Filovirus vaccine design and rationale

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Vaccines for Marburg and Ebola Viruses

Simplified, the Overarching Challenges in Vaccine Development are Proofs of:

1. Safety

and

2. Efficacy

… in humans

Complex Interactions Determine Outcome of Viral Infection

sensitive dependence on initial conditions

Host

innate immunity, resistance
germline immune system
acquired immunity
health, nutritional status
behavior

VACCINE

Virus

receptors, tropisms
subversion of host defenses
replication, spread
variability, mutability
coat stability

Environment

crowding, sanitation
toxic/debilitating factors
transmission barriers
dose, route of exposure
inactivation of agent
disease vectors

Figure 1A

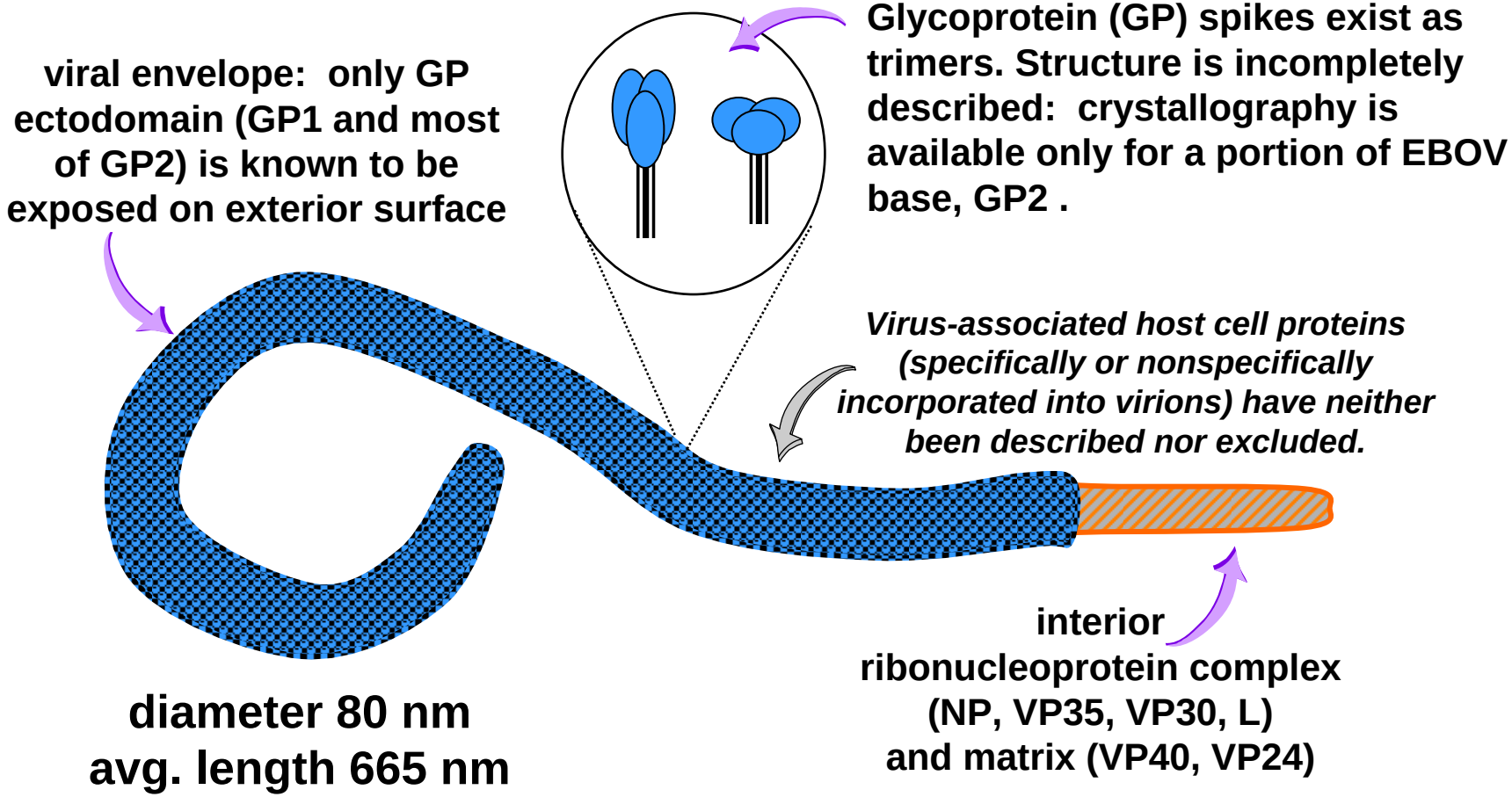
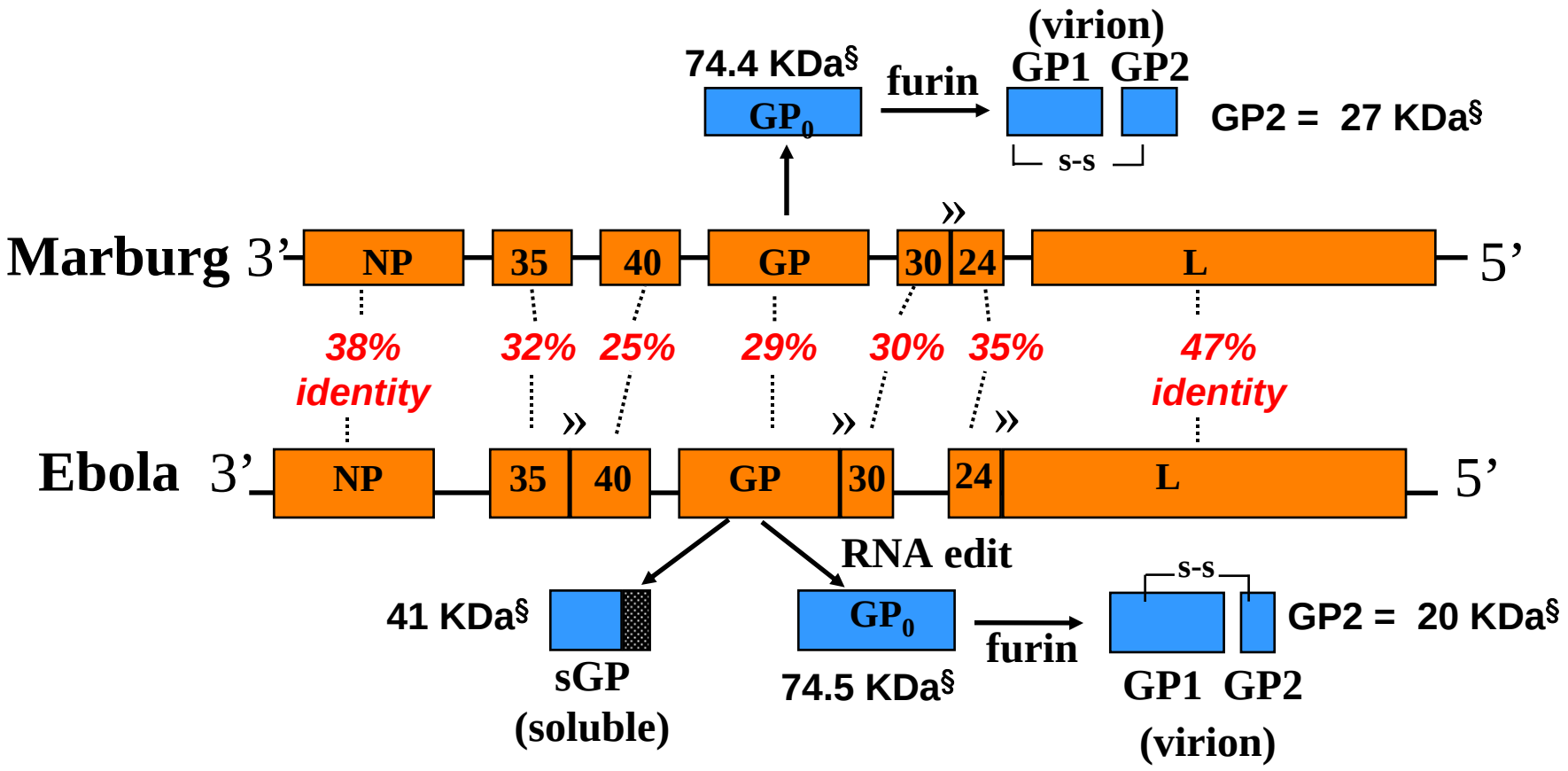

viral envelope: only GP ectodomain (GP1 and most of GP2) is known to be exposed on exterior surface
Glycoprotein (GP) spikes exist as trimers. Structure is incompletely described: crystallography is available only for a portion of EBOV base, GP2 .
Virus-associated host cell proteins (specifically or nonspecifically incorporated into virions) have neither been described nor excluded.
interior ribonucleoprotein complex (NP, VP35, VP30, L) and matrix (VP40, VP24)
diameter 80 nm
avg. length 665 nm

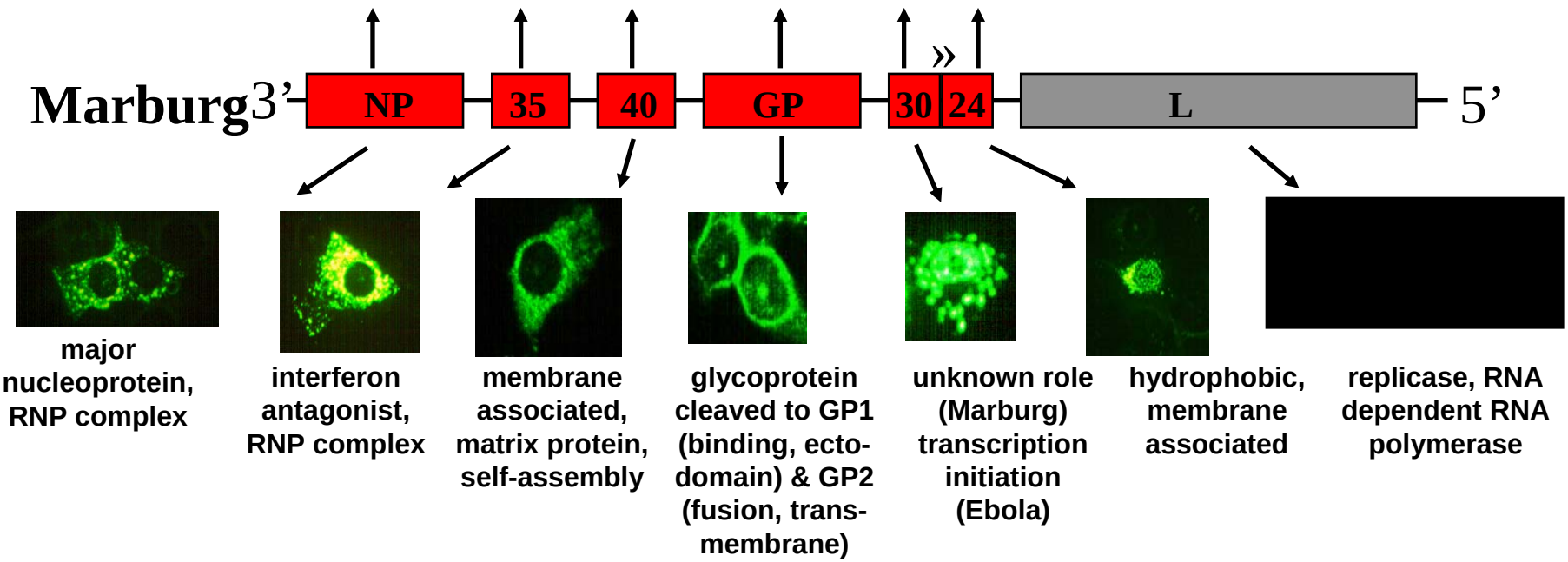
Figure 1B


74.4 KDa§
GP0
furin
(virion)
GP1 GP2
GP2 = 27 KDa§
s-s
Marburg 3'
NP
35
40
GP
30
24
L
5'
38% identity
32%
25%
29%
30%
35%
47% identity
Ebola 3'
NP
35
40
GP
30
24
L
5'
RNA edit
41 KDa§
sGP (soluble)
GP0
74.5 KDa§
furin
s-s
GP2 = 20 KDa§
GP1 GP2
(virion)

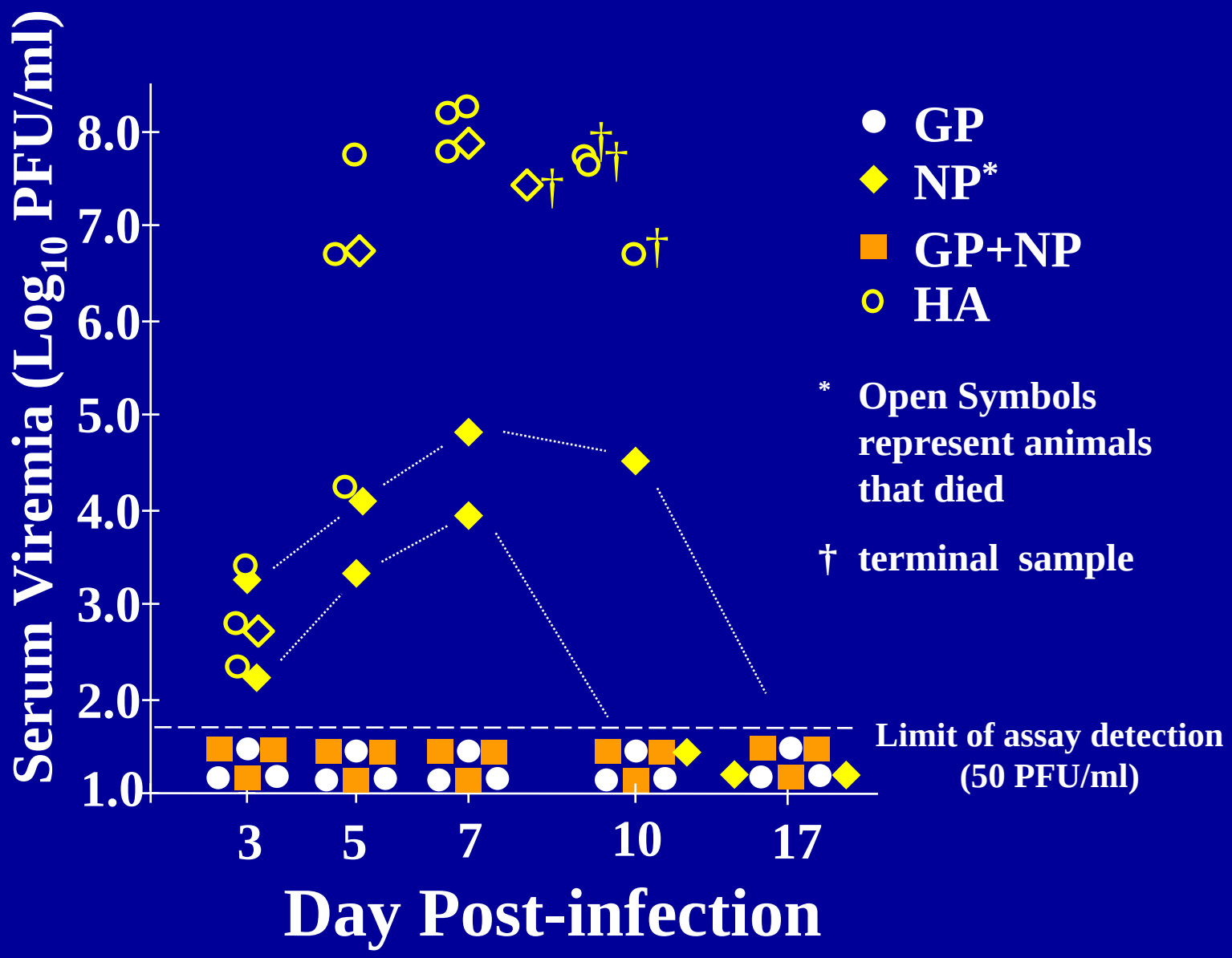
Candidate Antigens for Marburg Virus Vaccine

Evaluation in a Guinea Pig Model, Replicon-based Immunization

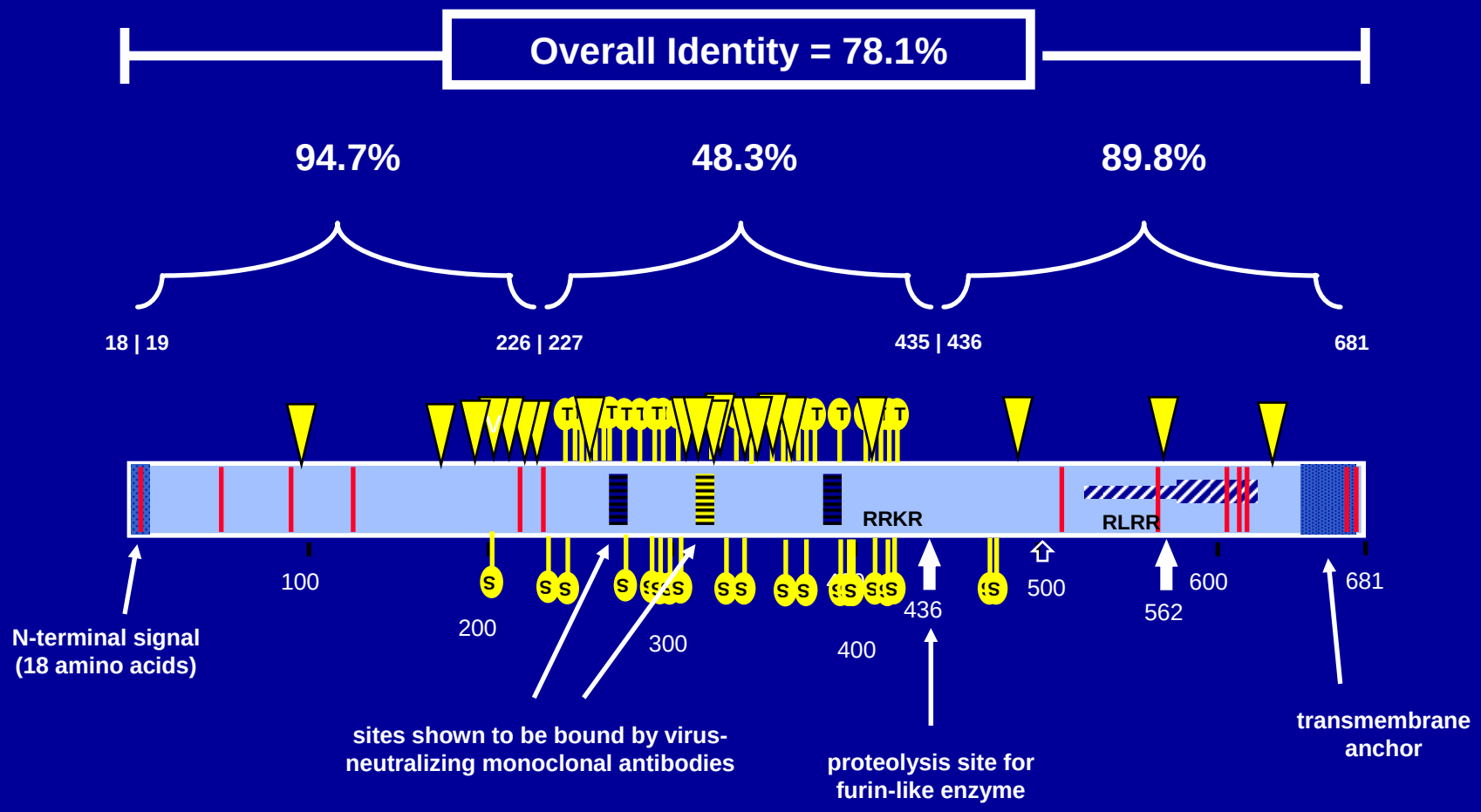
Guinea Pigs	Survivors/Total						Challenge (control S/T)
Strain 2	0/6	3/5	...	4/5	...	...	MBG-Musoke (0/6)
Hartley	4/6	1/6	...	11/11	...	...	MBG-Musoke (0/15)
Strain 13	16/18	13/18	1/6	18/18	0/6	1/6	MBG-Musoke (2/24)



Viremias in Replicon-Vaccinated Cynomolgus Macaques



MARBURG VIRUS GLYCOPROTEINS:
Amino Acid Identities between Musoke and Ravn



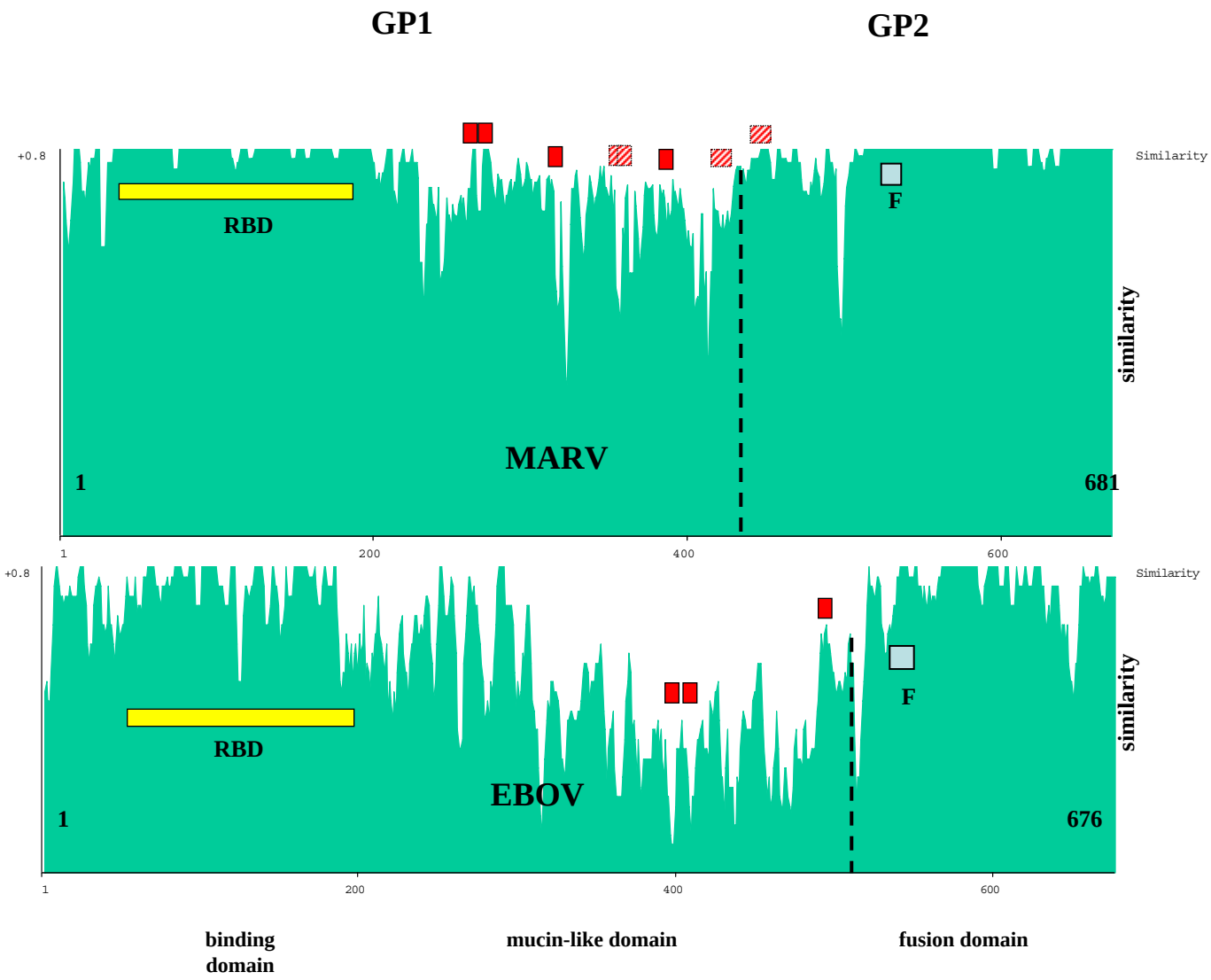
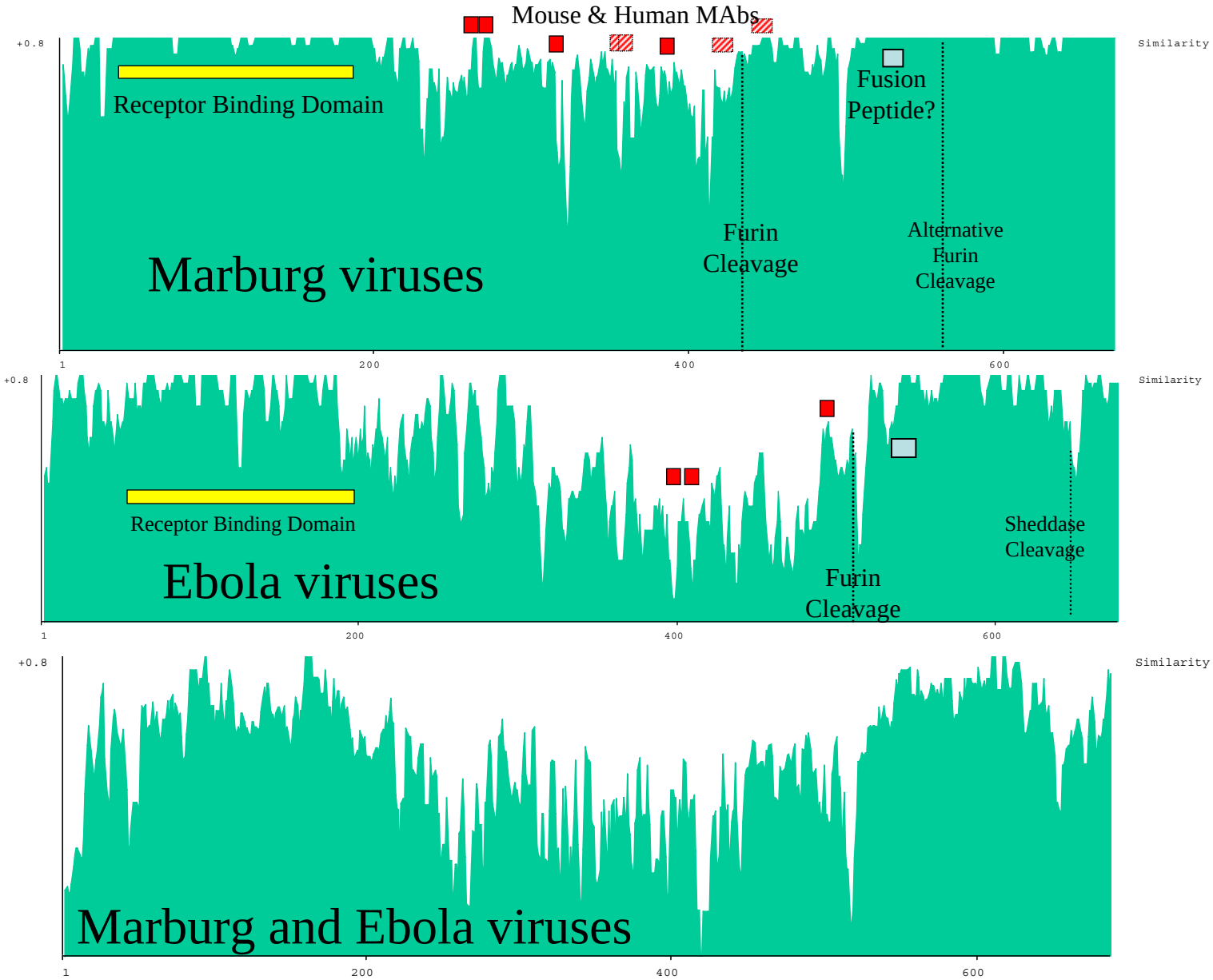

GP1
GP2
+0.8
Similarity
RBD
F
similarity
MARV
1
681
1
200
400
600
+0.8
Similarity
RBD
F
similarity
EBOV
1
676
1
200
400
600
binding domain
mucin-like domain
fusion domain

Figure 3

Similarity Plots - Filovirus Glycoproteins



Y2K Toolbox: Approaches to Viral Vaccines

EMPIRICAL/CLASSICAL ← → **RATIONAL/AVANT-GARDE**

naturally attenuated isolate

attenuate by passage & selection

random mutagenesis

inactivated virus

subunit/split vaccine

genome site-directed mutagenesis

defined reassortants

attenuated hybrids, chimeras

live vectored vaccines

Replication-defective vectors:
(RNA replicon, naked DNA, Adeno, Poxvirus)

purified expression product

peptides & chimeric Ags

anti-idiotype/pseudoantigen

REPLICATING ↑

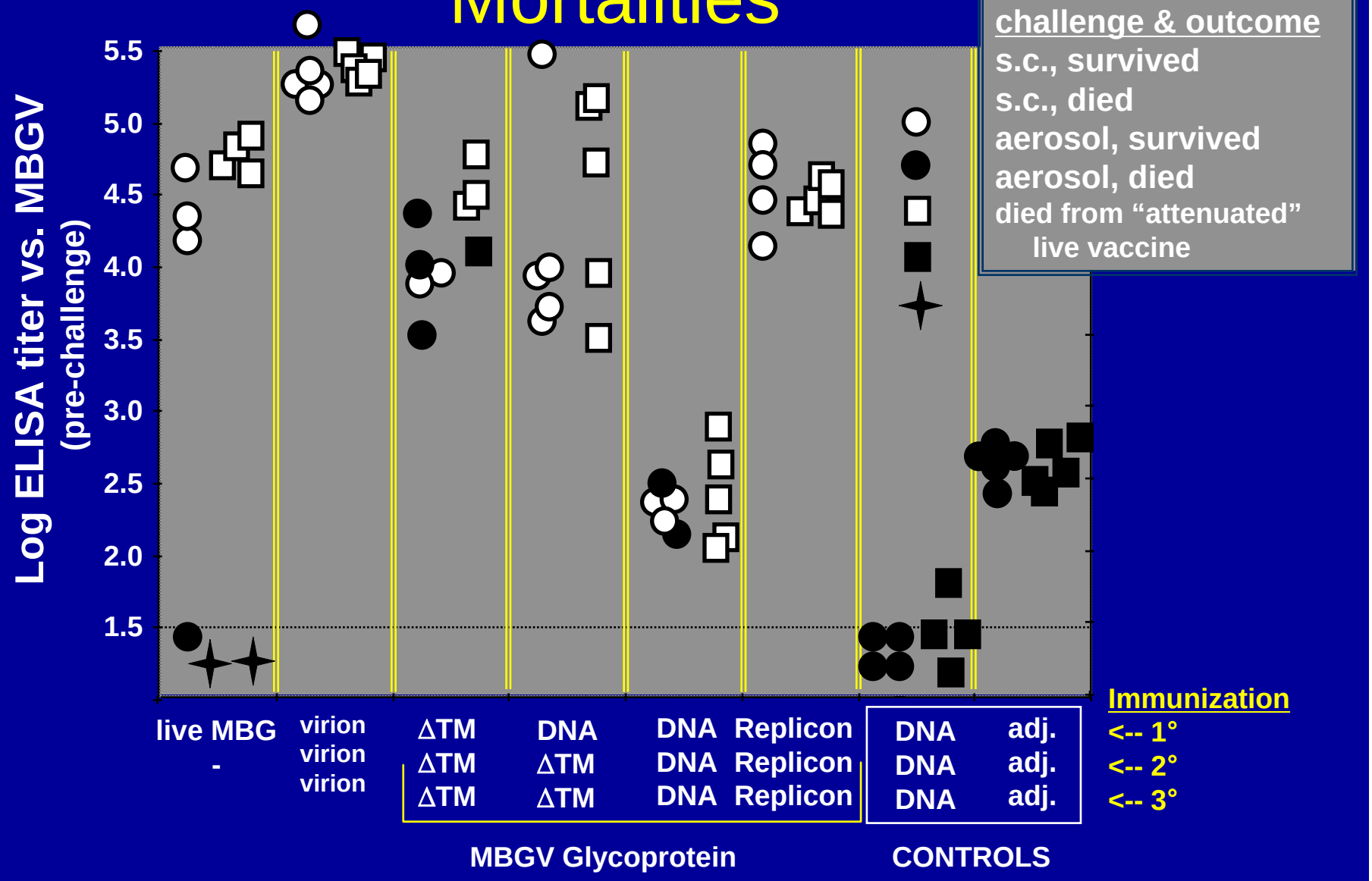
NON-REPLICATING ↓

GENERIC IMPROVEMENTS

oral/skin delivery **timed-release/microencapsulation**

classical adjuvants **immunomodulators** **cytokines**

Serum ELISA Titers and Mortalities



Marburg and Ebola Virus Vaccines containing Glycoprotein (GP): the Evolving Menu of Options (2007)

Vaccine type	Comments	Principal Concerns
killed filovirus	Early vaccine efforts and recent proofs of concept; inadequate efficacy[a] in NHP	safety[b]; potency[c]; observed disease exacerbation
live attenuated filovirus	Only as proofs of concept with natural or passaged viruses; high risk could theoretically be mitigated by reverse genetics approach	live vaccine safety; incomplete attenuation or reversion
live vaccinia vectored	Proof of concept, deprioritized along with other live pox-vectored vaccines	vaccinia safety; vector immunity[d]; potency
expressed protein, bacuolvirus	Incomplete efficacy in guinea pigs, no reported efficacy in NHP	potency, adjuvant requirement; altered glycosylation
defective VEE replicon	Excellent rodent efficacy, first demo of NHP efficacy against MARV, potency of 10^8 IU[f] near "tipping point" in NHP	vector immunity; safety at doses high enough to achieve potency
DNA	Adequate in rodents; incomplete NHP efficacy with MARV and none reported with EBOV; touted for immunological priming	potency
defective adenovirus	Excellent rodent and NHP efficacy at high doses (e.g. 10^{10} IU). First demo of NHP efficacy and single-shot efficacy with EBOV in NHP	vector immunity, safety at doses high enough to achieve potency
virus-like particles	Good rodent efficacy, no robust efficacy yet reported with NHP	potency; adjuvant requirement
live recombinant vesicular stomatitis virus, VSV	Excellent rodent and NHP efficacy with both MARV and EBOV. Single shot vaccine, rapid immunity. No overt illness from live vaccine itself. In recombinant, filovirus GP replaces VSV GP	live vaccine; balance of safety & potency; environmental release[e]
live recombinant parainfluenza	Good efficacy against Ebola in guinea pigs and NHP, contains both parainfluenza and EBOV glycoproteins	live vaccine; balance of safety & potency; environmental release

a. Here, vaccine efficacy for filoviruses is operationally defined by prevention of disease in a susceptible animal inoculated with an amount of virus (usually 10^2 to 10^3 PFU $\approx LD_{50}$) associated with ordinary exposure. b. Safety concerns may arise in manufacture itself (as with killed viruses) but more often refer to anticipated difficulties in achieving cGMP manufacture suitable for initial human trials and then an expectation of adequate safety in large trials and diverse populations. c. Potency is interpreted as the capacity to elicit a protective immune response as defined by some quantitative assay that correlates with protection. d. For live and replication-defective recombinant vaccines, vector immunity refers to pre-existing immunity to the vaccine vector, which can arise naturally or by successive vaccination with the same vector and acts to diminish the effective dose and thus potency of vaccine delivered. e. Environmental release is an additional concern for novel replicating vaccines, requiring assessment of possible consequences of vaccine transmission to persons or other animals including arthropods. f. IU refers to infectious units for replication-defective vectors that infect cells without further spread.

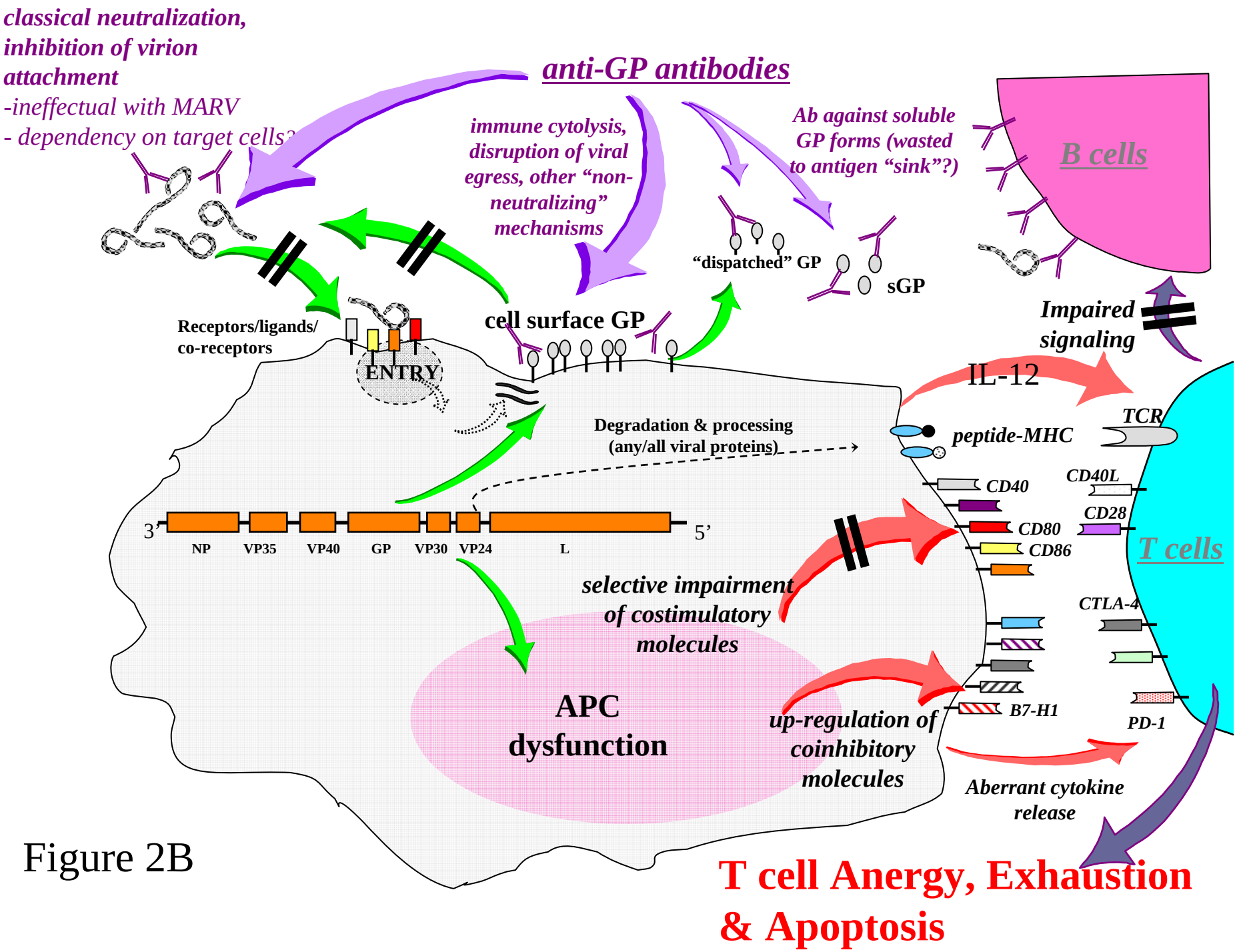
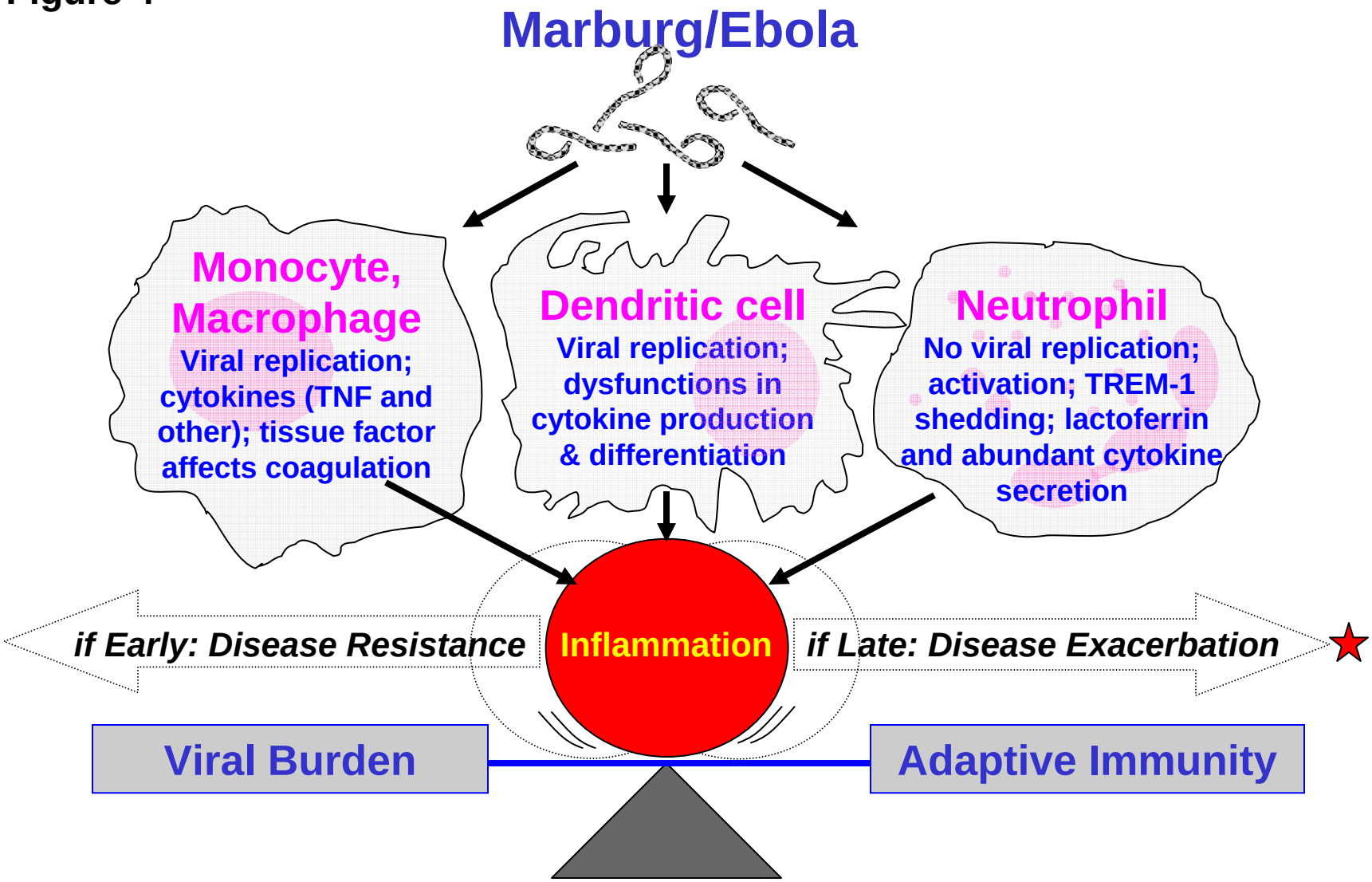

classical neutralization, inhibition of virion attachment
-ineffectual with MARV
- dependency on target cells?
anti-GP antibodies
immune cytolysis, disruption of viral egress, other "non-neutralizing" mechanisms
Ab against soluble GP forms (wasted to antigen "sink"?)
B cells
"dispatched" GP
sGP
Receptors/ligands/ co-receptors
ENTRY
cell surface GP
Impaired signaling
IL-12
Degradation & processing (any/all viral proteins)
peptide-MHC
TCR
CD40
CD40L
CD28
CD80
CD86
T cells
3'
5'
NP
VP35
VP40
GP
VP30
VP24
L
selective impairment of costimulatory molecules
CTLA-4
APC dysfunction
up-regulation of coinhibitory molecules
B7-H1
PD-1
Aberrant cytokine release
T cell Anergy, Exhaustion & Apoptosis

Figure 2B

Evasion and deception strategies available to filovirus glycoprotein

Observation	Comments	Immune consequences
antigenic variation	Variability between isolates and species is concentrated in the central ('mucin-like') portion of glycoprotein, which, after cleavage to GP1–GP2 becomes the distal aspect of the glycoprotein spike	Negligible cross-reactivities of antibodies and no cross-protection between viral species, limited cross-reactivities of antibodies among disparate isolates of MARV
abundant N- and O-linked glycosylation	Concentrated in mucin-like domain; variations in glycosylation, some due to cell type	Epitope masking; phenotypic variation; viral tropism for cells having lectin-like receptors (e.g. DCs and monocytes)
glycoprotein shedding	Failure of disulfide-bond formation results in GP1 shedding; cell proteases cause an alternative form of shedding of GP1–GP2 ectodomain	Soluble antigens compete for antibodies otherwise effective against viruses or infected cells
glycoprotein gene editing	Observed in EBOV, not MARV; genome encodes mRNA for a truncated, frame-shifted version of glycoprotein (sGP)	Similar to glycoprotein shedding, but with added twists of truncation, frame-shift and different glycosylation of sGP
structural masking of receptor-binding and fusion domains	Key functional domains are proximal to membrane, exposed only transiently upon entry	Antibodies underrepresented and possibly ineffective against important functional regions
promiscuous binding to cells	glycoprotein apparently binds multiple cell receptors with varying efficiencies; filamentous viral structure might favour effectiveness of low-affinity interactions	Problematic for antibodies to block initial interactions between viruses and a variety of cells and receptors
triggering of cytokine release	glycoprotein on viral particles is sufficient to trigger intracellular cascades, granule release, cytokines	Disorienting milieu for initiation of balanced immune response
toxicity of glycoprotein for cells	Might be less of a problem *in vivo* than with *in vitro* expression systems	Could affect performance or manufacture of genetic vaccines
immunosuppressive domain	Tenuous observation, with importance still neither confirmed nor refuted	Remains a possible explanation for some dysregulations now attributed to whole glycoprotein

Figure 4



To what degree (if any) does partial immunity have capacity for harm?

Vaccine Development is (among other things):

rational: *[relating to, based on, or agreeable to reason]*

empirical: *[relying on experience or observation alone often without due regard for system and theory; capable of being verified or disproved by observation or experiment]*

iterative: *[relating to or being a computational procedure in which replication of a cycle of operations produces results which approximate the desired result more and more closely]*

spiral: *[of or relating to the advancement to higher levels through a series of cyclical movements]*

Recommendations

1. Humility: do not pretend we know more than we know
2. Critical thinking: believe quality data, not someone else's optimism or bias
3. Prudence: define and manage risks, do not put all eggs in one basket
4. Realism: requires public funding of Phase 1 trials
5. Plain speech: do not be muzzled by political expediency and "realpolitik"

Recent Reviews, Meta-analyses

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Schmaljohn, A.L. and M. Hevey (2004) Chapter 11, Medical Countermeasures for Filoviruses and Other Viral Agents, in *Biological Weapons Defense: Principles and Mechanisms for Infectious Diseases Counter-Bioterrorism.* (Humana Press) L. Lindler, F. Lebeda, and G. Korch, Editors, Humana Press, Totowa, N.