Drug repurposing research offers new hope in the fight against RSV

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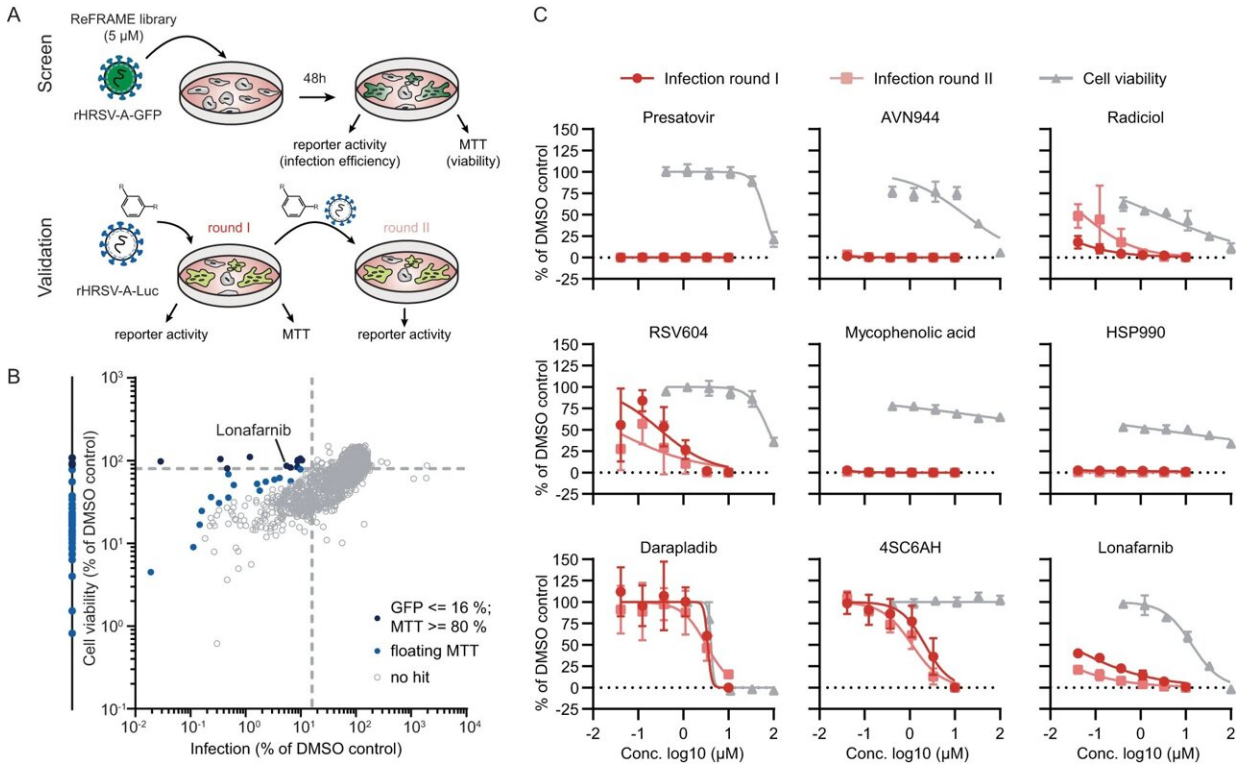

Identification of drug repurposing candidates. **A** Screening and validation procedure. **B** HEp-2 cells were infected with rHRSV-A-GFP[29] in presence of 5 µM compound. 48 hours later, infection and cell viability were quantified via GFP and MTT readouts. Dotted lines indicate primary hit criteria, and dots represent means of two technical replicates. **C** HEp-2 cells were infected with HRSV-A-Luc[29] at MOI 0.01 and treated with the indicated compound concentrations. 24 hours later, supernatant was transferred onto new cells for a second round of infection. Luminescence was quantified 24 hours post-inoculation of both infection rounds. Cell viability was measured via MTT

readout in treated but uninfected cells. Mean ± SD of three independent experiments. Known RSV inhibitors (F protein: presatovir; N protein: RSV604, IMPDH inhibitors (AVN944, mycophenolic acid), HSP90 inhibitors (radiciol, HSP990). 4-Sulfocalix[6]arene Hydrate (4SC6AH, unknown target). Source data are provided as a Source Data file. Credit: *Nature Communications* (2024). DOI: 10.1038/s41467-024-45241-y

Every year in the winter months, there are waves of infection with RSV. In healthy adults and adolescents, the infection is usually harmless. Not so with small children: Around 1% of them who are exposed to the pathogen for the first time become so seriously ill that they have to be hospitalized.

It can also cause serious illness in adults over the age of 65 due to pre-existing heart or lung conditions. Vaccines have been authorized for older people and pregnant women since 2023, but there is currently no direct antiviral therapy against the RS virus.

In order to discover new active substances against certain pathogens, researchers search through large collections of already known and clinically tested substances. This process is known as a "drug repurposing screen" and examines additional application areas for already-known pharmaceuticals.

A team from the Institute of Experimental Virology at TWINCORE, Center for Experimental and Clinical Infection Research, in Hanover, led by Thomas Pietschmann, used this method to search the ReFRAME Library of the Scripps Research Institute (U.S.) for potential new RSV drugs. This substance bank contains around 12,000 active substances that are in clinical development or have already been approved.

"To screen the library, we used a so-called reporter virus that is labeled with the fluorescent protein GFP," says Pietschmann. "A lack of fluorescence reaction in this test indicates an antiviral effect." At the same time, all substances were also analyzed for their toxicity. Only those that do not have a cell-damaging effect are shortlisted.

The tests were carried out automatically using a pipetting robot in collaboration with the Institute of Virology at Hannover Medical School. "Otherwise, it is almost impossible to sift through a collection of several thousand substances," says Sibylle Haid, a scientist at the Institute of Experimental Virology and co-corresponding author of the study.

From an initial 21 remaining candidates, the scientists focused on the active substance lonafarnib, which is approved for treating Hutchinson-Gilford progeria syndrome. People affected by this rare genetic disease age prematurely and die earlier, on average, at 14.5 years of age.

"Lonafarnib inhibits a specific maturation step of proteins in the cell," says Haid. In order to characterize the mechanism of action against the RS virus more precisely, the researchers tested another farnesylation inhibitor called tipifarnib and compared the results. "Tipifarnib does not work against RSV," says Haid. "From this, we were able to conclude that the antiviral effect of lonafarnib is probably not based on the inhibition of farnesylation."

With the help of cooperation partners Anna Hirsch from the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) and Thomas Krey from the University of Lübeck, the team was able to elucidate the molecular structure of the virus-drug complex. Lonafarnib binds to the fusion protein of RSV and thus prevents the virus from fusing with the membrane of the target cell. As a result, no new cells can be infected.

In cooperation with colleagues in France, a reduction in viral load has

already been demonstrated in the mouse model. "However, the dose of lonafarnib required for oral administration is very high, so we have also observed side effects," says Pietschmann. "It is conceivable that local application, for example, by inhalation, could improve the ratio between effect and side effect. This must be carefully examined in follow-up studies."

"With lonafarnib, we have identified an interesting candidate for the treatment of RSV," says Svenja Sake, first author of the study. "Because the drug has already undergone all clinical trials, approval for the new indication would be much easier, cheaper, and faster than for a completely new active substance," she says.

"This study is also another great example of teamwork, as is usual in science," says study leader Pietschmann. "We are networked with many of the cooperation partners in the RESIST Cluster of Excellence, for example."

The work is published in the journal *Nature Communications*.

More information: Svenja M. Sake et al, Drug repurposing screen identifies lonafarnib as respiratory syncytial virus fusion protein inhibitor, *Nature Communications* (2024). DOI: 10.1038/s41467-024-45241-y

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