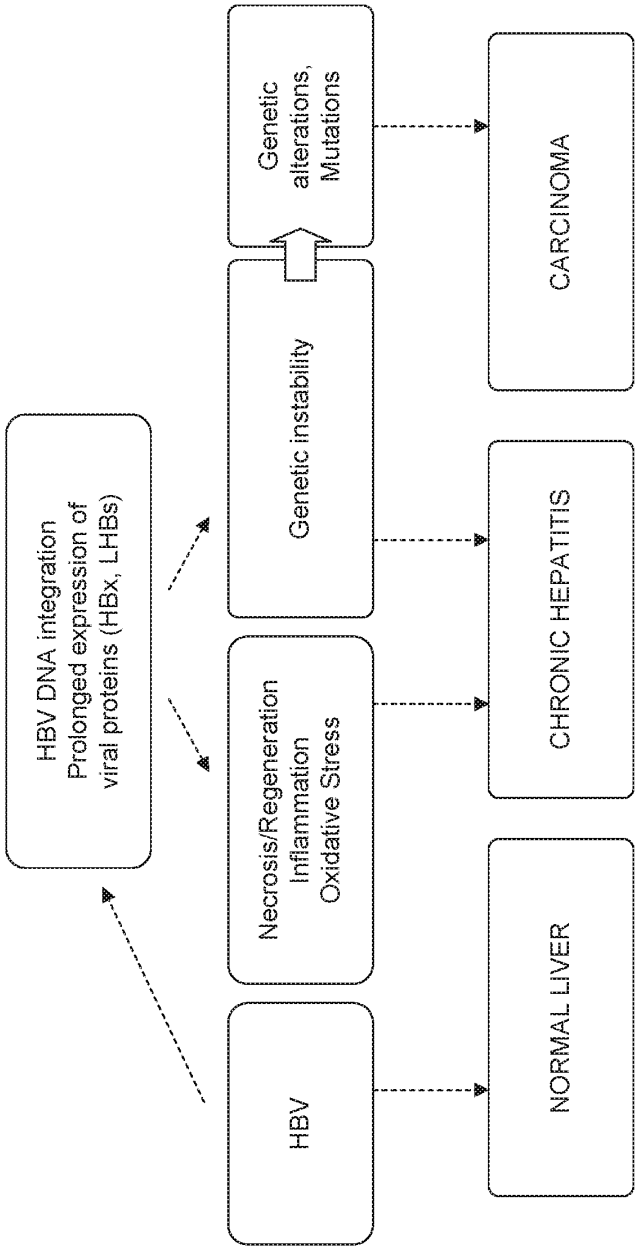

HBV DNA integration
Prolonged expression of
viral proteins (HBx, LHBs)
HBV
Necrosis/Regeneration
Inflammation
Oxidative Stress
Genetic instability
Genetic
alterations,
Mutations
NORMAL LIVER
CHRONIC HEPATITIS
CARCINOMA

FIG. 1

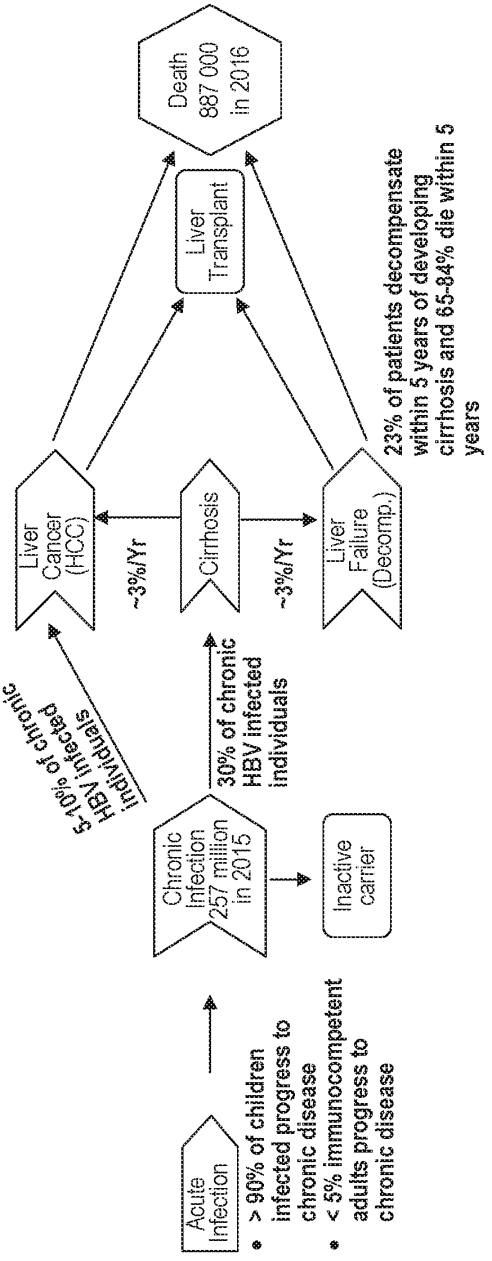

Acute Infection
• > 90% of children infected progress to chronic disease
• < 5% immunocompetent adults progress to chronic disease
Chronic Infection 257 million in 2015
Inactive carrier
5-10% of chronic HBV infected individuals
30% of chronic HBV infected individuals
Liver Cancer (HCC)
~3%/Yr
Cirrhosis
~3%/Yr
Liver Failure (Decomp.)
23% of patients decompensate within 5 years of developing cirrhosis and 65-84% die within 5 years
Liver Transplant
Death 887 000 in 2016

FIG. 2

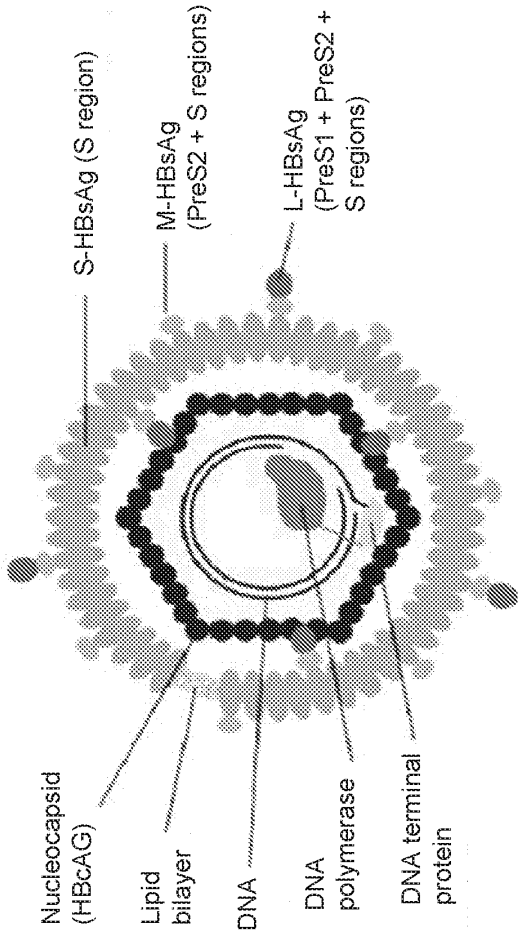

S-HBsAg (S region)
M-HBsAg (PreS2 + S regions)
L-HBsAg (PreS1 + PreS2 + S regions)
Nucleocapsid (HBcAG)
Lipid bilayer
DNA
DNA polymerase
DNA terminal protein

FIG. 3A

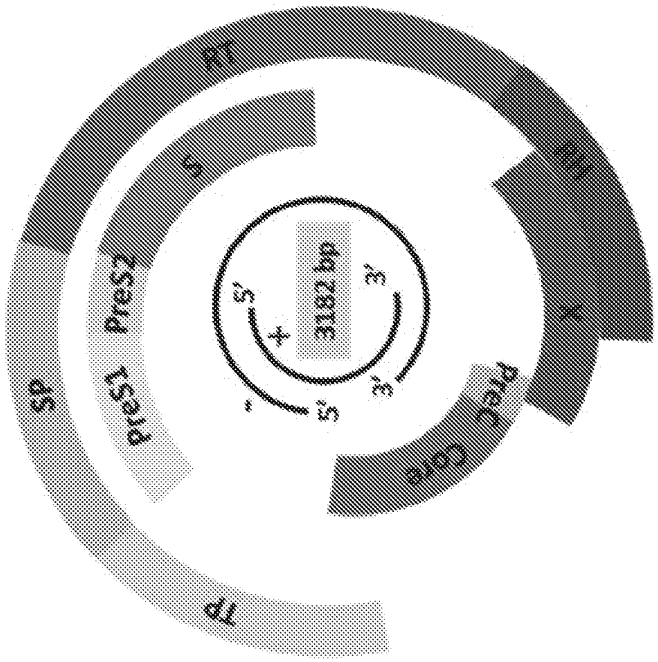


FIG. 3B

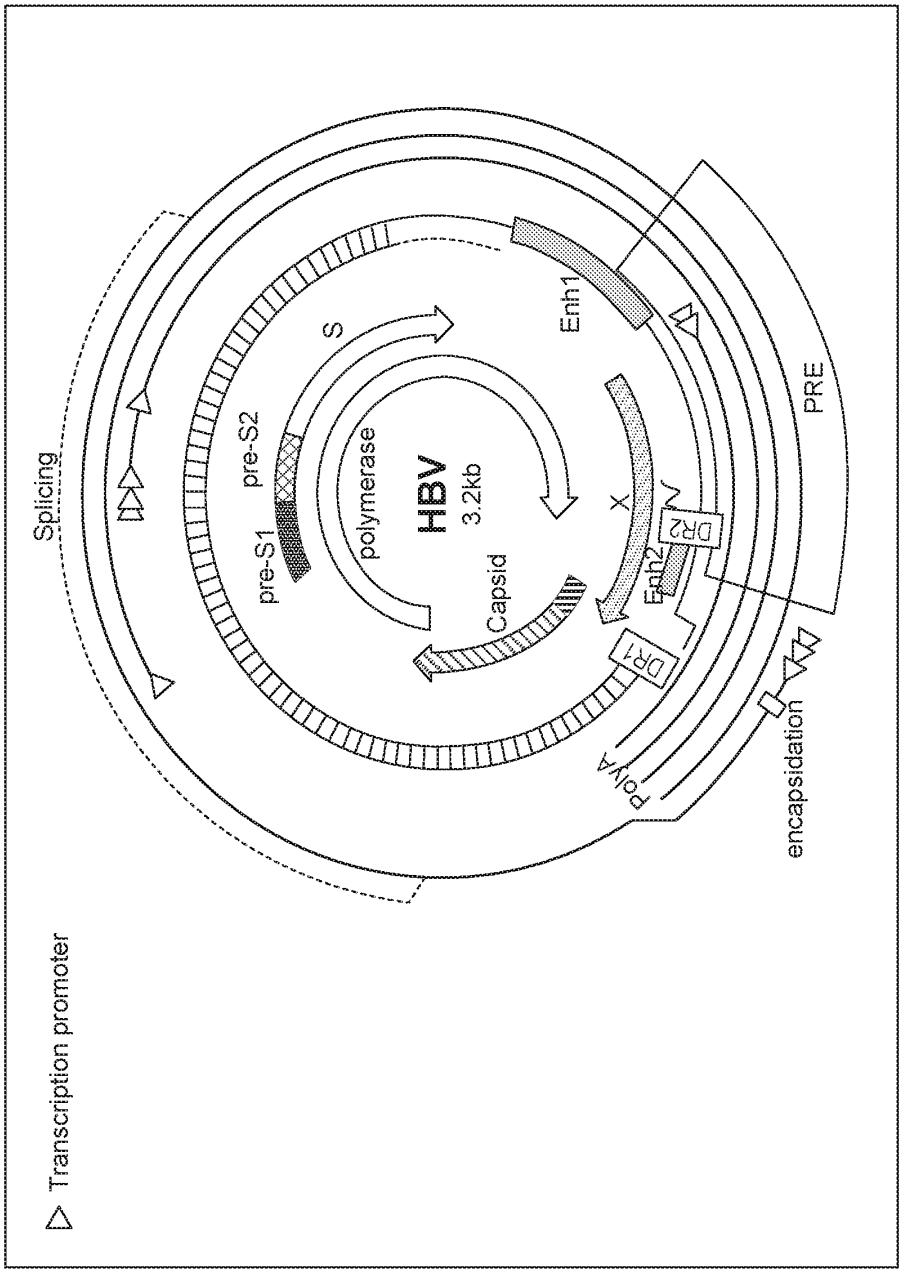

Transcription promoter
Splicing
pre-S1
pre-S2
S
polymerase
HBV
3.2kb
Capsid
Enh1
X
Enh2
DR2
DR1
PRE
PolyA
encapsidation

FIG. 3C

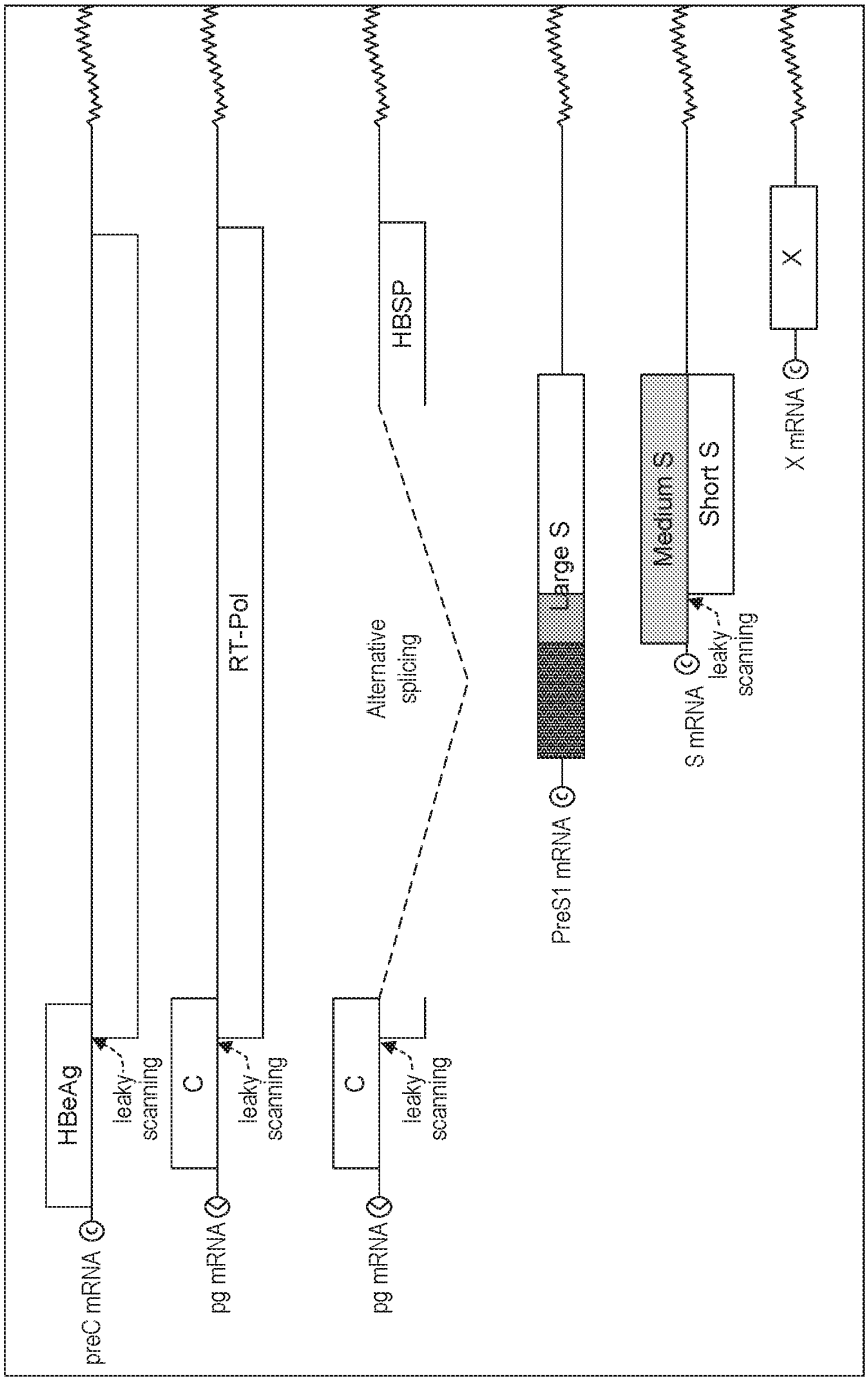

HBeAg
leaky scanning
preC mRNA
C
leaky scanning
pg mRNA
RT-Pol
C
leaky scanning
pg mRNA
HBSP
Alternative splicing
Large S
PreS1 mRNA
Medium S
Short S
leaky scanning
S mRNA
X
X mRNA

FIG. 3D

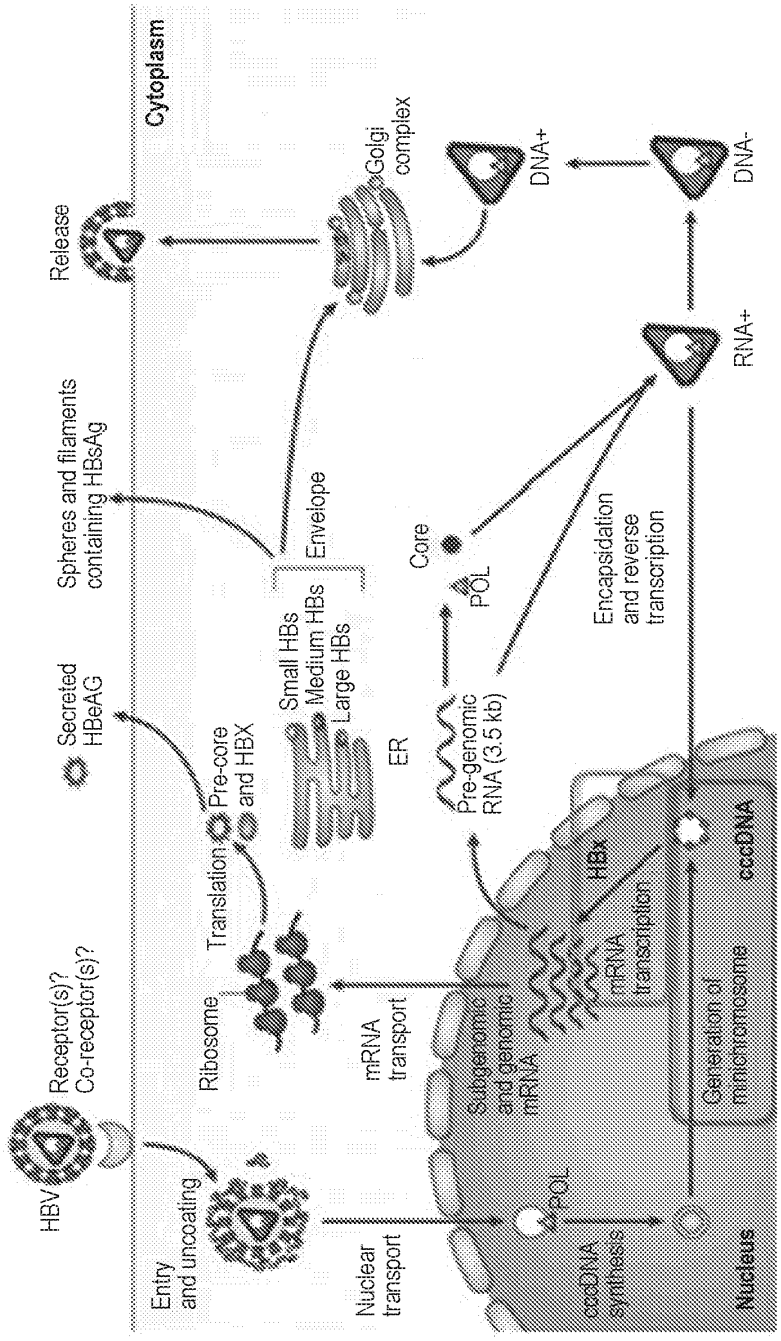

Cytoplasm
Release
Golgi complex
DNA+
DNA-
RNA+
Spheres and filaments containing HBsAg
Secreted HBeAG
Envelope
Small HBs
Medium HBs
Large HBs
ER
Core
POL
Pre-genomic RNA (3.5 kb)
Encapsidation and reverse transcription
Pre-core and HBX
Translation
Ribosome
mRNA transport
Receptor(s)? Co-receptor(s)?
HBV
Entry and uncoating
Nuclear transport
Subgenomic and genomic mRNA
HBx
mRNA transcription
cccDNA
Generation of minichromosome
POL
cccDNA synthesis
Nucleus

FIG. 4

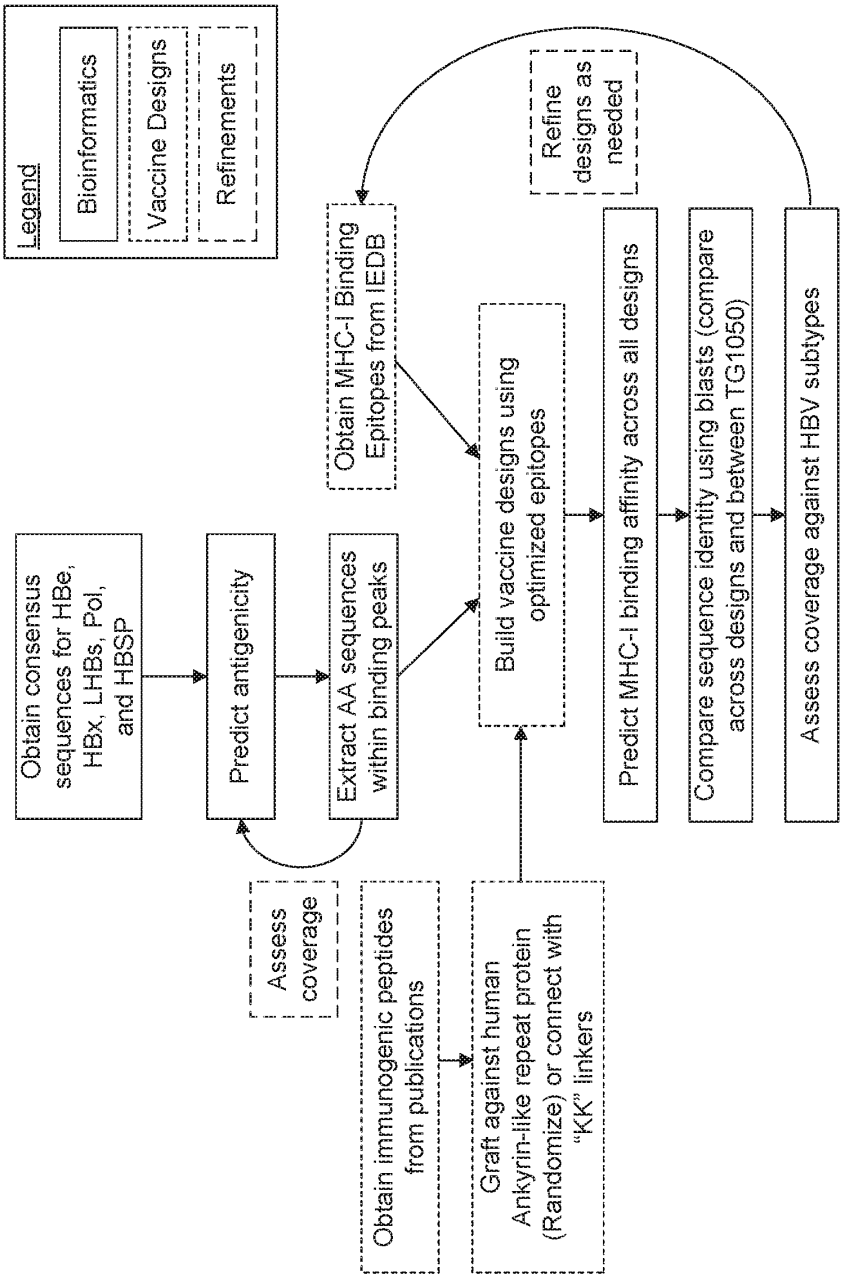

Legend
Bioinformatics
Vaccine Designs
Refinements
Obtain consensus sequences for HBe, HBx, LHBs, Pol, and HBSP
Predict antigenicity
Extract AA sequences within binding peaks
Obtain MHC-I Binding Epitopes from IEDB
Build vaccine designs using optimized epitopes
Predict MHC-I binding affinity across all designs
Compare sequence identity using blasts (compare across designs and between TG1050)
Assess coverage against HBV subtypes
Refine designs as needed
Assess coverage
Obtain immunogenic peptides from publications
Graft against human Ankyrin-like repeat protein (Randomize) or connect with "KK" linkers

FIG. 5

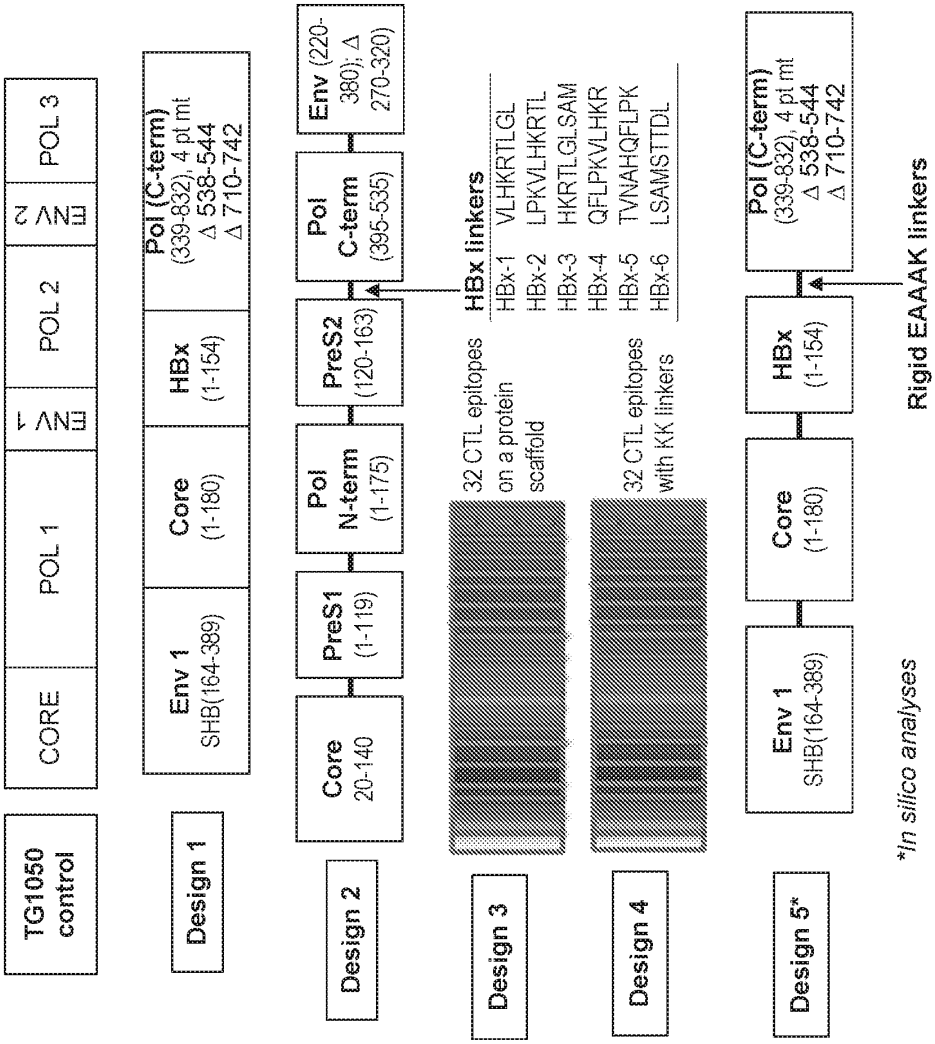

TG1050 control
CORE
POL 1
ENV 1
POL 2
ENV 2
POL 3
Design 1
Env 1 SHB(164-389)
Core (1-180)
HBx (1-154)
Pol (C-term) (339-832); 4 pt mt Δ 538-544 Δ 710-742
Design 2
Core 20-140
PreS1 (1-119)
Pol N-term (1-175)
PreS2 (120-163)
Pol C-term (395-535)
Env (220-380); Δ 270-320)
Design 3
32 CTL epitopes on a protein scaffold
HBx linkers
HBx-1 VLHKRTLGL
HBx-2 LPKVLHKRTL
HBx-3 HKRTLGLSAM
HBx-4 QFLPKVLHKR
HBx-5 TVNAHQFLPK
HBx-6 LSAMSTTDL
Design 4
32 CTL epitopes with KK linkers
Design 5*
Env 1 SHB(164-389)
Core (1-180)
HBx (1-154)
Pol (C-term) (339-832); 4 pt mt Δ 538-544 Δ 710-742
Rigid EAAAK linkers
*In silico analyses

FIG. 6

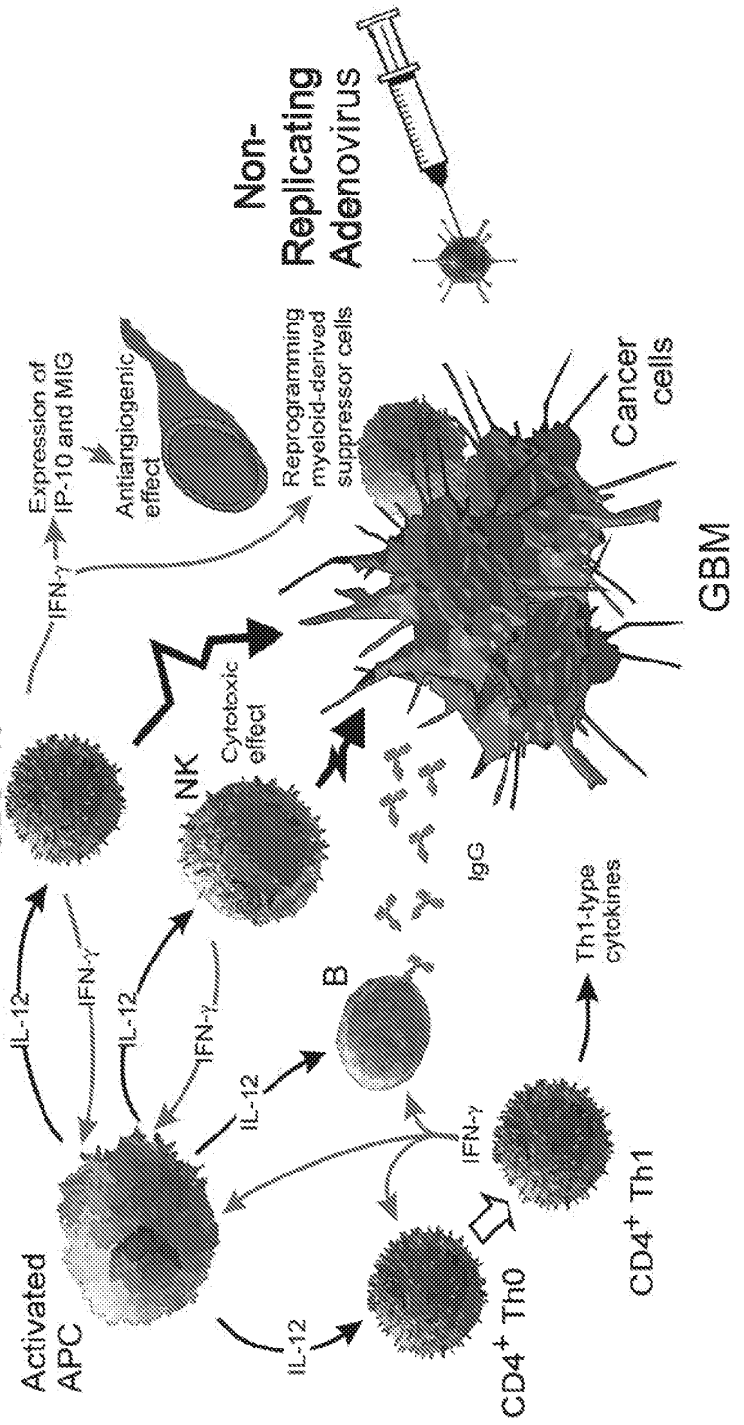

Non-Replicating Adenovirus
Expression of IP-10 and MIG
Antiangiogenic effect
Reprogramming myeloid-derived suppressor cells
Cancer cells
GBM
IFN-γ
CD8+ Tc
Cytotoxic effect
NK
IL-12
IFN-γ
IL-12
IFN-γ
IL-12
B
IgG
Th1-type cytokines
IFN-γ
Activated APC
IL-12
CD4+ Th0
CD4+ Th1

FIG. 7

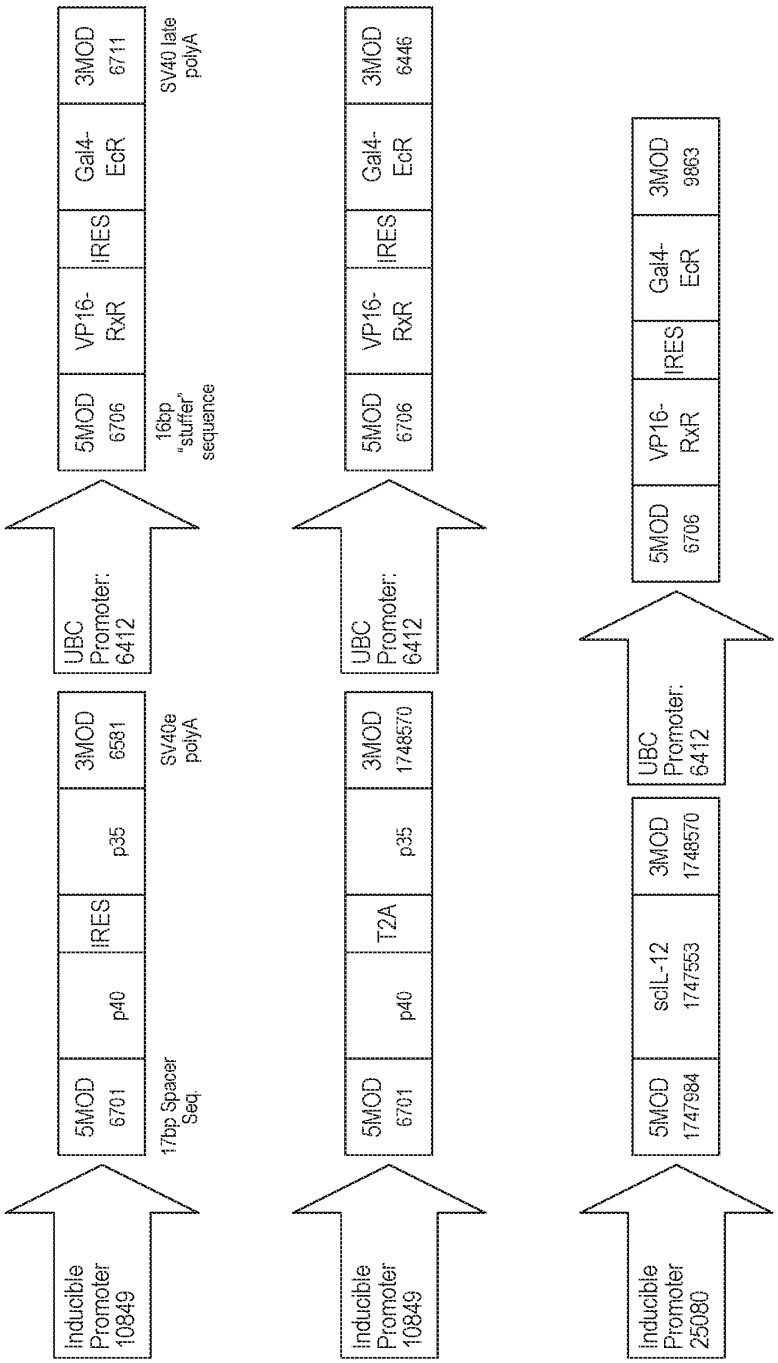

Inducible Promoter 10849
5MOD 6701
p40
IRES
p35
3MOD 6581
17bp Spacer Seq
SV40e polyA
UBC Promoter: 6412
5MOD 6706
VP16-RxR
IRES
Gal4-EcR
3MOD 6711
16bp "stuffer" sequence
SV40 late polyA
Inducible Promoter 10849
5MOD 6701
p40
T2A
p35
3MOD 1748570
UBC Promoter: 6412
5MOD 6706
VP16-RxR
IRES
Gal4-EcR
3MOD 6446
Inducible Promoter 25080
5MOD 1747984
scIL-12 1747553
3MOD 1748570
UBC Promoter: 6412
5MOD 6706
VP16-RxR
IRES
Gal4-EcR
3MOD 9863

FIG. 8

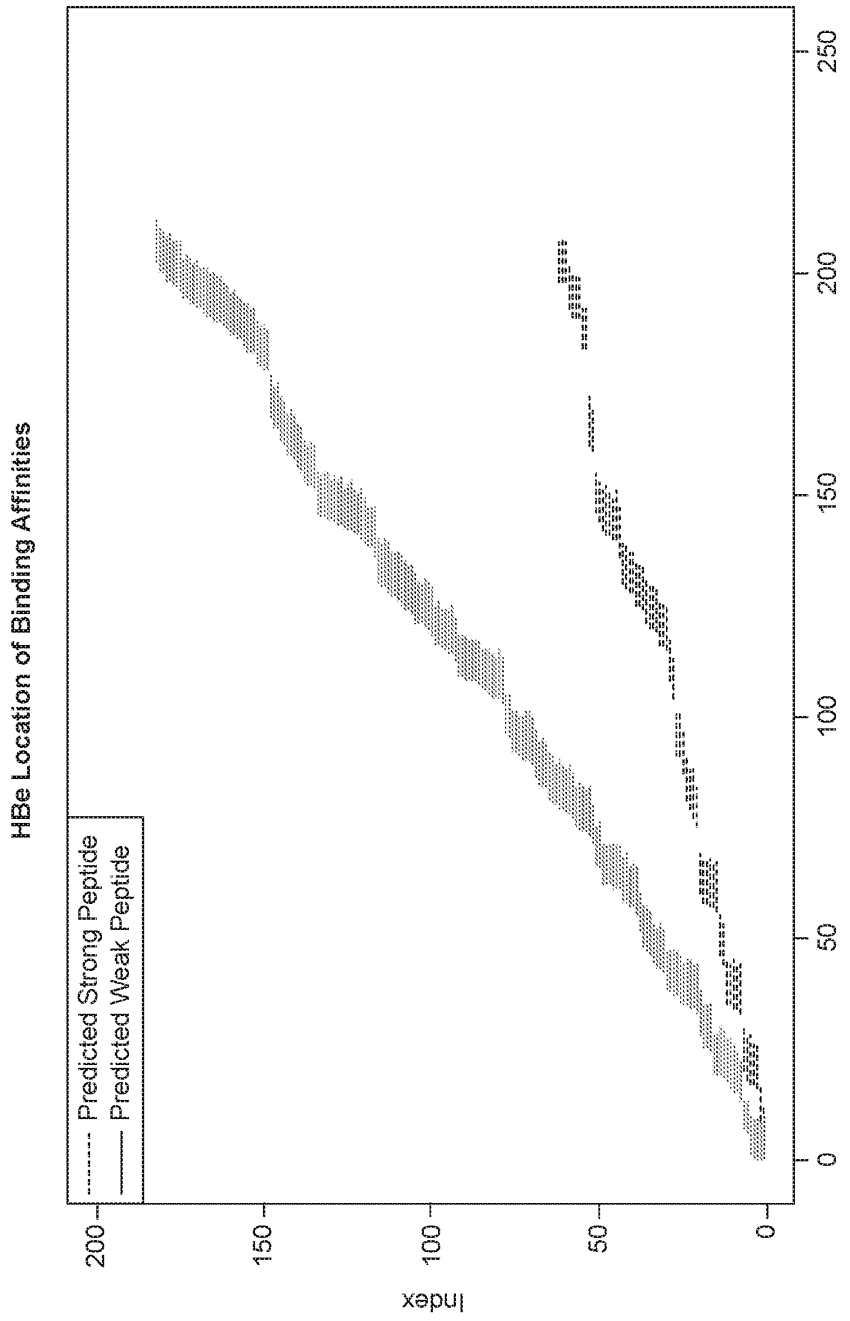

HBe Location of Binding Affinities
Predicted Strong Peptide
Predicted Weak Peptide
Index
Position
0
50
100
150
200
250

FIG. 9A

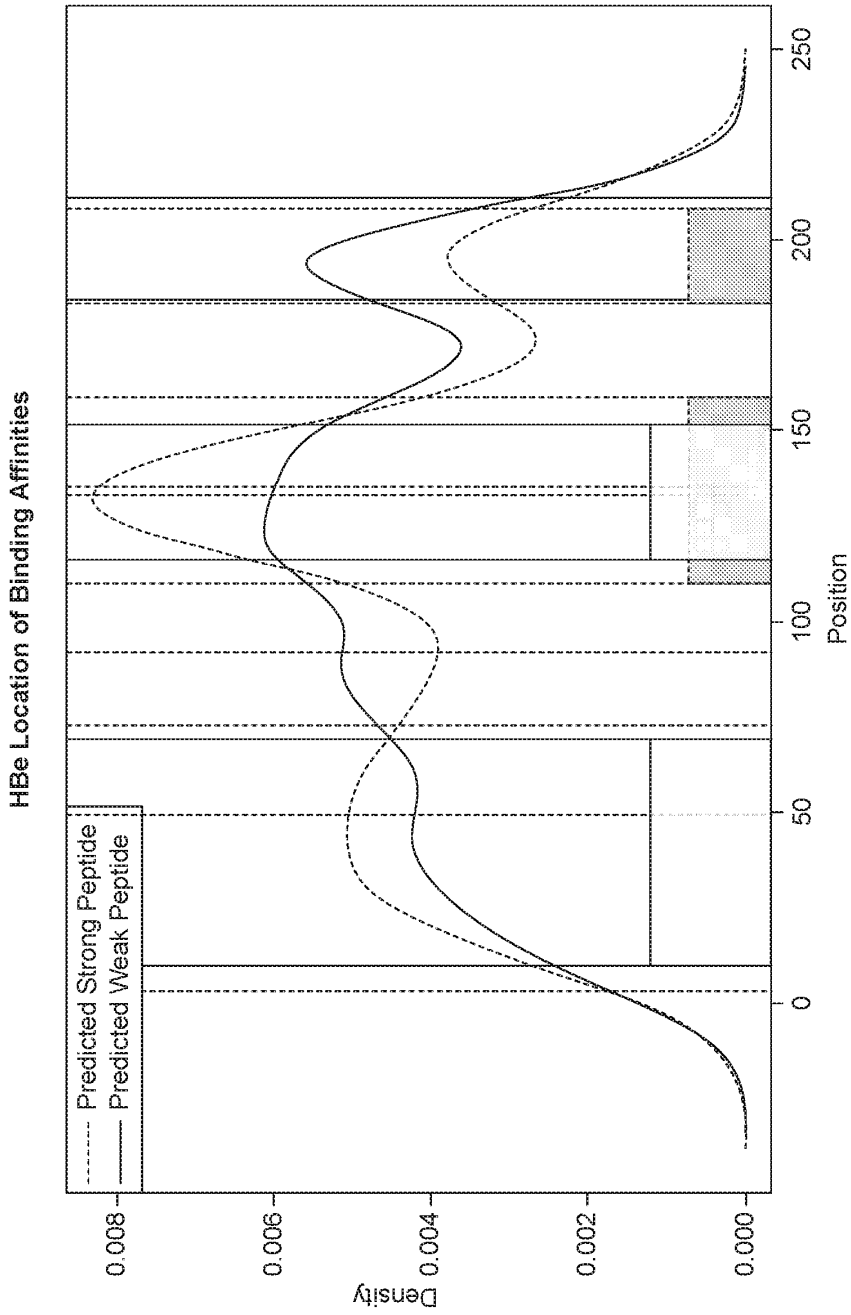

HBe Location of Binding Affinities
Predicted Strong Peptide
Predicted Weak Peptide
Density
0.000
0.002
0.004
0.006
0.008
0
50
100
150
200
250
Position

FIG. 9B

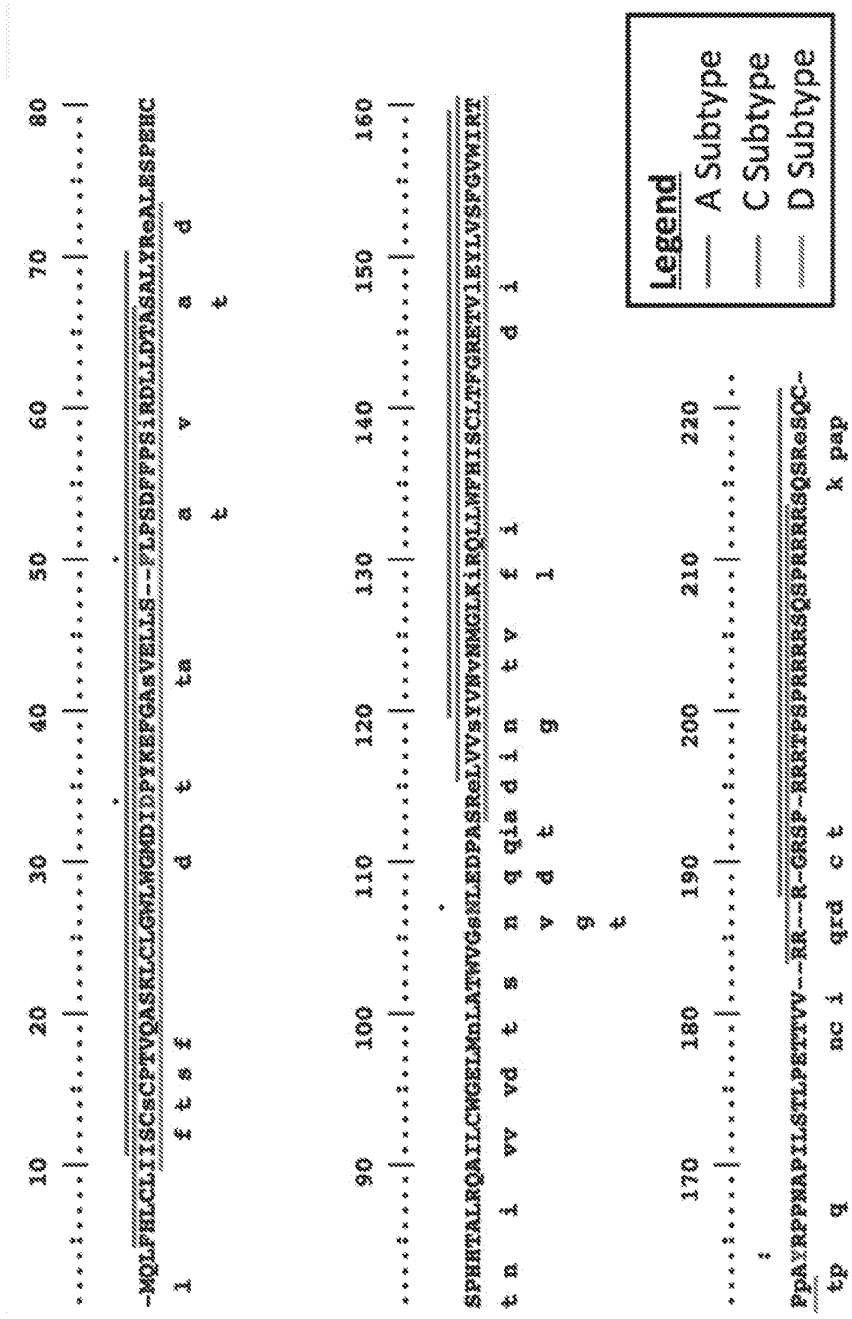


FIG. 9C

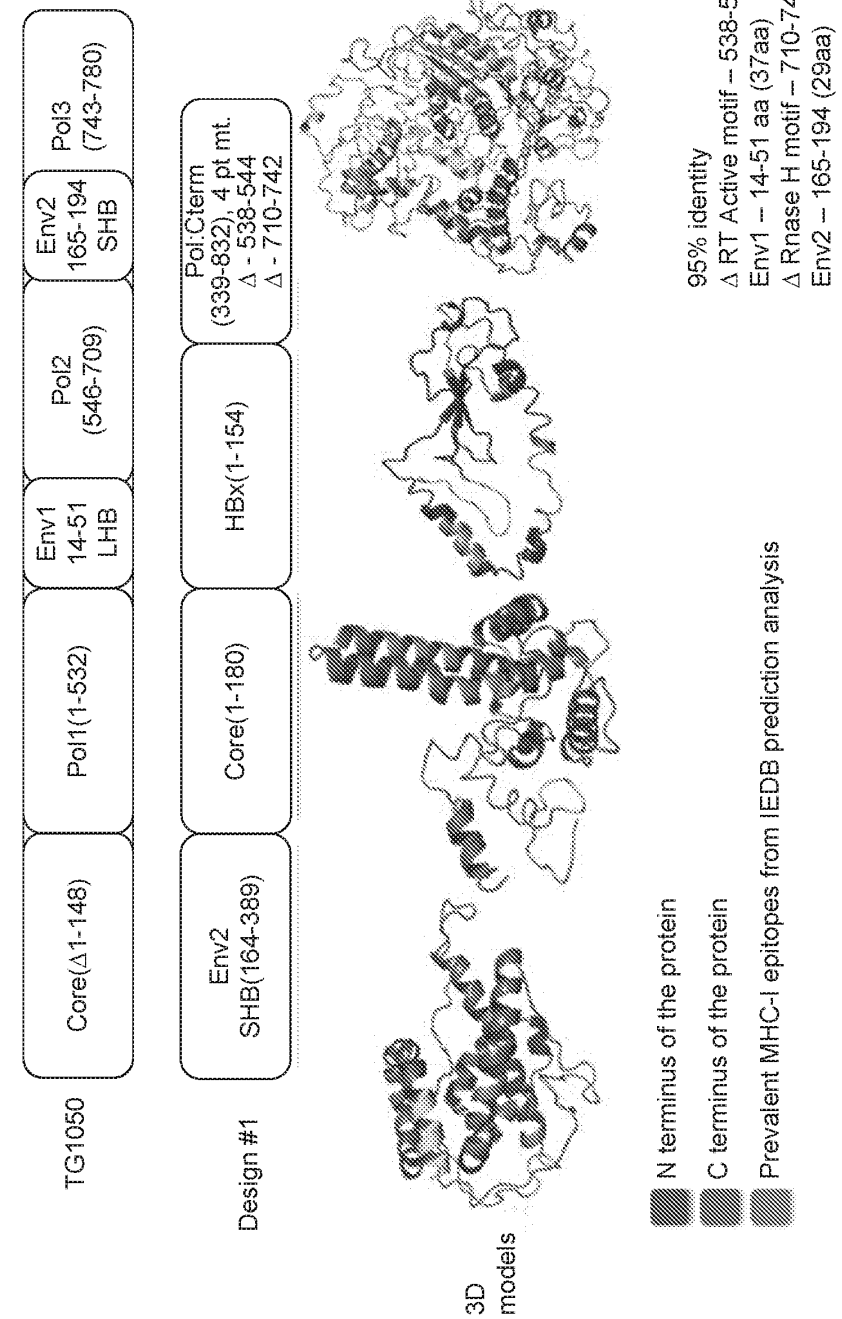
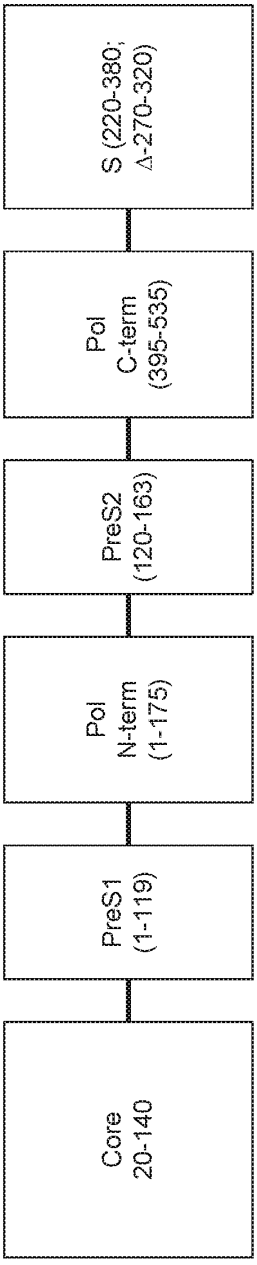

TG1050
Core(Δ1-148)
Pol1(1-532)
Env1 14-51 LHB
Pol2 (546-709)
Env2 165-194 SHB
Pol3 (743-780)
Design #1
Env2 SHB(164-389)
Core(1-180)
HBx(1-154)
Pol:Cterm (339-832), 4 pt mt. Δ - 538-544 Δ - 710-742
3D models
N terminus of the protein
C terminus of the protein
Prevalent MHC-I epitopes from IEDB prediction analysis
95% identity
Δ RT Active motif – 538-544 (6aa)
Env1 – 14-51 aa (37aa)
Δ Rnase H motif – 710-742 (32 aa)
Env2 – 165-194 (29aa)

FIG. 10



Linkers

HBx-1	VLHKRTLGL
HBx-2	LPKVLHKRTL
HBx-3	HKRTLGLSAM
HBx-4	QFLPKVLHKR
HBx-5	TVNAHQFLPK
HBx-6	LSAMSTTDL

FIG. 11

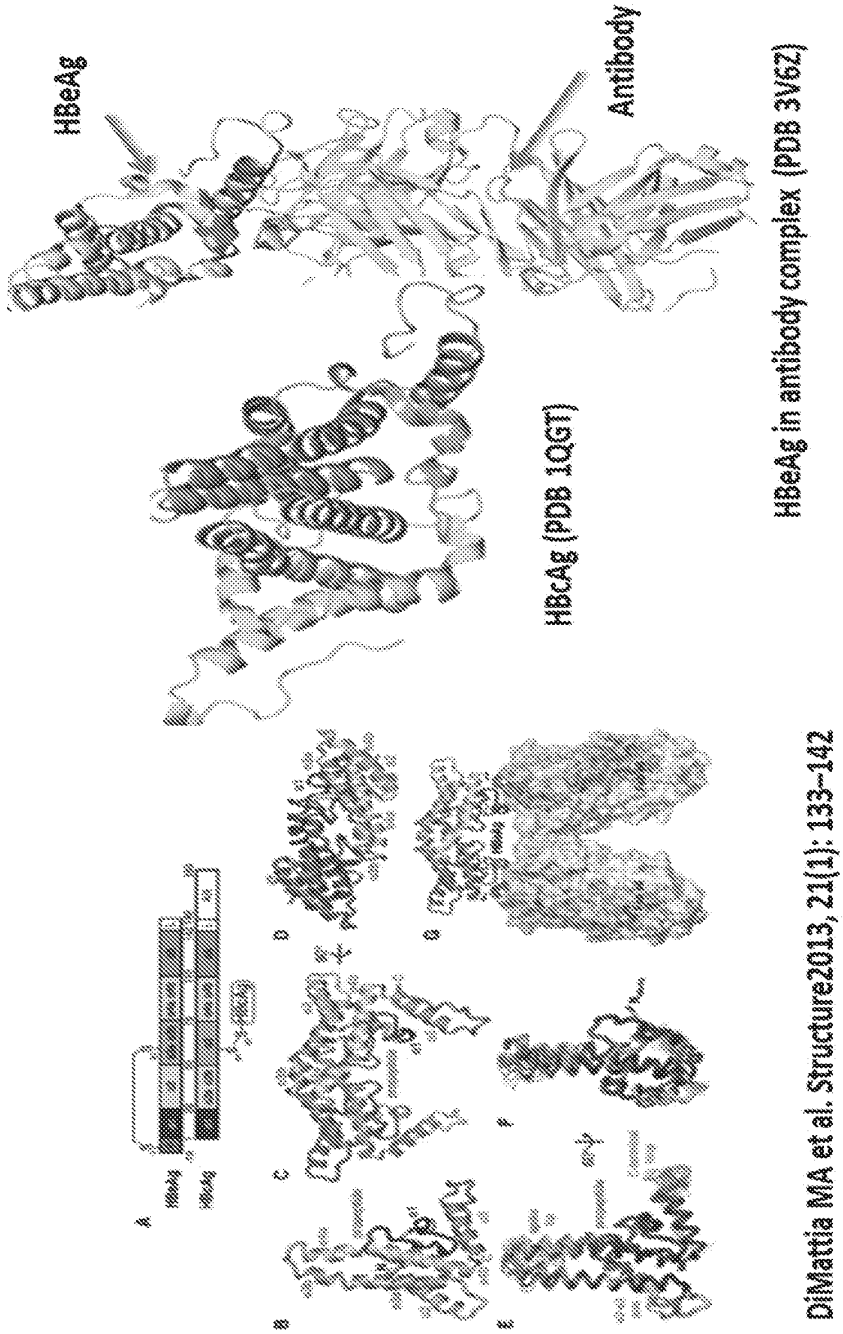

HBeAg
Antibody
HBcAg (PDB 1QGT)
HBeAg in antibody complex (PDB 3V6Z)
DiMattia MA et al. Structure2013, 21(1): 133–142

FIG. 12A

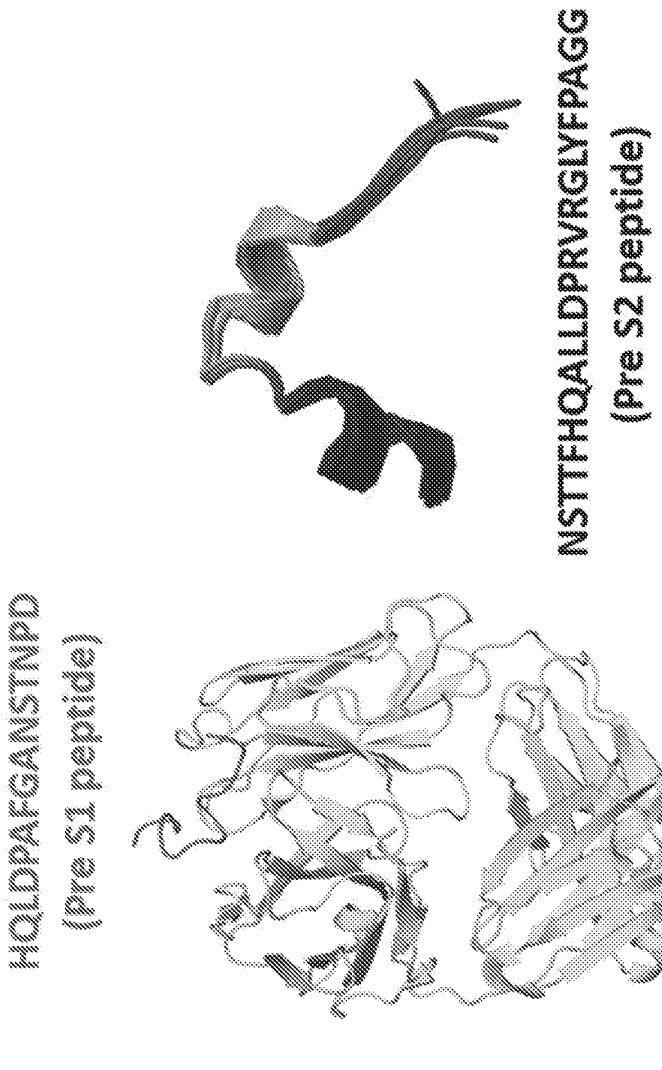
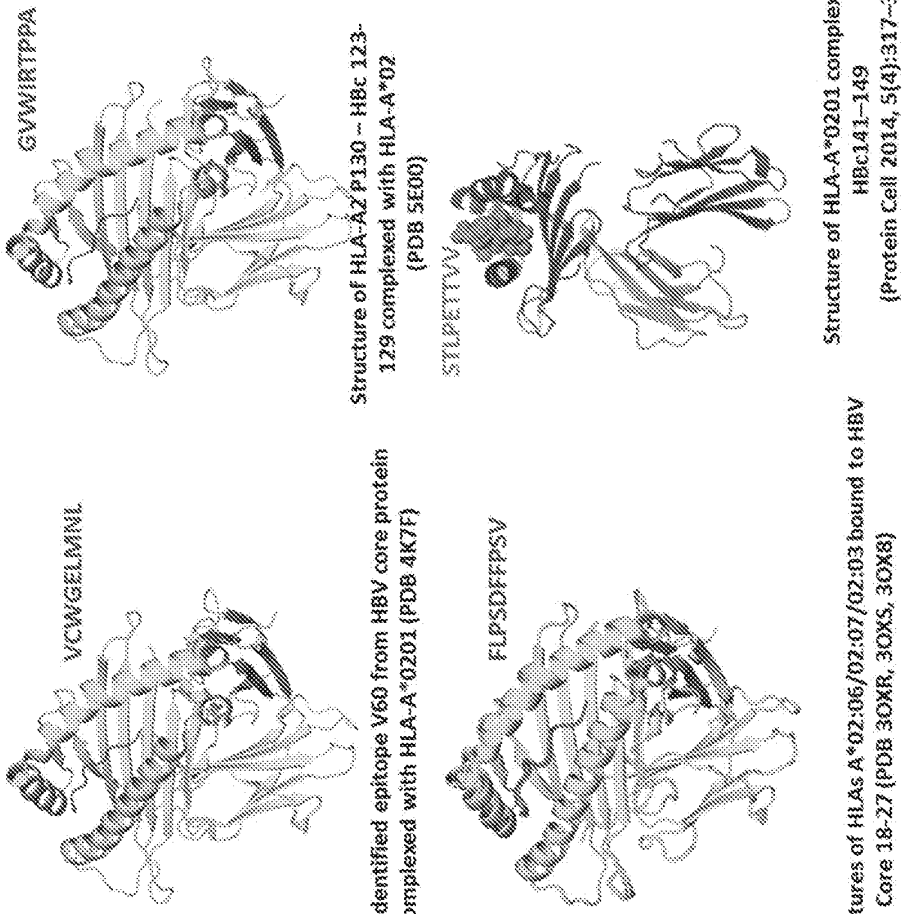

HQLDPAFGANSTNPD
(Pre S1 peptide)
NSTTFHQALLDPRVRGLYFPAGG
(Pre S2 peptide)
Antibody

FIG. 12B

Bcl-2 in complex with HBx-BH3 motif (PDB 3FCG)

DDB1 in Complex with the H-Box Motif of HBX (PDB 3I7H)

FIG. 12C



Newly identified epitope V60 from HBV core protein complexed with HLA-A*0201 (PDB 4K7F)

Structure of HLA-A2 P130 – HBc 123-129 complexed with HLA-A*02 (PDB 5E00)

Crystal structures of HLAs A*02:06/02:07/02:03 bound to HBV Core 18-27 (PDB 3OXR, 3OXS, 3OX8)

Structure of HLA-A*0201 complexed with HBc141–149 (Protein Cell 2014, 5(4):317–327)

FIG. 12D

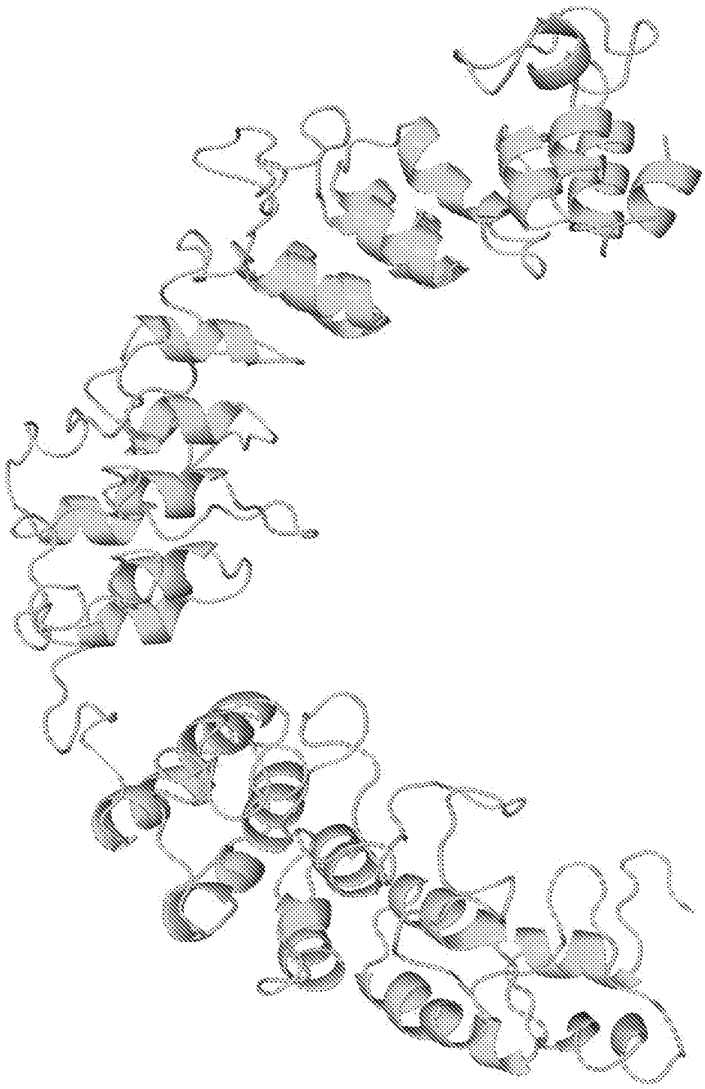


FIG. 13

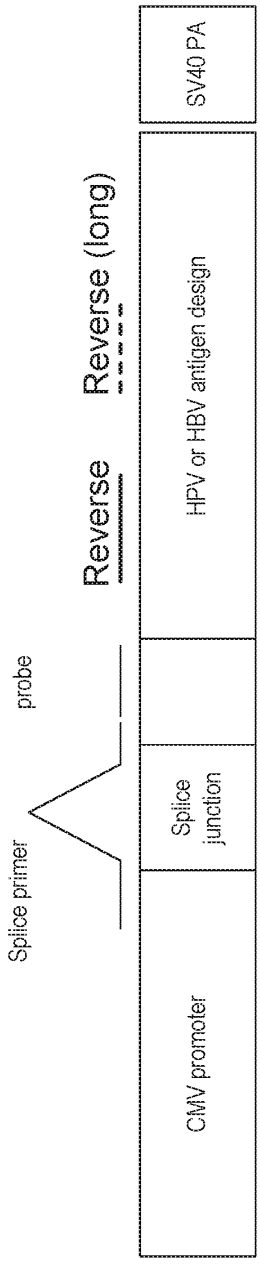

Splice primer
probe
Reverse
Reverse (long)
CMV promoter
Splice junction
HPV or HBV antigen design
SV40 PA

FIG. 14

```
TG1050 (MOD-1755595) HBV D Core   MDIDPYKEFGASVELLSFLPSDFFPSVRDLLDTATALYREALESPEHCTPHHTALRhvcL
TG1050 GB  Y07587.1               MDIDPYKEFGATVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRqaiL

TG1050 (MOD-1755595) HBV D Core   CWGELMnLATWVGtNLEDqaSRDLVVSYVNTNMGLKFRQLLWFHISCLTFGRDtVLEYLV
TG1050 GB  Y07587.1               CWGELMTLATWVGgNLEDpiSRDLVVSYVNTNMGLKFRQLLWFHISCLTFGREtVIEYLV

TG1050 (MOD-1755595) HBV D Core   SFGVWIRTPPAYRPsNAPILSTLPETTVmplsyqhfrrlllddeagpleeelprladeg
TG1050 GB  Y07587.1               SFGVWIRTPPAYRPpNAPILSTLPETTV----------------------------------

TG1050 (MOD-1755595) HBV D Core   lnrrvaedlnlgnlnvsipwthkvgnftglysstvpvfnphwktpsfpnihlhqdiikkc
TG1050 GB  Y07587.1               ----------------------------------------------------------------

TG1050 (MOD-1755595) HBV D Core   eqfvgpltvnekrrlqlimparfypnvtkylpldkgikpyypehlvnhyfqtrhylhtlw
TG1050 GB  Y07587.1               ----------------------------------------------------------------

TG1050 (MOD-1755595) HBV D Core   kagilykretthsasfcgspyswegklqhgaesfhqqssgilsrppvgsslqskhrksrl
TG1050 GB  Y07587.1               ----------------------------------------------------------------
```

FIG. 15A

Core 1-148AA
POL1
POL1
POL2
ENV1 14-51 AA
ENV2 165-194
Mutants
POL3
▼ Polymerase deletions – 538 – 544aa, 710-742aa

FIG. 15B

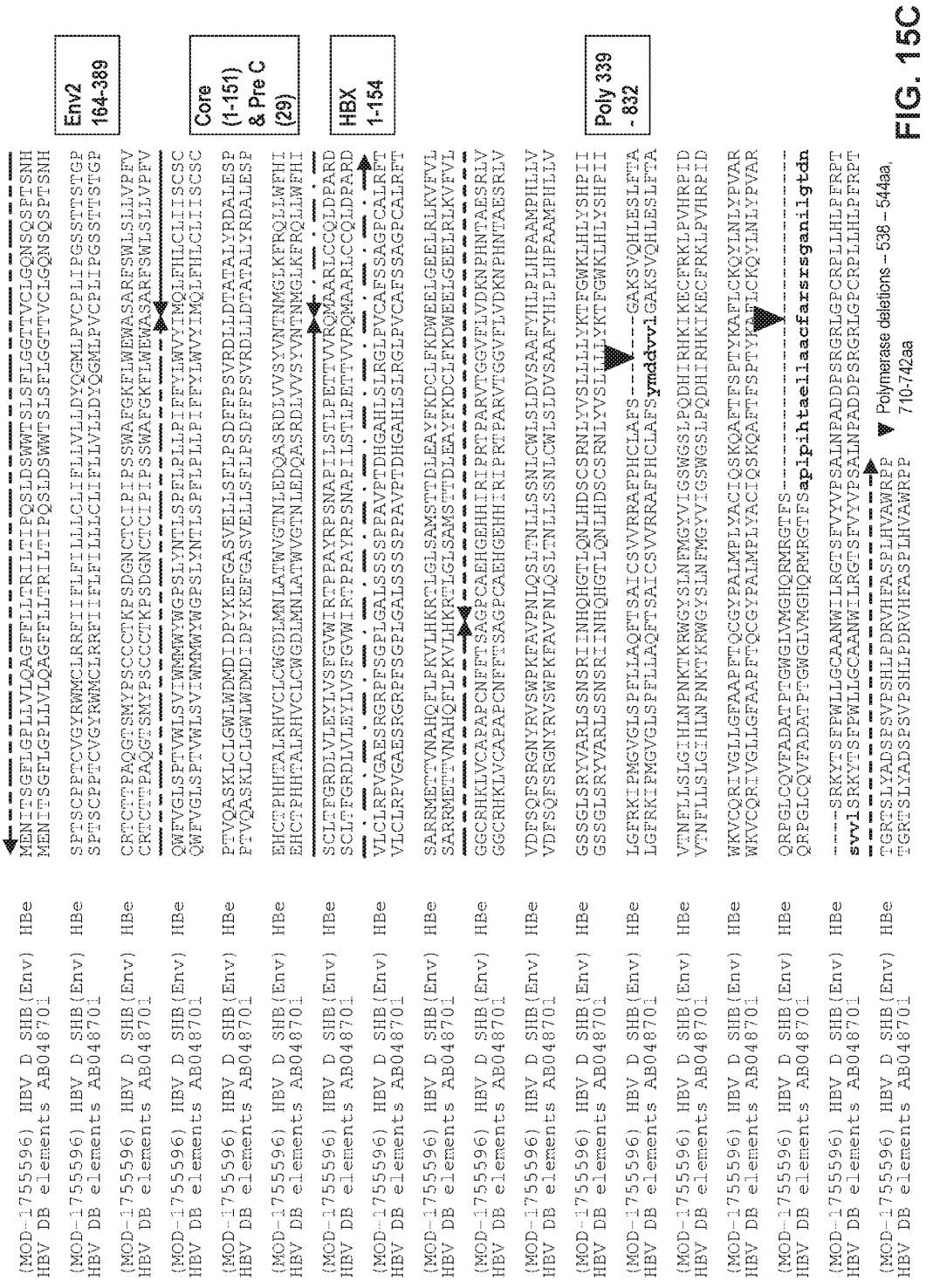

Env2
164-389
Core
(1-151)
& Pre C
(29)
HBX
1-154
Poly 339
- 832
(MOD-1755596) HBV D SHB(Env) HBe
HBV DB elements AB048701
Polymerase deletions – 538 – 544aa,
710-742aa

FIG. 15C

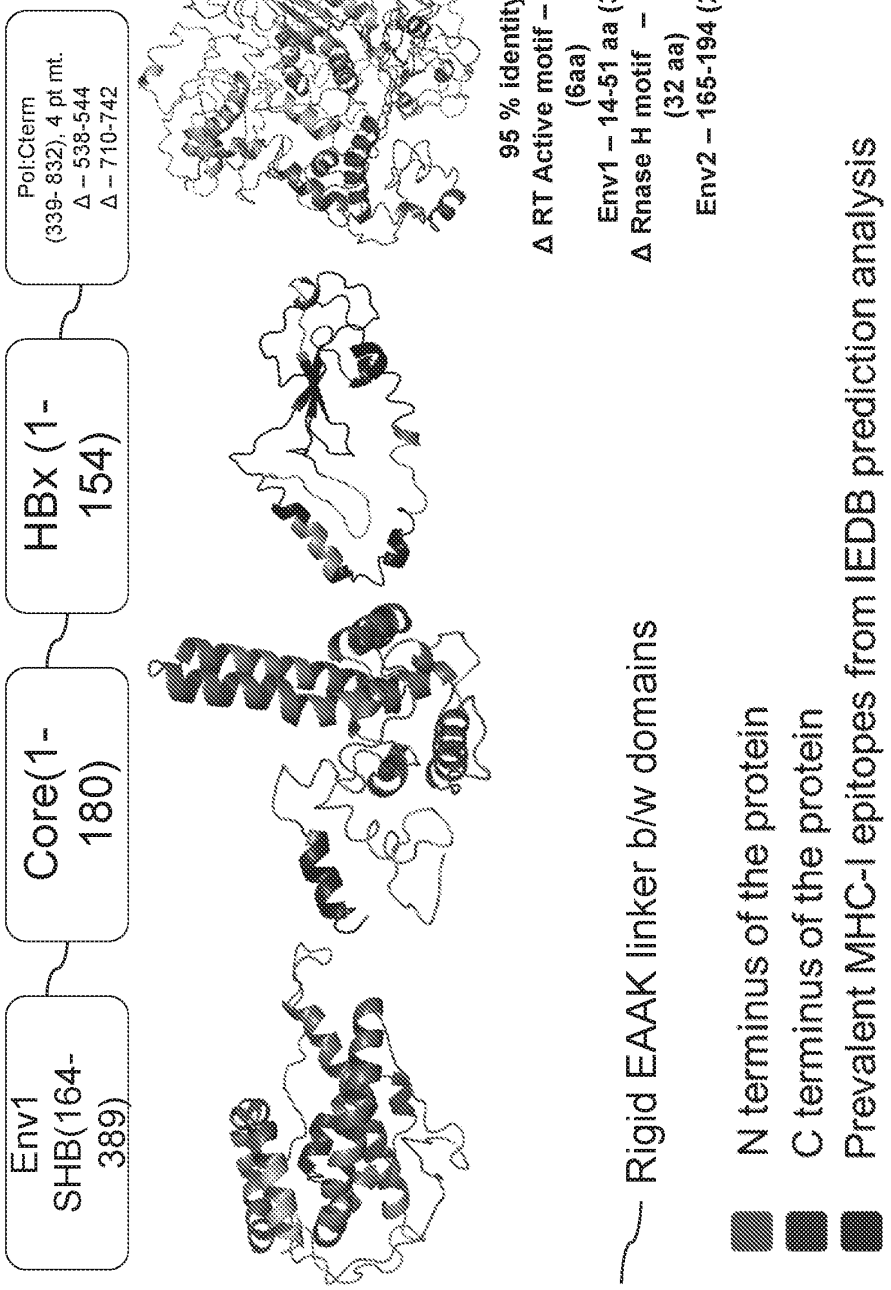

Env1 SHB(164-389)
Core(1-180)
HBx (1-154)
Pol:Cterm (339- 832), 4 pt mt. Δ – 538-544 Δ – 710-742
95 % identity
Δ RT Active motif – 538-544 (6aa)
Env1 – 14-51 aa (37 aa)
Δ Rnase H motif – 710-742) (32 aa)
Env2 – 165-194 (29aa)
Rigid EAAK linker b/w domains
N terminus of the protein
C terminus of the protein
Prevalent MHC-I epitopes from IEDB prediction analysis

FIG. 16