonenveloped viruses. In addition HIV envelope glycoproteins (gp120/41) are easily denatured. The low transmissibility of HIV is related, in part, to the unstable virus particle and its rapid loss of infectivity when extracellular.

**Slide 20:** But despite the low transmissibility, HIV has spread to become endemic.

HIV infects about 0.5% of world population; Sub-Saharan Africa remains the place where HIV infection levels are highest.

**Slide 21:** All of this HIV dispersal brings up questions of HIV diversity in different environments.

**Slide 22:** Even though HIV has entered into the human population (from chimpanzees) only about three times, the virus has had time to evolve in humans. These evolutionary trajectories have created strains. The strains are called M (for main) and O (for outlier). The M strains can be subdivided on the basis of
phylogenetic similarities. There are several clades. Clade B is in the USA and E is in Thailand. The reason why this is important is because vaccines and antiviral drugs have to be able to control all clades.

**Slide 23:** Going to the virus, note that the evolution of clade variation is in the surface glycoproteins. These surface projections are the variable parts of the virus.

**Slide 24:** A notable, and more proximal source of the HIV variation is within the individual infected patient. HIV undergoes variation and Darwinian selection within patients, principally in the gene encoding the envelope glycoproteins gp120/41.

Note that HIV infects cells in the bloodstream and lymphatics, specifically T cells and macrophages. Variations in gp120/41 correlate with changes in tropism. For example, HIV that typically enters via coital acts will infect macrophages, as it is macrophages that are in the vicinity. Macrophages express CD4 (green) and the chemokine receptor CCR5 (purple). The type of HIV gp120 that binds CD4 and CCR5 is termed macrophage – tropic. At later stages of HIV infection, perhaps as late as when AIDS diseases occurs, the HIV gp120 has evolved to also bind CXCR4, another chemokine receptor that is found on T cells. Such a later T – cell tropic virus contributes to T cell depletion and AIDS.

Evidence for the hypothesis that HIV normally transmits as a macrophage tropic virus includes the fact that individuals lacking the macrophage – prevalent CCR5 do not get HIV / AIDS, even though they may be repeatedly exposed to HIV.

**Slide 25:** HIV is pathogenic.

**Slide 26:** Pathogenicity is facilitated by several additional HIV genes (not on test, but may be on USMLE step 1). These genes encode proteins fostering virus growth in vivo.

The additional HIV genes are depicted here in the context of the provirus. Note the positions of gag, pol and env. The six additional genes are in between these core genes, sometimes overlapping and sometimes discontinuous (unified by RNA splicing). Many of the functions of these gene products are known. If there is a USMLE board question on these HIV genes, it would be about tat or rev. Tat promotes HIV gene expression, its like an accelerator for HIV expression. Rev promotes HIV RNA transfer from nucleus to cytoplasm, also improving HIV infection. Together these tat and rev will make HIV very robust.
Slide 27: The robustness of HIV can be witnessed both in cell cultures (at top) and in patient samples (at bottom). The infection by HIV is cytolytic to CD4+ T cells. Infection (at right) causes cells to apoptose, or fuse together. Abundant virus is released. In patients, one can (rarely) observe cells clumping together into syncytia in lymph nodes. This is caused by the envelope glycoproteins gp120/41, which catalyze cell-cell membrane fusions. This damages the patients’ ability to have well-functioning lymph nodes.

Slide 28: CD4 depletion is what causes AIDS. CD4 T cells are key regulatory T cells. They control the functions of other arms of the immune system by secreting cytokines such as IFN gamma and IL2. There are many effectors, including B cells, NK cells and macrophages. Without CD4 T cells, these effectors will dysfunction (for example, B cells may overproduce low-affinity antibodies, macrophages may lose their phagocytic activities). The overall effect is immune suppression, which then allows HIV to expand ever more.

Slide 29: Prolonged HIV infection can reach an irreversible state. Deposition of virus in lymph nodes, and infection in the nodes, can permanently destroy the delicate architecture in which dendritic cells present antigens to lymphocytes. With dissolution of the follicular dendritic cell networks, antigens cannot be presented, and the virus can flow freely through nodes and lymphatics. This compounds AIDS disease.

Slide 30: All of this can be understood in the context of an HIV infection time course.

Slide 31: This slide depicts the progression of HIV infection in the absence of any antiretroviral therapies. At top are CD4+ PBL (Peripheral Blood Lymphocytes). Normal ranges are about 1000 of these cells per microliter. Weeks after HIV infection, the CD4 T cell levels drop to about half this level and then they rebound. Thereafter the CD4 T cell levels inexorably decline. After many years, the CD4 levels drop below 500 per microliter and this correlates with AIDS and ARC (AIDS Related Complex). Death comes after profound immune suppression.

Note the long time scale. HIV is a retrovirus in the lentivirus subfamily. Lenti means “slow” and the virus is slow to elicit disease.

At bottom are the time courses of viremia (HIV in blood), spiking at the acute stage, then declining but never dropping to below detection limits. Low level viremia continues for years. During this long, largely asymptomatic phase, there are antibodies to HIV env (gp120/41) and to HIV p24 (a gag protein), and there are also CTL (Cytotoxic T Lymphocytes) specific for HIV infected cells. But none of these host responses are able to eliminate the virus, hence with immunosuppression, HIV levels increase and there is AIDS.
**Slide 32:** In the acute stage, there can be mono-like symptoms, swollen lymph nodes. The original name for HIV was Lymphadenopathy Virus (LAV), as the first HIV specimen to be analyzed was taken from a patient who came in with swollen lymph nodes. At this stage, the virus spreads to seed other lymphoid organs. The virus seeding includes depositing latent proviral DNAs (hard to get rid of).

**Slide 33:** In the clinical latency period, there are few if any clinical signs of disease, yet the virus continues to be present. There are cells in the patient that are truly latently infected with proviruses, but overall, the patient is not in a complete state of microbiological latency (there is a difference between microbiological latency (no viruses produced) and clinical latency (no disease). Some cells produce viruses, which circulate for days and can infect other T cells and macrophages, during the clinical latency period.

**Slide 34:** At the clinical disease stage, there are opportunistic infections. At 350 CD4+ per microliter, AIDS related symptoms appear, often mycobacterial infections. Tuberculosis is common in HIV+ patients.

At less than 200 CD4+ per microliter, the more rare opportunistic infections arise., protozoal and fungal infections. Also notable is AIDS dementia, which arises after HIV-infected macrophages traffic into the CNS and deposit infection there. HIV infects resident CNS glial cells, causing dementia. USMLE step 1 may ask you to remember that macrophages transport HIV to the CNS.

**Slide 35:** Photos of various conditions following opportunistic infection.

**Slide 36:** There will be future MHD or PHARM lectures on HIV therapeutics, so here we will only go through the essential features of ART.

**Slide 37:** HAART is applied continuously, lowering what is termed the “virologic set point”, and keeping viremia below detectable levels.

The indication to start HAART is HIV+ diagnosis, history of an AIDS defining illness, or a CD4 T cell count below 300 per microliter.
The central point of antiretroviral therapy (ART) is to lower the virus level during the clinical latency period. The lower the virus level during this stage, the longer the clinical latency period.

**Slide 38:** ART, or “Highly Active ART” (HAART) is intended to block HIV at more than one distinct infection stage, thereby achieving drug synergy. The two main stages are at replication, with RT inhibitors (usually nucleoside analogs) and at maturation, with protease inhibitors.

Note that HAART, similar to other antiviral therapies, has to be intense, because virus mutation and escape from drug action is easy to achieve. There are two stages in the infection cycle that introduce mutations. One is at reverse transcription of the viral genome, and the other is at forward transcription of the integrated provirus. There are so many mistakes during these stages that the viruses coming out of infected cells are not the same as those that came into the cell to infect it.

Another point about HAART is that it has to be maintained indefinitely. This is because latently infected cells are long lived. The time required to confidently clear infection is far greater than the normal human lifespan.

Another key point is that, even during the clinical latency period, an infected individual may produce \(10^{10}\) virions per day, and this has to be reduced with HAART.

**Slide 39:** HAART has side effects though. Notably AZT causes serious anemia. Other side effects of HAART are heart disease, diabetes, liver disease.

**Slide 40:** HIV comparison with HTLV

**Slide 41:** You may be aware of the fact that HIV is not the only medically relevant human retrovirus. There is also HTLV. HTLV-1 is the most prevalent, with 10 to 20 million infected individuals. Of these 10-20 million, 2 to 5% will develop acute T cell leukemia as an infection outcome. 0.2 to 2% will develop HTLV-1 associated myelopathy. Like HIV, HTLV-1 is distributed unevenly, endemic in Japan, SE Asia, Brazil, Iran, parts of Africa.

**Slide 42:** Note striking similarities between HIV and HTLV; zoonotic infections, CD4+ T cell tropic, prevalent viruses, poorly transmissible. However, HTLV is not directly cytotoxic but rather transforms CD4+ T cells and causes leukemias.
Slide 43: HTLV encodes a protein called tax. The tax protein, once made, can combine with host cell transcription factors to change the profile of genes that are expressed in the infected cell.

Amongst the profile of genes that become overexpressed after tax production are IL-2 and IL-2 receptor (IL-2R). These two products can form an autocrine growth loop, where secreted IL-2 binds back to IL-2R, setting up growth promoting signals. The T cell starts to grow without restrictions. Also Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) is made, which, with macrophages, sets up paracrine growth loops that include IL-1 from the macrophage. Thus the T cells grow, and this, with time, contributes to adult T cell leukemia, a severe and often fatal outcome of HTLV infection.

A sample question is on slide 44.