Research Article

VIRO-INFORMATICS: FINDING A HERBAL REMEDY FOR AIDS AND BLOCKING THE TRANSLATION PATHWAY OF HIV GLYCOPROTEINS BY RNAi TECHNIQUE

D M Shiva Reddy[1], Sinosh Skariyachan[2]*, Balaguru Palanivel[1], Dalal Tirth Anish[1], Jacob Kiran[1], Rahul Gopal[1]

1. CMR Institute of Technology, Bangalore, Karnataka, India
2. R & D Center, Department of Biotechnology, Dayananda Sagar College of Engineering, Bangalore, Karnataka, India

***Corresponding author's E-mail:** sinoshskariya@gmail.com

Accepted on: 29-05-2011; **Finalized on:** 25-08-2011.

ABSTRACT

Acquired Immune Deficiency Syndrome is an incurable and terminal disease of the human immune system caused by the Human Immunodeficiency Virus (HIV). It is associated with Envelope Glycoprotein (EGP) and Glycoprotein 120 (GP 120). 3D structures of these two proteins were generated using Homology Modeling. Active compounds of medicinal herbs- *Castanospermum australe* which may inhibit HIV replication and synctium formation induced by EGP and of *Ancistrocladus korupensis* which may act on HIV life cycle by inhibiting reverse transcriptase, cellular fusion and synctium formation were selected. Chemical structures of the active component of these herbs were drawn using chemsketch, combined & converted to .pdb. Both the proteins were successfully docked with the *Castanospermum australe* - *Ancistrocladus korupensis* active component combination. RNAi treatment was later employed to block the translation pathway. Our study concluded that the combination of plant alkaloids Michellamine B and Castanospermine could be used as the herbal remedy for HIV infection.

Keywords: AIDS, Homology modeling, *Castanospermum australe*, *Ancistrocladus korupensis*, plant alkaloids.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a clinical syndrome that is the result of infection with human immunodeficiency virus (HIV), which causes profound immunosuppression. It has been a serious, life-threatening health problem since the first case was identified in 1981 and is the most quickly spreading disease of the century. Since the epidemic began, more than 60 million people have been infected with the virus. HIV/AIDS is now the leading cause of death in Sub-Saharan Africa. Worldwide, it is the fourth biggest killer. According to recent reports of WHO and UNAIDS an estimated 40 million people (37.2 million adults and 2.2 million children) globally were living with HIV, out of which about 22 million had died. The most affected is Sub-Saharan Africa, where 3.1 million adults and children became infected with HIV and 2.3 million died. South Africa is reported to have the largest population living with the disease, at well over 5 million people infected, followed by Nigeria in second place and India being the third largest population of HIV infected with more than 2 million people reported[1-6]. Around 1.2 million people in Asia acquired HIV in 2009, bringing the number of people living with HIV to an estimated 8.2 million. The spread of HIV in India has been diverse, with much of India having a low rate of infection and the epidemic being most extreme in the southern states. As of now, 92% of all nationally reported AIDS cases have been found in 10 of the 28 states and 7 union territories. The greatest numbers were in Maharashtra and Gujarat in the west; Tamil Nadu and Andhra Pradesh in the south; and Manipur in the Northeast. In the southern states, the infections are mostly due to heterosexual contact, while infections are mainly found amongst injecting drug users in Manipur and Nagaland. A very high proportion of men and women infected with HIV virus are in their active reproductive ages and around half of the people who acquire HIV become infected before they turn 25. Of greater concern is the possibility of infected mothers transferring the disease to their babies[7-9]. Two major types of HIV have been identified so far, HIV-1 and HIV-2. HIV-1 is the cause of the worldwide epidemic and is most commonly referred to as HIV. It is a highly variable virus, which mutates readily. There are many different strains of HIV-1, which can be classified according to groups and subtypes; there are two groups, M and O. Within group M, there are currently known to be at least ten genetically distinct subtypes of HIV-1. These are subtypes A to J. In addition, Group O contains another distinct group of heterogeneous viruses. HIV-2 is much less pathogenic and occurs rarely; it is found mostly in West Africa HIV begins its infection of a susceptible host cell by binding to the CD4 receptor on the host cell. CD4 is present on the surface of many lymphocytes, which are a critical part of the body's immune system. It is now known that a co-receptor is needed for HIV to enter the cell. Following fusion of the virus with the host cell, HIV enters the cell. The genetic material of the virus, RNA, is released and undergoes reverse transcription into DNA. An enzyme in HIV called reverse transcriptase is necessary to catalyze this conversion of viral RNA into DNA. Once the genetic material of HIV has been changed into DNA, this viral DNA enters the host cell nucleus where it can be integrated into the genetic material of the cell. The enzyme integrase catalyses this process. Once the viral DNA is integrated into the genetic material of the host, it is possible that HIV may persist in a latent state for many years[10]. This ability of HIV to persist in certain latently

infected cells is the major barrier to eradication or cure of HIV. For this reason, based on current knowledge, patients must remain on anti-viral therapy for life. Several reviews on the natural products for chemotherapy of HIV infection have been reported. Matthee *et al.*[11] reported naturally occurring HIV reverse transcriptase inhibitors. Jung *et al.*[12] discussed anti-HIV agents according to their chemical classes. Yang *et al.*[13] reviewed natural products-based anti-HIV drug discovery and development facilitated by NCI development programme. Cos *et al.*[14] reviewed different plant substances as anti-HIV agents according to their mechanism of action.

MATERIALS AND METHODS

The softwares or web servers used for this study includes Modeller9v7, RASMOL, ACD/ChemSketch -version 11.00, RAMPAGE, ArgusLab 4.0.1, Genamics, Si RNA Design Tool and PATCHDOCK.

In silico modeling and docking simulations

The key protein responsible for HIV infection, envelope glycoprotein with accession number-ABH02539.1[15] was taken for this study. Human receptor protein to which HIV protein binds, gp120 with accession number AAF69492.1[16] was taken from NCBI GenBank database. Homology modeling was carried out using Modeller 9v7[17], for predicting 3 D structures for the above mentioned proteins. The best templates used for homology modeling were identified by similarity searching tool BLAST[18] and the best homologous protein structures from RCBS PDB were retrieved. The following templates were used:

Envelope glycoprotein- 3JWD, Chain A (Identity=100%, Source: Human immunodeficiency virus 1), 3JWO, Chain A (Identity=100%, Source- *Homo sapiens*), 2BF1, Chain A (Identity= 37%, Source: Simian immunodeficiency virus).

Gp120- 2BF1, Chain A (Identity= 37%, Source: Simian immunodeficiency virus), 1YYM, Chain G (Identity= 75%, Source: *Homo sapiens*), 1G9M, Chain G (Identity= 74% Source: *Homo sapiens*).

Five models of each of the above proteins were retrieved. The models were analyzed by Rampage Ramchandran plot server[19] and the best model of each was selected. Chemical structures of active component of *Ancistrocladus korupensis*[20], Michellamine B (Fig-1) and active component of *Castanospermum austral*[21], Castanospermine (Fig-2) were drawn and combined using ACD chemsketch[22] software (Fig-4). The combined structure was saved as .mol file and was later converted to .pdb file using Argus lab[23] software (Fig-4).

Each of the proteins used for this work, namely Envelope glycoprotein and gp120 was successfully docked with active components of *Ancistrocladus korupensis* and *Castanospermum australe* i.e. Michellamine B and Castanospermine combination. The docking study was conducted by PatchDock[24]. It is an automatic server for molecular docking and it uses molecular docking algorithm based on shape complementarity principles.

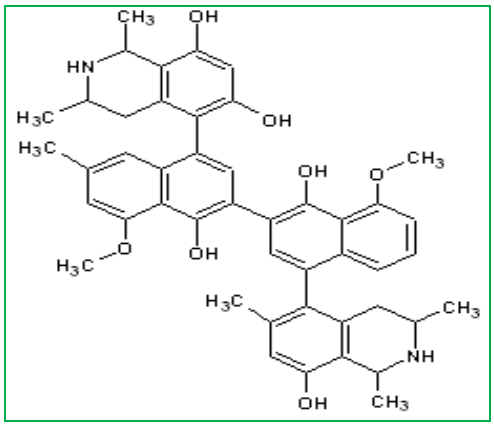

Figure 1: Chemical structure of Michellamine B.

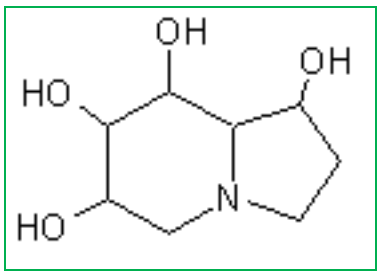

Figure 2: Chemical structure of Castanospermine.

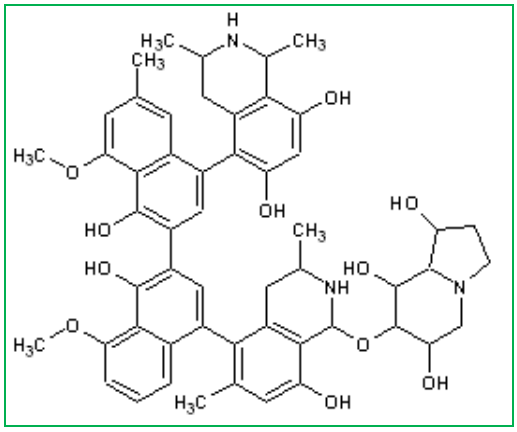

Figure 3: Combined Structure of Michellamine B and Castanospermine.

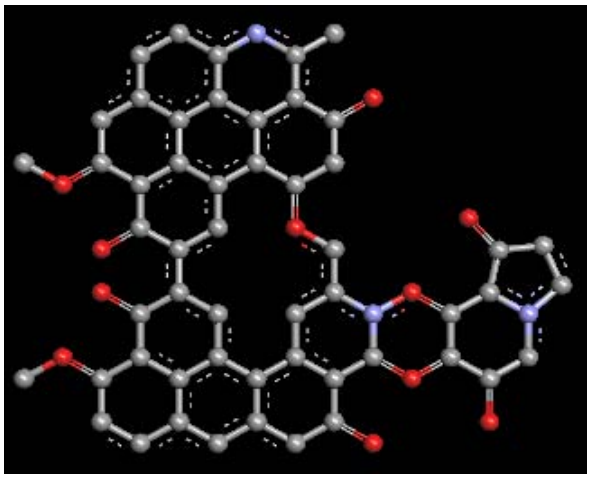

Figure 4: Combined structure of Michellamine B and Castanospermine visualized in Argus Lab.

RNAi treatment

RNA sequences of the proteins (i.e. envelope glycoprotein and gp120) were generated using the software GENAMICS and the fragments of the RNA sequence (Si RNA fragments) are generated using online Si RNA design tool from Microsynth.

RESULTS AND DISCUSSION

In silico homology modeling of target proteins

The first target protein identified in this study was the envelope glycoprotein of human immunodeficiency virus 1 with the Gene index number GI: 110893758. The protein consists of 455 amino acid residues. The next sequence used in our study was gp120 of Human immunodeficiency virus 1 with GI: 7769644. The sequence also consists of 455 amino acids. The 3D structure of envelope glycoprotein obtained by homology modeling was analyzed by RAMPAGE Ramchandran plot server. The results obtained for best model is good quality because almost all residues of the protein are located in to the allowed region of Ramchandran plot. (Fig-5) The steriochemical validation of the structure is follows:

Number of residues in favored region (~98.0% expected): 411 (90.7%)
Number of residues in allowed region (~2.0% expected): 37 (8.2%)
Number of residues in outlier region: 5 (1.1%)

Similarly the 3D structure of gp120 obtained by homology modeling was also analyzed by RAMPAGE Ramchandran plot server. This model also qualifies the quality as per the Ramchandran rule (Fig-6). The streriochemical validation of alpha carbon atoms of the model are given below:

Number of residues in favored region (~98.0% expected): 394 (87.0%)
Number of residues in allowed region (~2.0% expected): 47 (10.4%)
Number of residues in outlier region: 12 (2.6%)

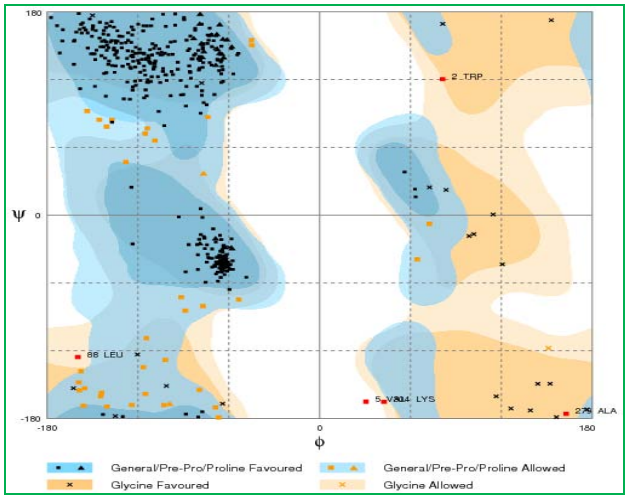

Figure 5: Ramachandran Plot analysis envelope glycoprotein

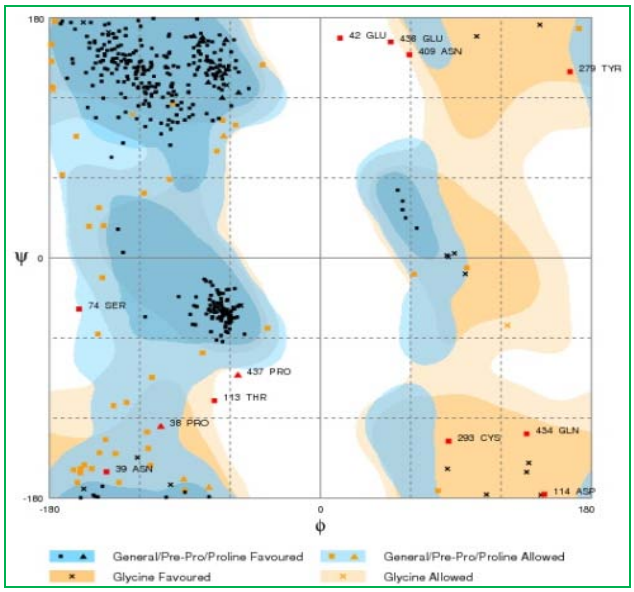

Figure 6: Ramachandran Plot analysis gp120

Docking simulations and *in silico* drug discovery

The docking studies were conducted with the modeled proteins (Envelope protein and gP 120) and the combinations of Michellamine B and Castanospermine. Michellamine B is a plant alkaloid isolated from *Ancistrocladus korupensis*. Michellamine B has exhibited significant *in vitro* activity against HIV-1 and HIV-2. Castanospermine is an indolizine alkaloid isolated from the seeds of *Castanospermum australe*. It is a potent inhibitor of some glucosidase enzymes and has antiviral activity.

The docking simulations were performed with PatchDock server. The docking mechanism in PatchDock relies on three concepts. First, molecular shape representation, the programme computes the molecular surface of the molecule followed by a segmentation algorithm for detection of geometric patches (concave, convex and flat surface pieces). The patches are filtered, so that only patches with 'hot spot' residues are retained. The second step is the surface patch matching, here we applied a hybrid of the geometric hashing and pose-clustering matching techniques to match the patches detected in the previous step. Concave patches are matched with convex and a flat patch with any type of patches. The final step is filtering and scoring. In this case the candidate complexes from the previous step are examined. The programme discards all complexes with unacceptable penetrations of the atoms of the receptor to the atoms of the ligand. Finally, the remaining candidates are ranked according to a geometric shape complementarity scores.

The PatchDock Server generated many best poses and the conformations which were stable with minimum energy. The envelop glycoprotein is bind to the inhibitors and produced stable protein-ligand complex (Fig-7). Similarly the combinations of inhibitors successfully docked with gp-120 protein with minimum binding energy and visualized by RASMOL (Fig-8). Docking scores for envelope glycoprotein was -8768 and gp120 was -9124. Our study

revealed that Michellamine B –Castanospermine complex could be used as a potential inhibitor against HIV infection as these ligands perfectly interacting with both the proteins.

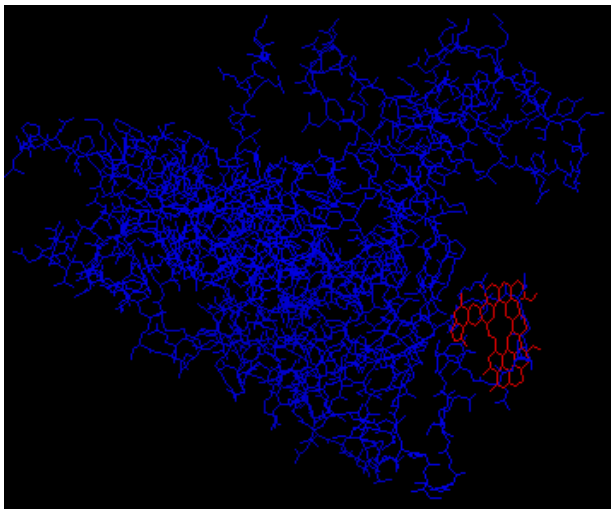

Figure 7: Docked structure envelope glycoprotein with Michellamine B and Castanospermine

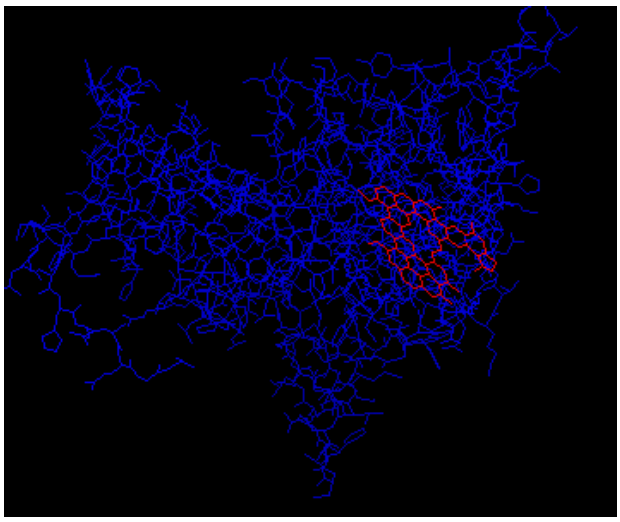

Figure 8: Docked structure gp120 with Michellamine B and Castanospermine.

Microsynth

siRNA Design Results

Candidates:

5'region	mRNA target sequence (= siRNA core sequence)	3'region	score	start	
AA	CAAGACAGUUGUACCAAUA	AC	9	678	blastn
AA	CAAGAAAUGUUGCGAUUGA	CC	9	1435	blastn
AA	CCAAACAAUGGUAAAUGAA	AA	8	159	blastn
AA	AAAGAAGUAUAGUGACACA	UG	8	276	blastn
AA	CUAUCAUUAGCUUGAAACAA	GC	8	620	blastn
AA	CAAGCAUUAUAAUCUCACA	AU	8	636	blastn
AA	CUAUGCGCCAUGCCAUAUA	AA	8	993	blastn
AA	UGAAAUUGCAAUACCACCAA	CG	7	68	blastn
AA	AAUAGUUCCUGUAUACGUA	AC	7	178	blastn
AA	AUAGUUCCUGUAUACGUAA	CG	7	179	blastn
AA	CGACAGCUGCGCAGGAUUA	GG	7	198	blastn
AA	CCAGACAUGUUACAUGAGA	CA	7	339	blastn
AA	GAUGUAAUGAUACCAACUA	CU	7	455	blastn
AA	GACAGUUGUACCAAUAACA	CU	7	681	blastn
AA	ACAUCCCAGGUACAAAGGA	AC	7	819	blastn
AA	CAUCCCAGGUACAAAGGAA	CC	7	820	blastn
AA	GGAACCAAUGACACAAGCA	AA	7	835	blastn
AA	UUUCUCUACUGCAACAUGA	CU	7	928	blastn
AA	UUAAUACCUGGCAUAAAGU	AG	7	1022	blastn
AA	AGUAGGGAAGAAUGUAUAU	UU	7	1038	blastn
AA	GUAGGGAAGAAUGUAUAUU	UG	7	1039	blastn
AA	CCAGCAUAAUUGCUAACAU	UG	7	1100	blastn
AA	CAUUACCUUUAGUGCAGAA	GU	7	1149	blastn
AA	AGCCUGACUGGGACAAUAU	GA	7	1595	blastn
AA	AUAUCAGUCAAGAUUUGGA	GC	7	1658	blastn
AA	AAAUUAGAUAGCUGGGAUA	UC	7	1720	blastn
AA	CUACUCAGGCUUUGAACCU	AA	6	471	blastn
AA	ACGCAAACUUCUACUUGGU	UU	6	535	blastn
AA	ACAAGACAGUUGUACCAAU	AA	6	677	blastn
AA	UAACACUUAUGUCAGGGCU	AA	6	695	blastn

Figure 9: Si RNA fragment for envelope glycoprotein's RNA.

RNAi treatment

The Si RNA fragment of envelop glycoprotein and gp120 were generated by the tools mentioned in materials and methods (Fig- 9 & 10). The translation pathways of envelope glycoprotein and gp120 proteins were blocked by the formation of Si RNA, thus inhibiting the disease. If RNAi treatment proves efficient in wet lab, the virus can be killed.

Microsynth

siRNA Design Results

Candidates:

5'region	mRNA target sequence (= siRNA core sequence)	3'region	score	start	
AA	GCAGGCUUGUCCAAAGAUA	UC	9	495	blastn
AA	CCAUAAUAGUGCACCUUAA	UA	9	737	blastn
AA	CCACAGACCUAAGAGAUAA	GC	8	371	blastn
AA	AAAUGUCAGCUCAGUACAA	UG	8	609	blastn
AA	AUAACAUGGUAGAGCAGAU	GC	7	170	blastn
AA	AGUCUAAAGCCAUGUGUAA	AG	7	220	blastn
AA	UAUAACAGAUGAGGUAAGA	AA	7	333	blastn
AA	UGUAACCACAGACCUAAGA	GA	7	366	blastn
AA	AGAUAUCCUUUGAUCCAAU	UC	7	509	blastn
AA	GAUAUCCUUUGAUCCAAUU	CC	7	510	blastn
AA	AUGGCAGUCUAGCAGAAGA	AG	7	674	blastn
AA	UGGCAGUCUAGCAGAAGAA	GA	7	675	blastn
AA	UCUCACAAACAAUGUCAAA	AC	7	717	blastn
AA	CAAUACAAGAACCAGUAUA	AC	7	792	blastn
AA	GAACAUAAGCUUUCAACCA	CC	7	951	blastn
AA	AUAUUACAGGGUUGCUAUU	AA	7	1181	blastn
AA	UAUUACAGGGUUGCUAUUA	AC	7	1182	blastn
AA	UGAGACCUUUAGACCUGGA	GG	7	1227	blastn
AA	GGACAAUUGGAGAAGUGAA	UU	7	1260	blastn
AA	AUCACAUGUGACAGAAGCA	CA	6	54	blastn
AA	GUCUAAAGCCAUGUGUAAA	GU	6	221	blastn
AA	AGCCAUGUGUAAAGUUAAC	UC	6	227	blastn
AA	CUCCUCUCUGCGUUACUUU	AA	6	245	blastn
AA	UUGUACCAAUGCUAAUUUG	AC	6	267	blastn
AA	UUUGACCAAUGCUAAUUUG	AC	6	282	blastn
AA	UUUGACCAAUGCUAAUAAC	AU	6	297	blastn
AA	GCAGCAGAAGGUCCAUGCA	CU	6	390	blastn
AA	GAAUAGUAGUGAUUAUAGG	UU	6	447	blastn
AA	UGGGACAGGGCCAUGUAAA	AA	6	591	blastn
AA	AAUGUCAGCUCAGUACAAU	GC	6	610	blastn

Figure 10: Si RNA fragments for gp120.

CONCLUSION

The *in silico* herbal work makes use of ayurvedic herbs in computer aided drug designing. The principle outlined in homology modeling is used to model the 3D structure of the proteins envelope glycoprotein and gp120. The docking studies of active components of the herbs *Ancistrocladus korupensis* and *Castanospermum australe* with the envelope glycoprotein and gp120 proteins provide a new insight to drug discovery process against HIV infection. Our study concluded that the successful docking of envelope glycoprotein and gp120 with Michellamine B and Castanospermine combination proves that the combination can be effective in the treatment of AIDS disease. Again, since the work is done in *in silico* platform, the combination (Michellamine B and Castanospermine) needs to go to clinical testing to establish its efficacy.

Acknowledgment: The authors would like to acknowledge the support and facilities provided by the R & D Centres of CMR Institute of Technology, Bangalore and Dayananda Sagar Institutions, Kumaraswamy Layout, Bangalore, Karnataka, India.

REFERENCES

1. Sepkowitz KA, AIDS-the first 20 years, *N Engl J Med*, 2001, 344 (23): 1764–72
2. Weiss RA, How does HIV cause AIDS? *Science*, 1993, 260 (5112): 1273–79
3. AIDS Epidemic Update, UNAIDS/WHO, 2004 (http:// www.unaids.org)

4. Karim SSA, Churchyard GJ, Karim QA, Lawn SD, HIV infection and tuberculosis in South Africa: an urgent need to escalate the public health response, *Lancet*, 2009, 374:921–33.
5. ASSA, AIDS and demographic model, Cape Town: Actuarial Society of South Africa, AIDS Committee, 2005 (http://aids.actuarialsociety.org.za/ASSA2003)
6. The National HIV and Syphilis Prevalence Survey South Africa, Cape Town: National Department of Health South Africa, 2008 (http://data.unaids.org/pub/Report)
7. Coffin JM, HIV population dynamics *in vivo*: Implications for genetic variation, pathogenesis and therapy, *Science*, 1995, 267:483–488
8. Yudkin PL, Burger EH, Bradshaw D, Groenewald P, Ward AM, Volmink J, Deaths caused by HIV disease under-reported in South Africa, *AIDS,* 2009, 23:1600–02
9. Nojilana B, Groenewald P, Bradshaw D, Reagon G, Quality of cause of death certification at an academic hospital in Cape Town, South Africa, *S Afr Med J,* 2009, 99:648–52.
10. De Clercq E, Antiviral therapy for human immunodeficiency virus infections, *Clin Microbiol Rev,* 1995, 8: 200–239
11. Matthee G, Wright AD, Konig GM, HIV reverse transcriptase inhibitors of natural origin, *Planta Med,* 1999, 65: 493–506.
12. Jung M, Lee S, Kim H, Recent studies on natural products as anti-HIV agents, *Curr Med Chem*, 2000, 7: 649–661.
13. Yang SS, Cragg GM, Newman DJ, Bader JP, Natural product-based anti-HIV drug discovery and development facilitated by the NCI developmental therapeutics program, *J Nat Prod,* 2001, 64: 265–277.
14. Cos P, Mees L, Berghe DV, Hermans N, Pieters L, Vlietinck A, Plant substances as anti-HIV agents selected according to their putative mechanism of action, *J Nat Prod*, 2004, 67:284–293.
15. Labrosse B, Morand-Joubert L, Goubard A, Rochas S, Labernardière JL, Pacanowski J, Meynard JL, Hance AJ, Clavel F, Mammano F, Role of the envelope genetic context in the development of enfuvirtide resistance in human immunodeficiency virus type 1-infected patients, *J Virol,* 2006, 80(17): 8807-19.
16. Phan KO, Callahan ME, Vanichseni S, Hu DJ, Raktham S, Young N, Choopanya K, Mastro TD, Subbarao SA, Comparison of full-length glycoprotein 120 from incident HIV type 1 subtype E and B infections in Bangkok injecting drug users with prototype E and B strains that are components of a candidate vaccine, *AIDS Res Hum Retroviruses*, 2000, 16 (14): 1445-50.
17. Sali A, Blundell TL, Comparative protein modelling by satisfaction of spatial restraints, *J Mol Biol*, 1993, 234:779-81.
18. Johnson M, NCBI BLAST: a better web interface. *Nucl Acids Res*, 2008, 36: 5-9.
19. Lovell SC, Davis IW, Arendall WB, Bakker P I, Word JM, Prisant MG, Richardson JS, Richardson DC, Structure validation by Calpha geometry: phi,psi and Cbeta deviation, *Proteins,* 2003, 50(3): 437-50.
20. McMahon JB, Currens MJ, Gulakowski RJ, Buckheit RW, Lackman-Smith Jr. C, Hallock YF, Boyd MR, Michellamine B, a novel plant alkaloid, inhibits human immunodeficiency virus-induced cell killing by at least two distinct mechanisms, *Antimicrobial Agents and Chemotherapy*, 1995, 39(2): 484-488.
21. Saul R, Chambers JP, Molyneux RJ, Elbein AD, Castanospermine, a tetrahydroxylated alkaloid that inhibits beta-glucosidase and beta-glucocerebrosidase, *Arch Biochem Biophys*, 1983, 221 (2):593-7.
22. OsterbergT, Norinder U, Prediction of drug transport processes using simple parameters and PLS statistics. The use of ACD/logP and ACD/ChemSketch descriptors, *Eur J Pharm Sci*, 2001, 12 (3):327-37.
23. Thompson MA, ArgusLab 4.0.1, Planaria Software LLC, Seattle, WA (http://www.arguslab.com).
24. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ, PatchDock and SymmDock: servers for rigid and symmetric docking, *Nucl Acids Res*, 2005, 33: W363-367.

****