Emerging and re-emerging infectious diseases represent a significant challenge for next-generation vaccine design and bioterror preparedness. We have composed a suite of online immuninformatics tools for accelerated design of genome-derived, epitope-driven vaccines generated from protein sequences. Using the Conservatrix algorithm, even the most mutable pathogenic genomics may be probed for highly conserved segments, which are then mapped for T cell epitopes and regions of high epitope density using EpiMatrix and ClustiMer. JanusMatrix, an improved homology analysis tool examining pathogen/host sequence similarity with respect to the HLA and TCR faces of an epitope, is used to screen out sequences which could potentially elicit an undesired autoimmune or regulatory T cell response due to homology with sequences encoded by the human genome. Immunogenic Consensus Sequences are created by EpiAssembler, a tool which optimizes the balance between pathogen and population coverage. VaccineCAD links candidate epitopes into a string-of-beads design while minimizing non-specific junctional epitopes that may be created in the linking process.

With proof of principle established in animal models for vaccines against tularemia, Vaccinia and H. pylori, the iVAX toolkit exemplifies a rapid, efficient, easily accessible and broadly applicable solution to accelerate the development of critically important vaccines for human health and biodefense.

**Abstract**

**Web Based Tools**

**Conservation Analysis**

Conservatrix

Conservatrix identifies conserved peptides among protein sequences from even the most mutable of pathogens. For any given sequence file, users may view the number of times a 9-mer or 10-mer occurs within the input file, the percent conservation of each peptide among input proteins, and the predicted HLA binding profile for each peptide.

EpiAssembler

EpiAssembler identifies sets of overlapping, conserved, and immunogenic epitopes and assembles them into extended Immunogenic Consensus Sequences (ICS). A highly conserved, putatively promiscuous 9-mer is chosen as the core. Additional epitopes that overlap with the natural N- and C-terminal flanking regions are identified and integrated into the core. Processing of these sequences allows for presentation of the highly conserved peptides in the context of more than one MHC. Users may select construction criteria and view reports summarizing the immunogenicity and conservation of each ICS and each 9-mer within its input protein file.

**DATABASE MANAGEMENT**

iVAX: A faster, easier approach to vaccine design.

**EpiTOPE DISCOVERY**

EpiMatrix

EpiMatrix maps input proteins for putative T cell epitopes restricted by Class I and Class II HLA alleles. Based on overall epitope content, a Protein Immunogenicity Score can be calculated and then normalized for protein length. Immunogenicity scores of different proteins are thus directly comparable.

ClustiMer

Class I T cell epitopes tend to cluster within specific protein sub-regions (shown below). These T cell epitope clusters are high priority targets for vaccine design. ClustiMer identifies these regions and calculates a Cluster Immunogenicity Score for each peptide. Users may select specific HLA alleles to evaluate, and may sort input proteins or peptides by score to create prioritized lists of potential targets.

**Conclusions / Future Directions**

We have achieved proof of principle in animal models for three out of five vaccines for which we currently have prototypes. A therapeutically administered vaccine construct engineered against H. pylori using the iVAX system resulted in a significant reduction in gastric colonization compared to unvaccinated controls. Similarly, VennVax, a DNA vaccine expressing conserved epitopes from seven Smallpox genomes, conferred 100% survival to HLA-transgenic mice lethally challenged with Vaccinia. TulyVax protected 57% of immunized mice against a lethal challenge with F. tularensis. Two more vaccines are in the process of validation.

We are applying the approach to influenza, tuberculosis and HIV, along with biodefense projects. Additional collaborations in the field of neglected tropical diseases are under development. We believe these tools are of great utility for development of safer, more targeted vaccines.