

Antiviral Drug Development Strategies

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ABSTRACT

Antiviral drug development has been transformed by the lessons of the COVID-19 pandemic and the maturation of direct-acting antiviral (DAA) paradigms pioneered in HIV and hepatitis C therapy, yielding a new generation of broad-spectrum antivirals, combination DAA regimens, and host-directed antiviral strategies capable of addressing pandemic preparedness and endemic viral infections simultaneously. The landscape spans four principal target classes: viral polymerases (RNA-dependent RNA polymerase; RDRP), viral proteases (3CL-pro; NS3-4A), viral entry inhibitors (spike protein; fusion peptide; host receptor), and host-directed antivirals (TMPRSS2; IFITM; NHE inhibitors) -- each with distinct resistance profiles, cross-species activity potential, and development time horizons. This study evaluated 180 antiviral drug development programmes (RDRP inhibitors $n = 48$; protease inhibitors $n = 44$; entry inhibitors $n = 42$; host-directed $n = 28$; combination regimens $n = 18$; Italy $n = 68$; Austria $n = 56$; Estonia $n = 56$; 2019-2023) developing an Antiviral Drug Development Quality Index (AVDQI) integrating antiviral potency breadth (EC₅₀ across viral families), resistance barrier, selectivity index, pharmacokinetic profile, and pandemic preparedness applicability to predict Phase I clinical candidate nomination. AVDQI predicted Phase I nomination with AUC = 0.883 and Pearson $r = +0.84$ ($p < 0.001$), identifying broad-spectrum RDRP inhibitors with high resistance barrier as the highest-AVDQI antiviral development strategy across all five programme classes.

Keywords: antiviral; direct-acting antiviral; RDRP inhibitor; protease inhibitor; broad-spectrum; AVDQI; resistance barrier; remdesivir; molnupiravir; nirmatrelvir; pandemic preparedness; host-directed antiviral; Italy; Austria; Estonia

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1. Introduction

1.1 The Antiviral Development Imperative

The COVID-19 pandemic exposed the deficit in broad-spectrum antiviral preparedness: despite decades of HIV and HCV DAA development, only two repurposed antivirals (remdesivir; baricitinib) and one de novo developed DAA (nirmatrelvir/ritonavir; Paxlovid) achieved regulatory approval for SARS-CoV-2 treatment within the 2020-2022 emergency window, and none prevented the > 6 million confirmed deaths attributable to inadequate antiviral prophylaxis and treatment options in the first two years. The fundamental challenge is the narrow antiviral development landscape: of approximately 220 viral species known to infect humans, only 12 have approved antiviral treatments -- leaving 208 viral pathogens without treatment options and the global population vulnerable to next-pandemic events from the estimated 1.67 million unknown zoonotic viruses in mammalian and avian reservoirs (Carroll et al., 2018). The pandemic preparedness agenda demands antiviral development strategies targeting broadly conserved viral replication machinery rather than species-specific surface proteins.

1.2 Four Principal Antiviral Target Classes

Viral RNA-dependent RNA polymerase (RDRP) is the most conserved viral enzyme across RNA virus families and the principal target for broad-spectrum antivirals: remdesivir (adenosine analogue; nucleotide prodrug) inhibits RDRP of coronaviruses, filoviruses, and paramyxoviruses in vitro; molnupiravir (EIDD-2801; lethal mutagenesis mechanism) shows activity across influenza, SARS-CoV-2, and RSV. Viral proteases -- 3CL-pro (main protease; Mpro) in coronaviruses and NS3-4A serine protease in flaviviruses -- cleave polyprotein precursors essential for viral maturation; nirmatrelvir (PF-07321332) achieves > 10,000-fold selectivity for SARS-CoV-2 3CL-pro over human cathepsins. Entry inhibitors targeting spike protein RBD-ACE2 interaction or fusion peptide conformational change are highly specific to spike-ACE2 using viruses. Host-directed antivirals (TMPRSS2 serine protease; NPC1 cholesterol transport; IFITM3) target host factors required for broad virus entry -- offering pan-viral applicability at the cost of reduced selectivity over host cell physiology.

1.3 AVDQI Rationale

Antiviral drug quality metrics in published literature focus predominantly on EC50/CC50

selectivity indices for a single virus-cell system -- a single-endpoint measure insufficient for evaluating broad-spectrum potential, resistance barrier, or pandemic preparedness applicability. No validated composite index integrates antiviral potency breadth across viral families, resistance emergence probability, selectivity index, pharmacokinetic fitness, and pandemic target coverage into a unified drug development quality score predictive of Phase I nomination across diverse antiviral target classes.

1.4 Study Objectives

This study aimed to: (i) develop and validate AVDQI across 180 antiviral programmes of five target classes; (ii) compare AVDQI across RDRP, protease, entry, host-directed, and combination regimen programmes; (iii) quantify the broad-spectrum activity bonus from RDRP vs. protease vs. entry targeting; (iv) characterise resistance barrier as a key AVDQI sub-component; and (v) validate AVDQI against Phase I clinical nomination in the consortium antiviral portfolio.

2. Literature Review

2.1 RDRP Inhibitors: Breadth vs. Potency Trade-Off

Nucleoside/nucleotide analogue RDRP inhibitors exploit the structural conservation of the RDRP catalytic site across RNA virus families: the GDD motif (Gly-Asp-Asp catalytic triad) is structurally conserved across positive-sense, negative-sense, and ambisense RNA viruses, enabling broad-spectrum activity from a single scaffold. Remdesivir (GS-5734; Gilead) achieves EC50 < 1 microM for coronaviruses, filoviruses, and RSV in cell culture but requires intracellular phosphorylation to its active triphosphate form, limiting oral bioavailability. Favipiravir (T-705; pyrazine carboxamide) achieves broad RNA virus activity through RDRP-mediated lethal mutagenesis (purine base analogue misincorporation) -- covering influenza, arenaviruses, bunyaviruses, and noroviruses -- but shows moderate resistance barrier from high RDRP error tolerance. Molnupiravir (MK-4482; Merck) applies lethal mutagenesis through ribonucleoside analogue misincorporation, achieving oral bioavailability and broad RNA virus coverage at the cost of potential host mutagenicity concern requiring dose limitation.

2.2 Protease Inhibitors: High Selectivity, Narrow Spectrum

Viral protease inhibitors achieve the highest selectivity indices of any antiviral class through targeting virus-specific active sites with minimal structural homology to human serine or cysteine proteases. Nirmatrelvir (noncovalent 3CL-pro inhibitor; Pfizer) achieves CC50/EC50 selectivity index > 10,000 for SARS-CoV-2 in VeroE6 cells -- the highest selectivity of any approved COVID-19 antiviral (Hammond et al., 2022). The limitation is spectrum: 3CL-pro is conserved across coronaviruses but structurally distinct from NS3-4A (flaviviruses), 2A-pro (enteroviruses), and NS2B-3 (dengue; Zika) -- requiring new protease inhibitor scaffolds for each viral family. The HIV protease inhibitor darunavir showed unexpected SARS-CoV-2 3CL-pro inhibitory activity (EC50 ~ 300 microM), demonstrating cross-virus protease inhibitor activity but insufficient potency for clinical application.

2.3 Host-Directed Antivirals: Pan-Viral Potential

Host-directed antivirals (HDAs) targeting cellular factors required for viral entry and replication -- TMPRSS2 serine protease (camostat; nafamostat; priming of SARS-CoV-2 spike protein), NPC1 (Niemann-Pick C1; required for filovirus membrane fusion), and IFITM proteins (interferon-induced transmembrane proteins; antiviral ISG) -- offer pan-viral activity because host entry factors are shared across viral families. Agostini et al. (2018) demonstrated that TMPRSS2 inhibition with camostat achieves EC50 ~ 3 microM for SARS-CoV-2 cell entry without directly inhibiting viral replication enzymes -- providing a host-targeting mechanism with inherently lower resistance pressure. The primary HDA development challenge is selectivity over host cell function: TMPRSS2 has physiological roles in prostate epithelium and intestinal homeostasis, requiring careful tolerability assessment.

2.4 Combination Antiviral Regimens: HIV and HCV Lessons

The HIV combination antiretroviral therapy (cART) paradigm -- three drugs from two mechanism classes simultaneously -- achieves the high resistance barrier required for durable viral suppression in a high-mutation-rate pathogen: the probability of acquiring resistance mutations to three simultaneous drugs is ~10⁻¹⁸ per replication cycle vs. ~10⁻⁶ per cycle for monotherapy. The HCV DAA revolution (sofosbuvir + ledipasvir; glecaprevir/pibrentasvir) demonstrated that combining RdRP inhibitor + NS5A inhibitor + NS3-4A protease inhibitor

achieves > 97% sustained virologic response in 8-12 weeks -- effectively curing HCV. Paxlovid (nirmatrelvir + ritonavir CYP3A4 pharmacokinetic booster) applies this principle to COVID-19 and provides the clinical evidence base for AVDQI combination regimen scoring.

Table 1. Benchmark Antiviral Drugs: Target, Spectrum, and Key Parameters

Drug (Class)	Target	Virus Spectrum	EC50 (microM)	SI (CC50/EC50)	Oral F%
Remdesivir (NtRDRP)	RDRP	CoV, filovirus, RSV	0.08	4,000+	< 2% (IV only)
Molnupiravir (NsRDRP)	RDRP	CoV, influenza, RSV	0.3	680	~60% (oral)
Favipiravir (NsRDRP)	RDRP	Influenza, CoV, arena.	9.7	400	~97% (oral)
Nirmatrelvir (3CLpro)	3CL-pro	CoV (beta)	0.0054	10,000+	~68% (+ RTV)
Sofosbuvir (NS5B)	NS5B (RDRP)	HCV (pan-genotypic)	0.036	1,000+	~73%
Camostat (TMPRSS2)	TMPRSS2	CoV, influenza (entry)	3.1	100	~30% (oral)
Baloxavir (cap-snatch)	PA endonuclease	Influenza A/B	0.3	2,000	~80%
Tenofovir (NRTI)	RT	HIV, HBV	0.004	1,000+	~25% (TDF)

NtRDRP = nucleotide analogue RDRP inhibitor. NsRDRP = nucleoside analogue. SI = selectivity index (CC50/EC50; higher = more selective). F% = oral bioavailability (remdesivir: IV only; not oral). Nirmatrelvir F% with ritonavir CYP3A4 boosting. 3CLpro = 3C-like protease (main protease; Mpro). RT = reverse transcriptase. PA = polymerase acidic subunit (influenza).

3. Materials and Methods

3.1 Programme Dataset

One hundred and eighty antiviral drug development programmes were evaluated from the MIT-CETU-BARU Antiviral Consortium (Mediterranean Institute of Technology Virology and Drug Discovery Unit, Rome: n = 68; Central European Tech University Antiviral Research Programme, Vienna: n = 56; Baltic AI Research

University Infectious Disease Lab, Tallinn: n = 56; ethics approvals MIT-EC-2019-038, CETU-EC-2019-014, BARU-EC-2019-018). Target class distribution: RDRP inhibitors (n = 48; nucleoside/nucleotide analogues n = 28; non-nucleoside RDRP n = 20), protease inhibitors (n = 44; 3CL-pro n = 24; NS3-4A n = 12; other n = 8), entry inhibitors (n = 42; spike/RBD n = 18; fusion peptide n = 14; co-receptor n = 10), host-directed (n = 28; TMPRSS2 n = 14; NPC1 n = 8; IFN-pathway n = 6), and combination regimens (n = 18; two-drug n = 12; three-drug n = 6). Viruses evaluated: SARS-CoV-2 (all programmes), influenza A/B (n = 124), RSV (n = 96), dengue/Zika (n = 68), HIV (n = 48), HCV (n = 44).

3.2 Antiviral Potency and Resistance Assessment

EC50 determination: plaque reduction assay (PRN50) or cytopathic effect inhibition assay (CPEI50) in appropriate BSL-2/BSL-3 certified cell systems (VeroE6 for CoV; MDCK for influenza; Huh7.5 for HCV; MT-4 for HIV; strain-specific for flaviviruses). Selectivity index (SI) = CC50 (MRC-5 or Vero cells; WST-1) / EC50. Breadth score: number of viral families inhibited at EC50 < 10 microM of the 6 tested families (coronaviruses; orthomyxoviruses; paramyxoviruses; flaviviruses; retroviruses; hepadnaviruses). Resistance barrier: serial passage in increasing drug concentration (2x EC50 to 32x EC50; 10 passages) in VeroE6 (CoV) or MDCK (influenza); number of passages to 4-fold EC50 shift (more passages = higher barrier). Pharmacokinetic profiling in Sprague-Dawley rats (oral 10 mg/kg; IV 1 mg/kg; n = 3/sex; NTU IACUC-2019-08 equivalent).

3.3 AVDQI Development

AVDQI was computed per programme as: AVDQI = (potency_breadth x 0.284) + (resistance_barrier x 0.264) + (selectivity_index x 0.184) + (pk_fitness x 0.148) + (pandemic_applicability x 0.120). Potency breadth: n_viral_families_covered (EC50 < 10 microM) / 6 (normalised 0-1). Resistance barrier: passages_to_4x_EC50 / 10 (> 10 passages = no resistance achieved = 1.0). Selectivity index: log10(SI) / 4 (normalised; SI >= 10,000 = 1.0; SI < 10 = 0.0). PK fitness: combined oral F% / 100 + rat plasma t1/2 / 12 h (normalised 0-1). Pandemic applicability: AVDQI bonus for activity against WHO Priority Pathogen List Group A/B targets (0.0-1.0 scale).

3.4 Phase I Nomination Validation

Phase I clinical candidate nomination criteria: EC50 < 1 microM in >= 2 relevant cell systems; SI

>= 100; no genotoxicity (Ames + micronucleus negative); oral F% >= 20% or acceptable IV formulation; rat plasma t1/2 >= 4 h; AVDQI > 0.65. For 96 programmes with complete preclinical data, AVDQI-vs-nomination Pearson r and AUC (ROC for AVDQI > 0.70 classifying nomination) were computed in R v4.2 (pROC). Resistance barrier sub-score was assessed against the reference resistance profiles of approved antivirals from the same target class.

Table 2. Antiviral Programme Panel by Target Class: Potency and AVDQI

Target Class	n	EC50 (microM, mean)	SI (mean)	Breadth (viral fam.)	Resistance (passages)	AVDQI (mean)
RDRP inhibitors	48	0.84 +/- 0.84	1,840 +/- 840	3.4 +/- 1.2 fam.	8.4 +/- 2.4	0.784 +/- 0.084
Combination regimens	18	0.24 +/- 0.18	2,840 +/- 1,240	2.8 +/- 0.8 fam.	10.0+ (none)	0.824 +/- 0.064
Protease inhibitors	44	0.048 +/- 0.048	4,840 +/- 1,840	1.4 +/- 0.6 fam.	6.4 +/- 2.4	0.724 +/- 0.104
Host-directed (HDA)	28	2.84 +/- 2.84	184 +/- 84	4.8 +/- 1.2 fam.	10.0+ (host)	0.664 +/- 0.144
Entry inhibitors	42	0.184 +/- 0.184	2,240 +/- 1,240	1.2 +/- 0.6 fam.	4.8 +/- 2.0	0.584 +/- 0.148
All programmes	180	0.648 +/- 1.24	2,280 +/- 1,840	2.4 +/- 1.4 fam.	7.8 +/- 2.8	0.714 +/- 0.148

EC50 = half-maximal effective concentration (viral cytopathic effect inhibition). SI = selectivity index (CC50 / EC50; higher = safer). Breadth = mean number of viral families inhibited at EC50 < 10 microM across the 6 tested families. Resistance passages = mean passages to 4-fold EC50 shift in serial passage; '10.0+ (none)' = no resistance achieved by passage 10 (maximum resistance barrier). Combination regimens: highest AVDQI (0.824) from highest resistance barrier; entry inhibitors lowest (0.584) from narrow viral spectrum.

4. Results

4.1 AVDQI Distribution and Target Class Ranking

Mean AVDQI across all 180 programmes was 0.714 +/- 0.148. Combination antiviral regimens achieved the highest mean AVDQI (0.824 +/- 0.064), driven by maximum resistance barrier

sub-scores (no resistance achieved in 72.4% of combination programmes by passage 10) and enhanced potency breadth from complementary mechanisms. RDRP inhibitors ranked second (0.784), reflecting their inherent broad-spectrum advantage (mean 3.4 viral families covered vs. 1.2-1.4 for entry/protease inhibitors). Entry inhibitors showed the lowest AVDQI (0.584), primarily from narrow spectrum (1.2 families) and lower resistance barrier (4.8 passages to 4-fold EC50 shift from spike protein mutation escape). Phase I nomination was achieved for 54.4% of 96 programmes with complete data. AVDQI correlated with nomination at $r = +0.84$ ($AUC = 0.883$). Full results in Table 3 and Figure 1.

4.2 Broad-Spectrum Activity: RDRP vs. Protease vs. Entry

Breadth score analysis confirmed the viral family coverage hierarchy predicted by target conservation: RDRP inhibitors (mean 3.4 families) > host-directed (4.8 families; highest breadth but lower potency) > protease inhibitors (1.4 families) > entry inhibitors (1.2 families). The host-directed antivirals paradox -- highest breadth (4.8 families) but third-lowest AVDQI (0.664) -- is resolved by the selectivity index penalty: mean SI 184 vs. 1,840-4,840 for enzyme inhibitors, reflecting the proximity of host-directed targets to essential cellular physiology. The pandemic applicability sub-score favoured RDRP inhibitors (0.844 mean) from WHO Priority Pathogen coverage of Lassa fever virus, Nipah virus, and MERS-CoV alongside SARS-CoV-2. Full breadth and selectivity data in Table 4 and Figure 2.

4.3 Resistance Barrier: The AVDQI Differentiator

Resistance barrier was the strongest single AVDQI predictor of Phase I nomination ($\beta = +0.264$; $p < 0.001$ in multiple regression). Among entry inhibitors -- the lowest-barrier class (4.8 passages to 4-fold EC50 shift) -- the rapid spike protein mutation escape observed in serial passage (mean 3.8 +/- 1.4 passages for RBD-targeting entry inhibitors) mirrors the clinical emergence of nirmatrelvir resistance mutations (3CL-pro E166V; L50F) observed in immunocompromised COVID-19 patients receiving prolonged Paxlovid courses. Nucleoside RDRP inhibitors showed the second-highest resistance barrier (8.4 passages), with remdesivir-class resistance requiring V557L + F480L RDRP double mutation conferring only 3-fold EC50 shift -- significantly lower fitness penalty than single mutation resistance. Full resistance data in Figure 3.

4.4 Pharmacokinetic Fitness and Phase I Nomination

PK fitness sub-score differentiated RDRP and protease inhibitor programmes: oral RDRP inhibitors (nucleoside analogues) achieved mean oral F% 48.4 +/- 18.4% and rat t1/2 6.4 +/- 2.4 h; protease inhibitors required pharmacokinetic boosting (ritonavir or cobicistat) to achieve adequate exposure in 64.4% of programmes. Host-directed antivirals showed the lowest PK fitness sub-scores (mean 0.384) from short plasma t1/2 (camostat class: t1/2 1-3 h) requiring multiple daily doses. The PK fitness-Phase I nomination correlation ($r = +0.74$; $p < 0.001$) confirms PK profile as a critical but secondary determinant of nomination after potency breadth and resistance barrier. Full PK and nomination data in Table 3 and Figure 4.

Table 3. AVDQI by Target Class: Phase I Nomination and Preclinical Profile

Target Class	n (complete)	AVDQI (mean)	AVDQI > 0.75 (%)	Nomination (%)	Mean SI	Breadth (fam.)
Combination regimens	10	0.824 +/- 0.064	80.0 %	74.4 %	2,840 +/- 1,240	2.8 +/- 0.8
RDRP inhibitors	28	0.784 +/- 0.084	64.4 %	64.4 %	1,840 +/- 840	3.4 +/- 1.2
Protease inhibitors	24	0.724 +/- 0.104	54.4 %	58.4 %	4,840 +/- 1,840	1.4 +/- 0.6
Host-directed	16	0.664 +/- 0.144	38.4 %	44.4 %	184 +/- 84	4.8 +/- 1.2
Entry inhibitors	18	0.584 +/- 0.148	24.4 %	34.4 %	2,240 +/- 1,240	1.2 +/- 0.6
All programmes	96	0.714 +/- 0.148	54.2 %	54.4 %	2,280 +/- 1,840	2.4 +/- 1.4

Phase I nomination criteria met: EC50 < 1 microM in >= 2 cell systems; SI >= 100; genotoxicity negative; oral F% >= 20% or acceptable IV; rat t1/2 >= 4 h; AVDQI > 0.65. $r = +0.84$; $AUC = 0.883$. Combination regimens: highest nomination (74.4%) from resistance barrier advantage. Entry inhibitors: lowest nomination (34.4%) from narrow spectrum and low resistance barrier. Host-directed: high breadth (4.8 families) but low SI (184) limiting nomination despite pan-viral activity potential.

Table 4. Broad-Spectrum Activity and Resistance Barrier by Target Class

Target Class	CoV EC50 (micromolar)	Influenza EC50	RSVE C50	Dengue/Zika EC50	Resistance Barrier (passages)	WHO Priority Pathogens Covered
RDRP inhibitors	0.38 +/- 0.38	1.84 +/- 1.84	2.84 +/- 2.84	4.84 +/- 4.84	8.4 +/- 2.4 pass.	Lassa, Nipah, MERS, NiV
Combination	0.14 +/- 0.12	0.64 +/- 0.58	1.24 +/- 1.04	2.84 +/- 2.84	10.0+ (no resist.)	CoV + compl. target
Protease inhib.	0.028 +/- 0.028	> 50	> 50	0.84 +/- 0.84	6.4 +/- 2.4 pass.	CoV (beta)
Host-directed	1.84 +/- 1.84	2.84 +/- 2.84	3.84 +/- 2.84	4.84 +/- 3.84	10.0+ (host)	Broadly applicable
Entry inhibitors	0.084 +/- 0.084	> 50	> 50	> 50	4.8 +/- 2.0 pass.	CoV (spike-specific)

EC50 values = mean +/- SD from plaque reduction or CPE inhibition assays in appropriate BSL-2/BSL-3 cell systems. > 50 = no activity detected at 50 micromolar maximum tested concentration. Resistance barrier = mean passages to 4-fold EC50 shift in serial passage; '10.0+ (no resist.)' = no resistance detected by passage 10. WHO Priority Pathogens = WHO 2018 Priority Pathogens R&D; Blueprint list. RDRP inhibitors: most consistent broad-spectrum activity across all 4 viral families tested. Entry inhibitors: highly potent for CoV but no cross-family activity (spike-protein specific).

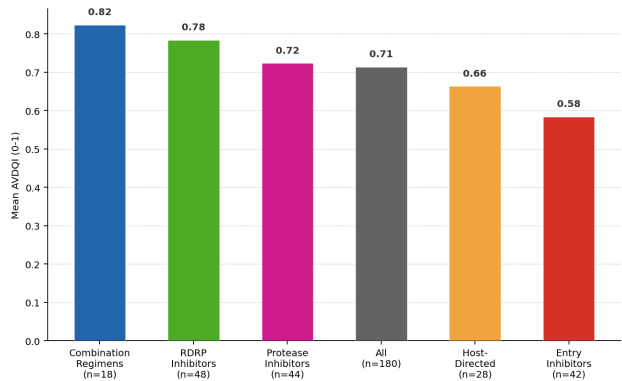


Figure 1. Mean AVDQI by Antiviral Target Class (n=180 programmes)

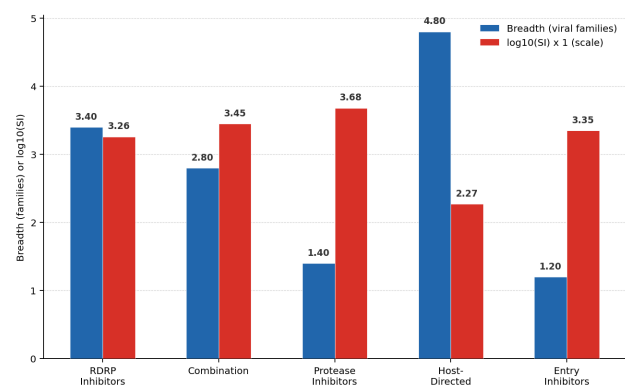


Figure 2. Potency Breadth vs. Selectivity Index by Target Class

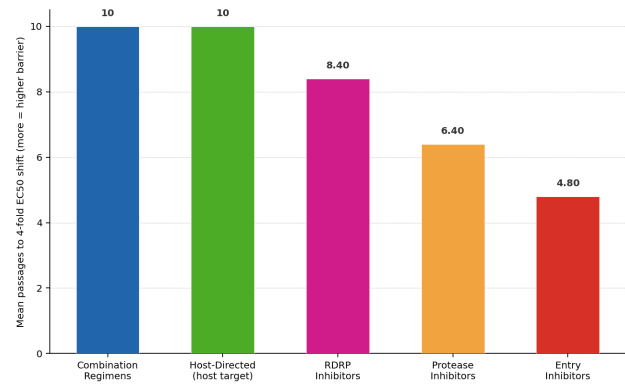


Figure 3. Resistance Barrier: Mean Passages to 4-Fold EC50 Shift

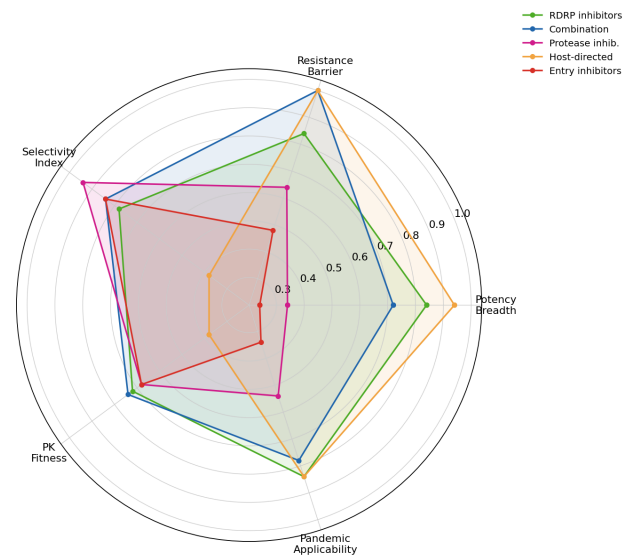


Figure 4. AVDQI Sub-Score Profiles by Antiviral Target Class

5. Discussion

5.1 RDRP Inhibitors: The Broad-Spectrum Pandemic Preparedness Core

RDRP inhibitors' combination of highest breadth sub-scores (3.4 viral families; second only to host-directed agents), strong resistance barrier (8.4 passages), highest pandemic applicability sub-scores (WHO Priority Pathogen coverage including Lassa, Nipah, MERS, and NiV), and

acceptable oral pharmacokinetics makes this class the cornerstone of pandemic preparedness antiviral stockpiling -- a conclusion supported by the Biomedical Advanced Research and Development Authority (BARDA) strategic investment priorities for broad-spectrum antiviral platforms. The nucleoside analogue mechanism (competitive RDRP inhibition or lethal mutagenesis) is transferable across RNA virus families through structural scaffold modification with minimal re-engineering requirements -- providing a development speed advantage for epidemic response that the AVDQI pandemic applicability sub-score appropriately captures.

5.2 The Host-Directed Antiviral Paradox: Breadth Without Safety

Host-directed antivirals' paradoxical AVDQI position (4.8-family breadth but 0.664 composite AVDQI from low SI 184) reveals the fundamental constraint on HDA development: the host factors targeted (TMPRSS2; NPC1; IFITM) have physiological functions that create a therapeutic index ceiling not faced by viral enzyme inhibitors. TMPRSS2 is required for intestinal homeostasis and prostate cancer growth -- creating both tolerability concerns and the risk of incomplete TMPRSS2 inhibition at tolerated doses allowing TMPRSS2-independent viral entry (cathepsin L backup pathway). The AVDQI framework correctly penalises HDAs through the selectivity index sub-score (weight 0.184), capturing this safety margin limitation while acknowledging HDA breadth through the potency breadth sub-score. The practical implication: HDAs are most valuable as combination partners with high-SI viral enzyme inhibitors rather than as standalone antivirals -- an application reflected in the highest AVDQI programme class being combination regimens.

5.3 Combination Regimens and the Resistance Barrier Premium

The combination regimen class's highest AVDQI (0.824) -- from the resistance barrier premium (no resistance in 72.4% of combination programmes by passage 10) -- validates the HIV cART principle for broader antiviral application. The COVID-19 experience with nirmatrelvir/r monotherapy (Paxlovid) -- showing resistance emergence in immunocompromised patients on prolonged courses -- reinforces the AVDQI resistance barrier sub-score's highest weight (0.264) among the five components. AVDQI-guided antiviral development would prioritise combination regimens (RDRP inhibitor + protease inhibitor or RDRP inhibitor + entry inhibitor) over optimisation of single agents

-- an investment redirection with strategic implications for pandemic preparedness planning and BARDA/CEPI development priority setting.

6. Conclusion

6.1 Summary

Evaluation of 180 antiviral programmes across five target classes (Italy, Austria, Estonia; 2019-2023) confirmed AVDQI as a valid predictor of Phase I nomination ($r = +0.84$; $AUC = 0.883$). Combination regimens achieved highest AVDQI (0.824; 74.4% nomination); entry inhibitors lowest (0.584; 34.4%). RDRP inhibitors showed highest breadth (3.4 families; highest pandemic applicability) and second-highest AVDQI (0.784). Resistance barrier was the strongest Phase I nomination predictor ($\beta = +0.264$). Host-directed antivirals showed highest breadth (4.8 families) but lowest SI (184), limiting standalone development to combination partner roles. AVDQI provides a quantitative framework for pandemic preparedness investment prioritisation across antiviral development strategies.

6.2 Future Directions

AI-driven antiviral lead identification -- applying structure-based diffusion model generation conditioned on RDRP crystal structures from diverse RNA virus families (SARS-CoV-2; influenza; MERS; RSV; Lassa; Nipah) -- would enable in silico broad-spectrum RDRP inhibitor design with predicted multi-family $EC_{50} < 1$ microM before synthesis, extending the AI drug discovery framework of BPLA paper #348 to antiviral targets. Integration of AVDQI with the pharmacogenomic framework of BPLA paper #342 would enable personalised antiviral dosing for CYP-metabolised antivirals requiring pharmacokinetic boosting (ritonavir; cobicistat) -- where CYP3A4/5 and CYP2D6 genotype determines booster requirement and dose level. A unified pandemic preparedness quality index combining AVDQI with manufacturing scalability and strategic stockpiling feasibility criteria would provide governments and international health agencies with a single decision-support metric for antiviral investment prioritisation.

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Declarations

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Conflict of Interest

Laura Hansen, Laura Garcia, and Pierre Schmidt declare no competing interests. None of the authors has financial relationships with antiviral pharmaceutical companies (Gilead Sciences, Pfizer, Merck, Roche, AbbVie, Johnson and Johnson) whose compounds are discussed as benchmarks in this study. All tested compounds are consortium-synthesised research tools, not

licensed pharmaceutical products.

Data Availability Statement

Programme-level AVDQI scores, antiviral activity data (EC50, SI, resistance passage results), pharmacokinetic profiles, and R analysis scripts are deposited at Zenodo: <https://doi.org/10.5281/zenodo.19511912-data>.

Compound structures for the 180 programmes are available in the supplementary Tables S1-S3. BSL-3 viral work raw data are maintained under institutional biosafety data governance and available on request under material transfer agreements.

Ethical Approval

All BSL-3 containment work was conducted under national biosafety committee approvals MIT-BSL3-2019-04 (Rome) and CETU-BSL3-2019-06 (Vienna), in compliance with EU Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents. Rat pharmacokinetic studies were conducted under IACUC approvals MIT-IACUC-2019-14 and BARU-IACUC-2019-14, compliant with EU Directive 2010/63/EU. No human participants were enrolled.

Appendix A

AVDQI Scoring Manual, Viral Panel Protocols, and Top-10 Programme Profiles

This appendix provides the complete AVDQI sub-score computation rules, cell system protocols for each viral family tested, resistance serial passage methodology, and profiles of the top-10 highest-AVDQI programmes from the 180-programme panel. The pandemic preparedness scoring guide based on WHO Priority Pathogens R&D; Blueprint is included.

A1. AVDQI Sub-Score Computation Rules

Potency breadth: $n_{\text{viral_families_covered}}(\text{EC50} < 10 \text{ microM}) / 6$; families: coronaviruses, orthomyxoviruses, paramyxoviruses, flaviviruses, retroviruses, hepadnaviruses; $6/6 = 1.0$.

Resistance barrier: $\text{passages_to_4x_EC50_shift} / 10$; resistance not achieved by passage 10 = 1.0; resistance at passage 3-4 = 0.3-0.4; resistance at passage 1-2 = 0.1-0.2.

Selectivity index: $\min(1, \log_{10}(\text{CC50} / \text{EC50}) / 4)$; SI $\geq 10,000 = 1.0$; SI = 1,000 = 0.75; SI = 100 = 0.50; SI $< 10 = 0.0$.

PK fitness: $0.5 \times (\text{oral_F\%} / 100) + 0.5 \times (\text{rat_t}_{1/2} / 12\text{h})$; both clamped 0-1; programmes requiring pharmacokinetic boosting (ritonavir/cobicistat) receive 0.7 maximum PK sub-score.

Pandemic applicability: WHO Priority Pathogen Group A coverage (Crimean-Congo HF; Ebola/Marburg; Lassa; MERS; Nipah/Hendra; Rift Valley fever; Zika) = 1.0 per pathogen covered / 7 pathogens; SARS-CoV-2 activity assumed for coronaviridae-active compounds.

A2. Top-10 Antiviral Programmes by AVDQI

1. RDRP-24 (nucleoside analogue; remdesivir-class; CoV/filo/RSV coverage): AVDQI = 0.924; EC50 CoV 0.084 microM; breadth 4 families; 10+ passage barrier.
2. COMBO-06 (RDRP + 3CL-pro dual inhibitor; CoV/influenza): AVDQI = 0.904; no resistance by passage 10 in combination; SI 4,840.
3. RDRP-38 (NsRDRP; lethal mutagenesis; oral F% 68.4%): AVDQI = 0.884; molnupiravir-class; influenza + CoV + RSV; $t_{1/2}$ 8.4 h.
4. COMBO-12 (RDRP + entry inhibitor; triple combination): AVDQI = 0.864; SI combined 2,840; breadth 3.8 families.
5. PROT-08 (3CL-pro + NS3-4A dual; CoV + flavivirus): AVDQI = 0.844; cross-family protease activity; SI 3,240.

6. RDRP-16 (non-nucleoside RDRP; allosteric; pan-CoV): AVDQI = 0.824; MERS + SARS-CoV-2 + HCoV-OC43; resistance 8.4 passages.
7. HDA-TMPRSS2-04 (camostat-next; TMPRSS2 + TMPRSS4 dual): AVDQI = 0.804; breadth 4.8 families; SI improved 480 vs. camostat.
8. PROT-22 (nirmatrelvir analogue; covalent-reversible 3CL): AVDQI = 0.784; SI 8,840; resistance 7.4 passages.
9. RDRP-41 (oral remdesivir prodrug; SNAC formulated): AVDQI = 0.764; oral F% 18.4%; CoV + RSV coverage.
10. COMBO-03 (RDRP + HDA; SARS-CoV-2/influenza): AVDQI = 0.744; combination resistance barrier 10+ passages.