Tricking the Trickster: How to Kill Any Virus

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Viruses are nature's ultimate cheaters. A tiny strip of genetic material can trick our cells into molecular servitude. Yet they have one potentially fatal weakness that can be exploited…

A virus's two essentials: Copying and packaging

A virus is a molecular self-replicating machine that consists of a nucleic acid containing the blueprint, and a protective shell around it that is capable of introducing this nucleic acid into a host cell. The virus then takes possession of the cell's metabolism to build first a system for copying its nucleic acid, then new shells into which the copies are packaged. When the progeny thus formed are released, the cycle continues.

Thus, despite the astonishing variety of viral shapes and structures, merely two elements are essential: The copying mechanism and the shell. Large viruses may comprise hundreds of additional genes, but these play only supporting roles, e.g. warding off the host's immune system. All the resources lacking in the viral genome are stolen from the host cell, such as the biochemistry for producing precursors of nucleic acids and proteins.

Currently only two weapons: Vaccines and inhibitors

This simplicity makes viruses harder to target than bacteria – both for the immune system and for drugs. As viruses "have less self" than cells do, there is less there to attack. At the same time, this enables extremely high reproduction rates, which in turn permit viruses to tolerate high mutation rates, resulting in high adaptability.

Some viruses have quirks in their machinery that can be exploited – e.g., the copying system of many herpesviruses can be disabled by the drug acyclovir –, but most simply do not have enough "self" for antiviral therapy to flourish in analogy to antibiotic therapy. Thus, today's antiviral medicine is mostly preventive, relying strongly on vaccination. However, many viruses have mechanisms for defeating the immune system, and the high mutation rates of many viruses further limit immunological approaches (many viruses "drift" and "shift" structurally until the vaccine is obsolete). Moreover, from distribution logistics to side effect profiles, preventive drugs are more demanding than curative ones are.

There is thus an **unmet need** for a general curative treatment approach that can be adapted across viral taxa, applied only where actually required, and quickly tuned to mutant strains.

The needle-in-the-haystack problem: A closer look at the two core functions

The main problem a virus faces upon entry into a cell is quantitative, in that its nucleic acid is vastly outnumbered by the cellular nucleic acid ("needle in the haystack"). Thus, the virus must be able to **copy and package its own nucleic acid selectively** – whereas copying and packaging the host cell's nucleic acid would at best be a waste of scarce resources and in the worst case destroy the host before completion of the viral cycle.

As early as 1965, Haruna & Spiegelman identified small portions of the genomes of bacteriophages Qβ and MS-2 that were specifically detected by each phage's copying system (replicase). A fragment of Qβ containing its recognition sequence became known as "Spiegelman's Monster" due to its ability to out-replicate the native viral nucleic acid. This work was later expanded by many researchers, and today we know recognition sequences from a few hundred base pairs in some DNA viruses down to hexanucleotides in citrivirus (Vives &al. 2002).

While viruses exhibit a variety of architectures, most vertebrate-hosted viruses use a single linear RNA strand with such a recognition sequence and its mirror image at the ends, whereby the replicase produces first a mirrored copy (complementary strand) and then a mirrored copy of the mirrored copy.

The same holds true for packaging; in his medical doctoral thesis, one of us demonstrated that in polyomaviruses the replication and packaging signal sequences overlap to form a block of about 190 base pairs sufficient for replication and, within the size limits of the shell, packaging (Flaig 1997).

Stealing back from the thief: Mimicking of hallmark sequences

We now propose a **nucleic acid** comprising

- an **imitation of a replicase recognition sequence** (or a pair thereof as described above),
- optionally also a **packaging signal sequence**,
- and/or optionally an antiviral effector

as a basis for a curative antiviral treatment.

The idea is to **turn the virus's own functions against it**.

As demonstrated by Spiegelman's Monster, the replicase recognition sequence will enable the curative nucleic acid to out-compete the longer viral nucleic acid (what we call **stolen recognition**), ideally already suppressing viral replication. In the case of RNA viruses, this may also cause an interferon response before the virus has a chance to suppress it.

Similar phenomena have been described but the implications not fully understood by other authors, e.g. Zhong &al. 1992, identifying "defective RNAs" of FHV that appear in about 1% of host cells and make them resistant to the virus.

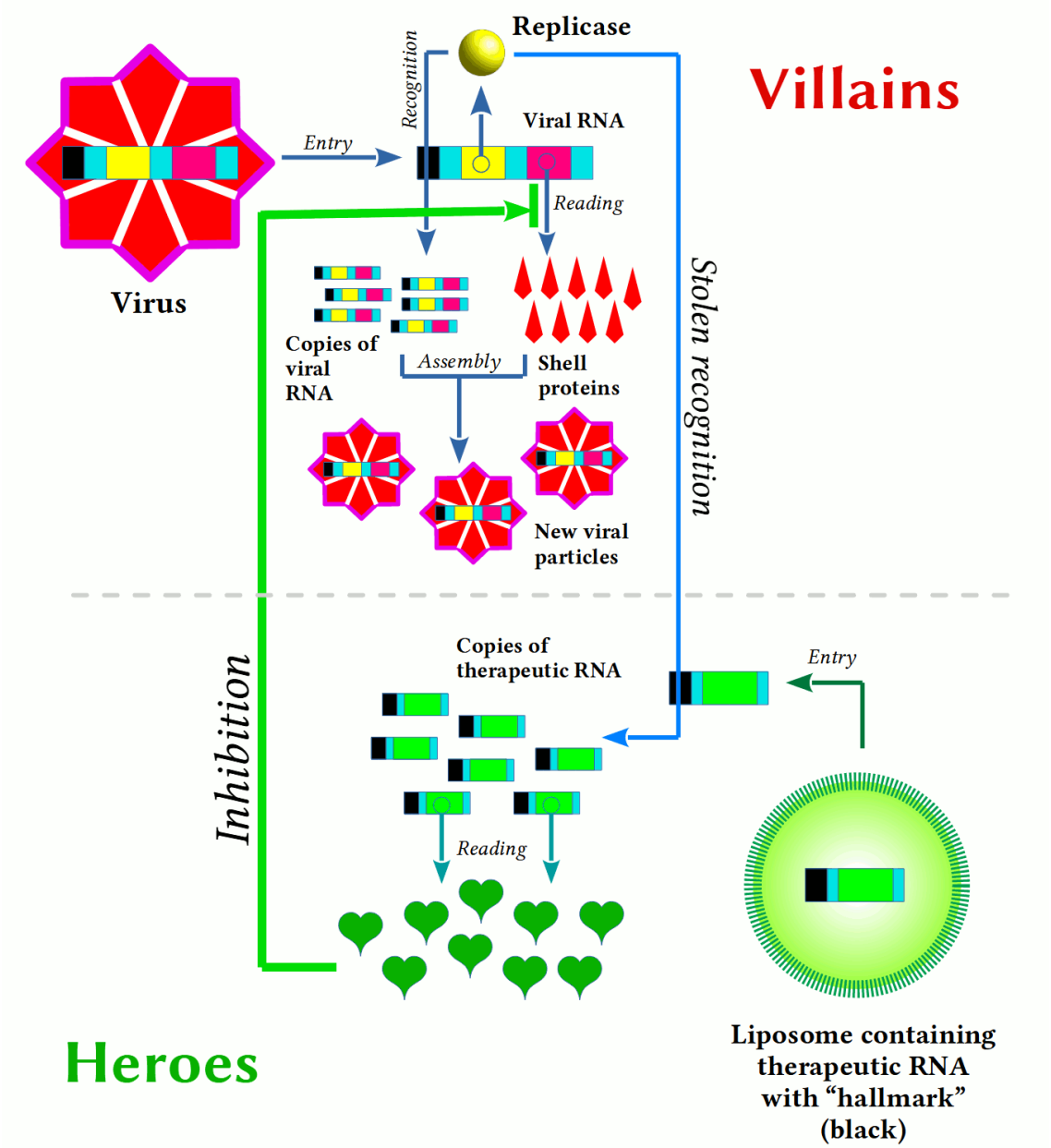
A packaging recognition sequence will allow the curative nucleic acid to effectively compete with the viral nucleic acid for shells – thereby either preventing formation of infectious progeny or, ideally, even commandeering the new viral shells and spreading the protection through the organism. Intracellular accumulation of incompletely assembled shells will also facilitate raising of an immunological alarm.

While these two elements alone can be expected to have strong antiviral effect (supported by our in silico studies), this can be enhanced by including something harmful to the virus. Keep in mind that the **hallmarked constructs become active only when copied by the viral system**. For RNA viruses, effectors can be easily presented in complementary-sequence form, ensuring that only the virus-made mirror copies can be translated.

Thus, we can use a wide range of aggressive effectors:

- antisense structures of all kind (it is just fine if an interferon response is triggered in the process),
- immunomodulators (including, counter-intuitively, presentation suppressors to induce killing by NK cells),
- degrading enzymes (RNAses and proteases) of low specificity,
- release inhibitors (membrane modulators),
- apoptosis inducers,
- immunological tags to be included into the new viral shells in order to alert the immune system,
- and many others...

Tit for tat – the virus steals resources from the cell, we steal replicase from the virus to hit back.



*EXEMPLARY EMBODIMENT: The **upper part** depicts the natural life cycle of an RNA virus as described – the viral RNA encodes replicase and shell, the replicase is produced and copies (starting at the hallmark) the RNA, which is then packaged into the shells –, while the **lower part** illustrates its disruption by administration of a curative RNA comprising the hallmark and an effector.*

Rules of engagement: Applications from agriculture to medicine

The diversity of viral designs leads to a corresponding diversity of possible implementations.

Agriculture: A straightforward yet economically important application will be to equip crops with hallmarked constructs to confer resistance to specific viruses – offering the additional benefit that the GM crops can be designed in such a way that their proteome remains unaltered, which will be politically advantageous.

Medicine: Here, nanoparticle-based transport systems are an obvious choice, as discussed in Flaig's pharmaceutical Ph.D. thesis (2001). For treatment of the many important viruses that enter via the airways, conventional liposome suspensions may be administered by inhalation. As the effect is amplified by the virus itself, the quantitative aspects of such administration can be expected to be secondary. Ideally, reaching a small

number of target cells will be enough to generate a reaction that uncloaks the virus to the immune system, which can then do the clearing.

Compared to prevention by vaccination, such curative treatment confers a distinct second-mover advantage. It is not only that much smaller numbers of people will need to be treated – the virus has less opportunity to develop "escape mutations." And even if it does, the curative treatment can be adapted quickly. Thus, where it comes to emergent diseases, the benefits are evident.

There are further ramifications conceivable, such as replicase-based test kits, which will however not be discussed here for the time being.

Now and then: Current status and perspectives

Feasibility of the approach is suggested by established scientific knowledge and our own wet lab work, and supported by simulations and "sudden making sense" of incidental findings in the literature. Unfortunately, here maverick spirit meets a culture obsessed with vindication of existing knowledge, and like Flaig's previous research this approach has so far been assiduously ignored by the mainstream.

Keep in mind that this is not another vaccine, nor a drug in the conventional sense. This is a conceptual shift – a platform approach applicable across viral taxa, from phages and coronaviruses right up to Ebola, beyond the ken of the "bandwagoners."

We have filed patents and drafted a research plan that progresses incrementally, from a "toolkit" in model organisms through full demonstration of feasibility to the R&D pipeline proper, and are now looking for a partner with reach *and* the wisdom and courage to venture into currently uncharted territory. If you are ready to rethink antivirals, let us talk!