•综述•

新型冠状病毒 (2019-nCoV) 的靶向药物研究策略

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摘要: 新型冠状病毒尚无特效药。新型冠状病毒靶向药物研发面临诸多挑战, 其药物研发策略包括筛选广谱抗病毒药, 老药新用, 以及开发特异的全新药。药物既可抑制病毒靶点 (如蛋白酶、合成酶、树突蛋白及病毒壳膜), 又可靶向宿主 (如病毒受体抑制剂、病毒内吞和跨膜蛋白酶抑制剂等)。最近核糖核酸合成酶抑制剂瑞德西韦在孤例重症患者表现出良好疗效, 广谱病毒蛋白酶抑制剂克力芝也在临床上应用。 这两种药刚启动III期临床试验, 以评价其安全性和有效性。多种药物联用也是当前针对新型冠状病毒的一个主要策略, 但应遵从科学依据和临床需求。通过大量文献和多种数据库检索, 针对病毒和宿主细胞的关键成药靶点, 作者挑选出75个临床在研的靶向药物, 包括20个上市药, 以助力临床前、临床试验研究和药物改良。

关键词: 新型冠状病毒; 新型冠状病毒肺炎; 靶向治疗; 联合用药; 瑞德西韦; 克力芝

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Strategies for the development of drugs targeting novel coronavirus 2019-nCoV

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Abstract: There is no specific drug that has been approved for 2019-nCoV. There are a number of factors that pose major challenges in their development. Approaches to the development of anti-2019-nCoV include screening existing broad-spectrum antiviral drugs, repositioning of readily available clinical compounds, and *de novo* development of novel and specific agents for 2019-nCoV. Candidate compounds can be developed either to inhibit virus-based targets, such as RNA proteases, polymerase, spike glycoproteins, and viral envelop and membrane proteins, or to inhibit host-based targets, such as receptors and proteases that are utilized by virus for viral entry and endocytosis. Recently, the RNA polymerase remdesivir had demonstrated clinical efficacy in one patient with severe novel coronavirus pneumonia (NCP). The broad-spectrum viral protease inhibitor Kaletra® is also recommended in the current NCP clinical practice. Both drugs had lately been proceeded into multiple controlled phase III clinical trials to test their safety and efficacy in NCP. Combinational therapies consisting of multiple drugs provide other viable options against 2019-nCoV, based on scientific and clinical rationales. Using bioinformatics and database analysis, we have identified 75 clinically compounds, including 20 marketed compounds, that are efficacious in inhibiting key targets in virus- and host-based approaches, which may facilitate the development of new therapeutic options for 2019-nCoV.

Key words: novel coronavirus 2019-nCoV; novel coronavirus pneumonia; targeted therapy; combination therapy; remdesivir; Kaletra

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蔓延中国并有扩散全球趋势的新型冠状病毒(2019-nCoV)肺炎, 已证明人传人的传染性很强, 引起的重症病例和死亡率较高, 是近年来急性传染病中罕见的, 被世界卫生组织宣布为国际突发公共卫生事件。但到目前为止还没有针对新型冠状病毒的上市特效药, 一些抗病毒药物和中药已用于临床治疗这种新型冠状病毒肺炎, 但疗效并不确切。因此, 根据冠状病毒结构, 寻找治疗该病毒的靶向、高效、低毒的药物, 是目前中国和国际医药界的当务之急。在通过多种相关数据库的检索分析、加工整理后, 本文作者根据正在临床研究的治疗冠状病毒药物对2019-nCoV肺炎的试用情况与其他抗病毒药物的比较, 以及抗新型冠状病毒的可能靶点及新药开发策略进行综述, 以期对此类药物的研究开发提供一些可借鉴的思路。

1 已在新型冠状病毒肺炎患者试用的药物

目前已经报道在新型冠状病毒患者身上试用的药物, 主要有两大类: 第一类是由吉利德 (Gilead) 公司原本为埃博拉 (Ebola) 冠状病毒开发的广谱抗病毒药瑞德西韦 (remdesivir); 第二类在临床使用的药物, 是由艾伯维 (Abbvie) 公司原本为艾滋病毒开发的克力芝 (Kaletra, 洛匹那韦联用利托那韦, lopinavir/ritonavir, LPV/r)。

1.1 瑞德西韦 来自武汉的美国首例新型冠状病毒重症肺炎患者在住院第7天接受了瑞德西韦静脉输注后, 肺部X-射线阴影消失, 血氧饱和度恢复正常, 临床症状改善显著[1]。瑞德西韦是一种核苷酸类似物前药。体外实验和动物模型数据表明, 瑞德西韦通过抑制核糖核酸依赖的核糖核酸合成酶 (RNA-derived RNA polymerase, RdRp) 从而抑制包括非典型肺炎 (SARS冠状病毒)、中东呼吸综合征 (MERS冠状病毒)、埃博拉冠状病毒和其他多种冠状病毒[2]。体外细胞实验表明, 瑞德西韦对新型冠状病毒的半数有效浓度 (IC_{50}) 为0.77 μmol·L^{-1} [3]。但是, 瑞德西韦尚未在任何国家获得批准上市, 其有效性和安全性还没有被III期临床试验证实, 针对埃博拉病毒只进行到II期临床试验[4]。而且在这个随机分组和严格对照控制的II期临床试验的半程数据分析发现, 瑞德西韦治疗组的死亡率要远高于再生元 (Regeneron) 公司开发的靶向病毒树突蛋白 (spike protein, S protein) 的单克隆抗体MAb114治疗组和REGN-EB3治疗组, 所以不得不在试验中途停用瑞德西韦而改用单克隆抗体。瑞德西韦在美国同情用药 (compassionate use) 的这一孤例患者属于重症肺炎, 而中国和武汉多数患者为轻度和中度肺炎。重症患者与轻度和中度患者在病理上有很大差异。最后, 瑞德西韦对新型冠状病毒的有效性尚缺乏动物模型支持。所以, 为了评估该药的有效性和安全性, 瑞德西韦对治疗轻度、中度[5]和重度[6]新型冠状病毒肺炎患者进行随机分组, 双盲和安慰剂对照的两个III期临床试验已于2020年2月初启动。

1.2 克力芝 克力芝是洛匹那韦与利托那韦的复方制剂。洛匹那韦是在利托那韦的基础上改良的病毒复制酶抑制剂, 利托那韦通过抑制细胞色素P450 (CYP) 3A来增加洛匹那韦半衰期, 从而增进洛匹那韦的药代动力学[7]。体外实验表明, 洛匹那韦或利托那韦单用可以抑制SARS冠状病毒, 联用可产生协同作用[8]。在非随机分组的临床试验中, 克力芝联用广谱抗病毒药利巴韦林的疗效 [急性呼吸窘迫综合征 (acute respiratory distresssyndrome, ARDS) 和死亡] 要优于利巴韦林治疗[8]。武汉金银潭医院隔离收治的99名新型冠状病毒患者中, 75名接收了克力芝及其他抗病毒药, 如奥司他韦 (oseltamivir) 和更昔洛韦 (ganciclovir) 治疗[9]。国家卫生健康委员会和中医药管理局也推荐克力芝进入新型冠状病毒治疗手册[10]。但是, 克力芝对新型冠状病毒的有效性尚缺乏临床前数据, 对临床患者的有效性既没有明确报道, 又缺乏对照组而难以进行临床统计。《柳叶刀》披露了克力芝正在中国开展随机分组、有对照组的临床试验 (chictr2000029308)[11]。

1.3 瑞德西韦和克力芝比较 瑞德西韦和克力芝对MERS冠状病毒有过临床前疗效对比[12]。在细胞培养实验上, 瑞德西韦要优于克力芝的抗病毒活性; 在小鼠动物模型实验上, 预防性和治疗性瑞德西韦均可改善肺功能和肺部病理, 并减少病毒负荷。相反, 预防性克力芝联用干扰素只会略微降低病毒载量, 而不会改善其他疾病参数。治疗性克力芝联用干扰素虽然可以改善肺功能, 但既不减少病毒复制也不改善严重肺部病理。因此, 临床前数据支持, 瑞德西韦治疗MERS冠状病毒感染有可能优于克力芝。

2 新型冠状病毒靶向治疗药物研发策略

针对SARS冠状病毒和MERS冠状病毒的药物研发策略可以作为新型冠状病毒参考[13]。这些研发策略包括筛选现有的广谱抗病毒药物和化合物库, 开发针对新型冠状病毒全新的小分子药物、小干扰RNA (small interfering RNA, siRNA) 及中和抗体等。药物即可靶向病毒, 也可靶向宿主。

2.1 靶向病毒的药物 主要是抑制病毒复制周期的各种底物, 酶及病毒表面蛋白。包括: ① 抑制核苷、核酸和核苷酸合成的广谱抗病毒药, 如霉酚酸 (mycophenolic acid)[14]; ② 抑制病毒复制蛋白酶, 如木瓜蛋白酶 (papain-like protease, PLpro) 抑制剂巯嘌呤 (mercaptopurine)[15]、3C类似蛋白酶 (protease 3C-like, 3CLpro) 抑制剂洛匹那韦、RdRp抑制剂瑞德西韦和利巴韦林 (ribavirin)[16], 以及ATP依赖性解旋酶 (ATP-

dependent helicase) 抑制剂伊维菌素 (ivermectin)[17]; ③ 靶向病毒树突状蛋白的siRNA、多肽和单克隆抗体[18], 网络报道不少公司正在开发新型冠状病毒树突状蛋白特异的单抗, 以及从红藻中提取的广谱抗冠状病毒蛋白Griffithsin[19]; ④ 靶向病毒包装、宿主整合, 降低宿主免疫功能的辅助蛋白, 如病毒包膜、膜蛋白、核衣壳和孔蛋白等, 如病毒孔蛋白抑制剂六亚甲基氨氯 (hexamethyleneamiloride)[20]。

2.2 靶向宿主的药物 主要是通过提升宿主自身免疫能力, 阻断病毒进入宿主细胞。包括: ① 免疫增强剂干扰素和干扰素诱导剂硝唑尼特 (nitazoxanide); ② 抑制新型冠状病毒的受体血管紧张素转化酶2 (angiotensin-converting enzyme 2, ACE2)[21,22], 如 *N*-(2-aminoethyl)-1 aziridine-ethanamine[23]、多肽P4 (ACE2氨基酸22-44) 和P5 (ACE2氨基酸22-57)[24]; ③ 细胞内吞抑制剂氯喹 (chloroquine)[3,25]、盐酸氯丙嗪 (chloropromazine)[25]和哇巴因 (ouabain)[26]; ④ 阻断病毒在宿主细胞表面激活和释放RNA进入细胞的步骤, 如跨膜蛋白酶丝氨酸 (transmembrane protease, serine 2, TMPRSS2) 抑制剂卡莫司他 (camostat)[27]。

2.3 多药联用 多药联用是抗新型冠状病毒的重要策略。激素、干扰素和抗病毒药联用在SARS和MERS冠状病毒临床试验中。金银潭医院的新型冠状病毒肺炎患者多接受了多种抗病毒药, 包括奥司他韦、更昔洛韦和克力芝与抗生素的联用[9]。克力芝本身就是洛匹那韦和利托那韦固定剂量联用。联用广谱抗冠状病毒药瑞德西韦与病毒树突蛋白特异性单抗也被看好[28]。在MERS冠状病毒体的体外细胞实验和动物模型实验上, 瑞德西韦联用干扰素要优于克力芝与干扰素的联用[12]。在SARS冠状病毒的动物模型上, 联用丝氨酸和色氨酸蛋白激酶来阻断病毒在宿主细胞表面激活和RNA进入宿主细胞的效果, 要优于单用蛋白激酶抑制剂[29]。鉴于RNA病毒的高突变性, 联用树突蛋白不同位点的单克隆抗体也将降低病毒突变而产生抗药性。鉴于冠状病毒利用内吞和蛋白激酶激活两种方式进入宿主细胞, 联用细胞内吞抑制剂和蛋白激酶抑制剂将更有效地阻断病毒侵入。联合用药的另一个重要考量因素是病症轻重程度。重症患者自身的一系列严重免疫反应可能将超过病毒感染, 成为治疗的首要问题。干扰素-β和抗病毒药, 如克力芝的联用也被临床专家所推荐[30]。

3 靶向病毒的临床在研药物

由于不同的冠状病毒有特异的树突状蛋白, 靶向新型冠状病毒树突状蛋白的特异性抗体目前正处在研发初期。但是不同病毒的蛋白激酶和底物有较高的相似性, 同样的蛋白激酶抑制剂在不同的病毒上可能有广谱作用。在冠状病毒感染宿主细胞的几个关键步骤中, 病毒蛋白激酶3CLpro和PLpro剪切病毒的转座子而产生RdRp, 后者催化病毒的RNA信息在宿主细胞内复制。根据信息学和大数据分析, 现将靶向这3个重要靶点的临床药物的体外筛选结果归纳于表1, 包

Table 1 Representative compounds that target essential viral enzymes in *in vitro* testing. 3CLpro: Protease 3C-like; PLpro: Papain-like protease; RdRp: RNA-derived RNA polymerase; HCV: Viral hepatitis C; N/A: Not available

Target	Drug	Material	Method	IC_{50} (-log mol·L^{-1})
3CLpro	Betulinic acid	Coronavirus (SARS-associated)	Spectrophotometric assay	5
3CLpro	Cryptotanshinone	Coronavirus (SARS-associated)	Fluorescent assay	3.6
3CLpro	Curcumin	Coronavirus (SARS-associated)	Spectrophotometric assay	4.4
3CLpro	Hesperetin	Vero African green monkey kidney cells	Luciferine/luciferase assay	5.1
3CLpro	Lopinavir	Human immunodeficiency virus type-1 protease	Fluorescent assay	10.9
3CLpro	Niclosamide	Coronavirus (SARS-associated)	Spectrophotometric assay	4.4
3CLpro	Rupintrivir	Transmissible gastroenteritis coronavirus (TGEV)	Fluorescence resonance energy transfer (FRET) assay	<4.3
PLpro	Cryptotanshinone	Coronavirus (SARS-associated)	Ubiquitin-7-amino-4-methylcoumarin as substrate	4.1
PLpro	Cryptotanshinone	Coronavirus (SARS-associated)	Arg-Leu-Arg-Gly-Gly-7-amino-4-methylcoumarin as substrate	5
PLpro	Curcumin	Coronavirus (SARS-associated)	Arg-Leu-Arg-Gly-Gly-7-amino-4-methylcoumarin as substrate	5.2
PLpro	Mercaptopurine	Coronavirus (MERS-associated)	Fluorescent assay, ubiquitin-7-amino-4-trifluoromethylcoumarin as substrate	4.6
PLpro	Mycophenolic acid sodium salt	Coronavirus (MERS-associated)	Fluorescent assay, ubiquitin-7-amino-4-trifluoromethylcoumarin as substrate	3.6
PLpro	Psoralen	Coronavirus (SARS-associated)	Arg-Leu-Arg-Gly-Gly-7-amino-4-methylcoumarin as substrate	<3.8

Continued

Target	Drug	Material	Method	IC_{50} (-log mol·L^{-1})
PLpro	Tioguanine	Coronavirus (MERS-associated)	Fluorescent assay, ubiquitin-7-amino-4-trifluoromethylcoumarin as substrate	4.9
RdRp	ALS-008112	Respiratory syncytial virus	Radioactivity assay	7.7
RdRp	CBR-2092	Staphylococcus aureus	N/A	7.5
RdRp	IDX-20963	Human enzyme (mitochondrial)	N/A	<3.3
RdRp	Nevirapine	Human immunodeficiency virus type-1	Poly(rA)-oligo(dT) as template primer	6.6
RdRp	Remdesivir	Mycobacterium tuberculosis	N/A	<3.7
RdRp	Remdesivir	Respiratory syncytial virus	N/A	6
RdRp	Ribavirin	HEK293 human embryonic kidney cells (influenzavirus A (H1N1)-infected)	Luciferine/luciferase assay	5.1
RdRp	Ribavirin	Human norovirus	N/A	4.2
RdRp	Rifalazil	Mycobacterium tuberculosis	N/A	>8.0
RdRp	Rifampicin	Escherichia coli	N/A	7.9
RdRp	Rifampicin	Mycobacterium tuberculosis	N/A	7.5
RdRp	Rifampicin	Mycobacterium tuberculosis	N/A	7.8
RdRp	Rifampicin	Staphylococcus aureus	N/A	7.8
RdRp	Suramin sodium	Human norovirus	Fluorescent assay	7.6
RdRp	Suramin sodium	Murine norovirus	Fluorescent assay	7.2
RdRp NS5B	2'-C-Methylguanosine	HCV	N/A	6.9
RdRp NS5B	ACH-3422	HCV	N/A	5.9
RdRp NS5B	AG-21541	HCV	Radioactivity assay	7.7
RdRp NS5B	AL-335	HCV	N/A	6.9
RdRp NS5B	Beclabuvir	Bovine viral diarrhea virus	Radioactivity assay	<4.6
RdRp NS5B	Beclabuvir	HCV	Poly(rA)-oligo(dT) as template primer	8.3
RdRp NS5B	Cordycepin	HCV	N/A	4.7
RdRp NS5B	Danoprevir	HCV	N/A	8.3
RdRp NS5B	Deleobuvir sodium	HCV	N/A	7.3
RdRp NS5B	Digitoflavone	HCV	Radioactivity assay	4.9
RdRp NS5B	Filibuvir	HCV	N/A	8
RdRp NS5B	GS-6620	HCV	RNA as template primer	>5.0
RdRp NS5B	GSK-2485852	HuH7 human liver cancer cells	N/A	7.3
RdRp NS5B	GSK-625433	HCV	N/A	8.5
RdRp NS5B	HCV-371	HCV	N/A	6.7
RdRp NS5B	mCyd	HCV	N/A	5.3
RdRp NS5B	mCyd	HCV	N/A	5.5
RdRp NS5B	MK-0608	HCV	Polymerase assay	7
RdRp NS5B	Naringenin	Dengue virus	Radioactivity assay	<4.3
RdRp NS5B	Nesbuvir	HCV	Radioactivity assay	7.4
RdRp NS5B	Nesbuvir	HCV	N/A	7.5
RdRp NS5B	Pinocembrin	Dengue virus	Radioactivity assay	<4.3
RdRp NS5B	PSI-6130	HCV	Radioactivity assay	5.4
RdRp NS5B	PSI-6130	HCV	N/A	5.7
RdRp NS5B	Quercetin	Dengue virus	Radioactivity assay	5.5
RdRp NS5B	Raltegravir potassium	HCV	N/A	<4.3
RdRp NS5B	Ribavirin	HCV	N/A	4.5
RdRp NS5B	Tegobuvir	HCV	RNA as template primer	7.1
RdRp NS5B	TMC-647055	HCV	Transcription assay	7.5
RdRp NS5B	Vaniprevir	HCV	N/A	8
RdRp NS5B	Vidarabine	HCV	N/A	4.3
RdRp NS5B	VX-135	HCV	N/A	6.2
RdRp NS5B-1a	Beclabuvir	HCV	Radioactivity assay	8.5
RdRp NS5B-1a	Dasabuvir	HCV	N/A	8.7
RdRp NS5B-1a	Dasabuvir	HCV	N/A	>8.0
RdRp NS5B-1a	GS-6620	HCV	N/A	6.7
RdRp NS5B-1a	IDX-184	HCV	Radioactivity assay	7.1

Continued

Target	Drug	Material	Method	IC_{50} (-log mol·L^{-1})
RdRp NS5B-1a	IDX-375	HCV	N/A	7.8
RdRp NS5B-1a	JTK-853	HCV	Uridine incorporation assay	7.8
RdRp NS5B-1a	Nesbuvir	HCV	N/A	7.4
RdRp NS5B-1a	Ursolic acid	HCV	Poly(rA)-oligo(dT) as template primer	5
RdRp NS5B-1a	VX-135	HCV	N/A	>8.5
RdRp NS5B-1b	Beclabuvir	HCV	Radioactivity assay	8.4
RdRp NS5B-1b	BILB-1941ZW	HCV	Uridine incorporation assay	7.2
RdRp NS5B-1b	Dasabuvir	HCV	N/A	8.5
RdRp NS5B-1b	Dasabuvir	HCV	N/A	>5.0
RdRp NS5B-1b	Deleobuvir sodium	HCV	N/A	7.3
RdRp NS5B-1b	Filibuvir	HCV	Radioactivity assay	7.1
RdRp NS5B-1b	Filibuvir	HCV	Radioactivity assay	<7.0
RdRp NS5B-1b	Filibuvir	HCV	N/A	8.2
RdRp NS5B-1b	GS-6620	HCV	N/A	6.3
RdRp NS5B-1b	IDX-184	HCV	Radioactivity assay	6.5
RdRp NS5B-1b	IDX-375	HCV	N/A	8.3
RdRp NS5B-1b	JNJ-54257099	HCV	Uridine incorporation assay	6.9
RdRp NS5B-1b	Lomibuvir	HCV	Radioactivity assay	7.3
RdRp NS5B-1b	Lomibuvir	HCV	Radioactivity assay	8.2
RdRp NS5B-1b	MK-3281	HCV	Uridine incorporation assay	5.2
RdRp NS5B-1b	Nesbuvir	HCV	N/A	7.4
RdRp NS5B-1b	PSI-352938	HCV	Uridine incorporation assay	6
RdRp NS5B-1b	PSI-6130	HuH7 human liver cancer cells	Viral replication assay	7.2
RdRp NS5B-1b	Radalbuvir	HCV	Radioactivity assay	7.4
RdRp NS5B-1b	Silybin	HCV	Radioactivity assay	3.1
RdRp NS5B-1b	Silymarin	HCV	Radioactivity assay	3.5
RdRp NS5B-1b	Taxifolin	HCV	Radioactivity assay	<2.8
RdRp NS5B-1b	Tegobuvir	HCV	Radioactivity assay	<4.0
RdRp NS5B-1b	VX-135	HCV	N/A	7.9
RdRp NS5B-2a	Dasabuvir	HCV	Uridine incorporation assay	4.8
RdRp NS5B-2a	GS-6620	HCV	N/A	6.2
RdRp NS5B-2a	IDX-184	HCV	Radioactivity assay	6.9
RdRp NS5B-2a	IDX-375	HCV	N/A	<6.0
RdRp NS5B-2a	JTK-853	HCV	Uridine incorporation assay	<5.0
RdRpNS5B-2a	PSI-352938	HCV	Uridine incorporation assay	5.3
RdRp NS5B-2a	Ursolic acid	HCV	Poly(rA)-oligo(dT) as template primer	5.1
RdRp NS5B-2a	VX-135	HCV	N/A	6.7
RdRp NS5B-2b	Beclabuvir	HCV	Radioactivity assay	6.8
RdRp NS5B-2b	IDX-184	HCV	Radioactivity assay	6.9
RdRp NS5B-2b	VX-135	HCV	N/A	7
RdRp NS5B-3a	Beclabuvir	HCV	Radioactivity assay	8.7
RdRp NS5B-3a	GS-6620	HCV	N/A	7.3
RdRp NS5B-3a	IDX-375	HCV	N/A	<6.0
RdRp NS5B-3a	JTK-853	HCV	Uridine incorporation assay	6.6
RdRp NS5B-3a	PSI-352938	HCV	Uridine incorporation assay	5.9
RdRp NS5B-3a	VX-135	HCV	N/A	6.9
RdRp NS5B-4a	GS-6620	HCV	N/A	7
RdRp NS5B-4a	JTK-853	HCV	Uridine incorporation assay	6.7
RdRp NS5B-4a	PSI-352938	HCV	Uridine incorporation assay	5.4
RdRp NS5B-4a	VX-135	HCV	N/A	6.4
RdRp NS5B-5a	Beclabuvir	HCV	Radioactivity assay	8.3
RdRp NS5B-5a	GS-6620	HCV	N/A	6.9
RdRp NS5B-5a	VX-135	HCV	N/A	7.3
RdRp NS5B-6a	Beclabuvir	HCV	Radioactivity assay	7.2
RdRp NS5B-6a	GS-6620	HCV	N/A	7
RdRp NS5B-6a	VX-135	HCV	N/A	7.4
RdRp PB2	ACH-3422	Polymerase PB2	N/A	<4.0

括检测系统、实验方法和IC_{50}。受篇幅所限, 参考文献暂不列出。其中lopinivir和ribavirin是已上市的广谱抗病毒药, 对SARS和MERS冠状病毒都有抑制作用。

4 靶向宿主的临床在研药物

ACE2是新型冠状病毒树突蛋白的受体。蛋白激酶Furin和TMPRSS2剪接、激活和释放RNA进入宿主细胞。表2列举了靶向这3个蛋白的临床药物的体外筛选结果, 包括检测系统、实验方法和IC_{50}。受篇幅所限, 参考文献暂不列出。其中camostat是已经上市的治疗慢性胰腺炎药物, 对SARS和MERS冠状病毒都有抑制作用。

5 上市药物分析

表1和表2列出了75个临床在研药物, 其中有20个已经按单药上市, 12个按固定剂量联合用药上市。20个上市药物是: 比卡鲁胺 (bicalutamide)、甲磺酸卡莫司他 (camostatmesylate)、卡托普利 (captopril)、丹诺普韦 (danoprevir)、达塞布韦 (dasabuvir)、恩杂鲁胺 (enzalutamide)、巯嘌呤 (mercaptopurine)、霉酚酸钠盐 (mycophenolic acid sodium salt)、奈韦拉平 (nevirapine)、氯硝柳胺 (niclosamide)、丙泊酚 (propofol)、槲皮素 (quercetin)、拉替拉韦 (raltegravir)、利巴韦林 (ribavirin)、利福平 (rifampicin)、水飞蓟素 (silymarin)、苏拉明钠 (suramin sodium)、硫鸟嘌呤 (tioguanine)、伐尼瑞韦 (vaniprevir) 和阿糖腺苷 (vidarabine)。现随机选取恩杂鲁胺作为代表列出其作用机制、在研适应症、终止适应症、给药方式、研发单位、药代动力学参数、治疗范围和商标名等, 见表3。

6 挑战和展望

研发抗病毒药面临着诸多困难: ① 单链RNA病毒变异快, 冠状病毒亚型众多, 既使得已有特异的冠状病毒单抗和疫苗难以对新型冠状病毒有效, 又使得广谱的抗病毒药难以有强效, 如广谱抗病毒药克力芝在抗新型冠状病毒临床应用中就面临药效弱的问题; ② 若广谱药物所需的有效治疗浓度过高, 会导致有效剂量和血药浓度比率 (EC_{50}/C_{max}) 过高, 这对于药物的体内药代动力学和毒理学都有较高的要求, 如正在新型冠状病毒上进行临床前测试的氯喹的EC_{50}高达3 μmol·L^{-1} [3], 将来在临床应用中可能会面临EC_{50}/C_{max}过高的问题; ③ 冠状病毒的外切核糖核酸酶nsp14-ExoN有RNA校对功能, 使得多种核苷类抗病毒药效减弱或易产生抗药性[31]; ④ ACE2转基因小鼠模型和灵长类的动物模型对于新型冠状病毒的感染能力尚需

Table 2 Representative compounds that target host receptor and proteases utilized by CoV for viral entry, and *in vitro* testing results. ACE2: Angiotensin-converting enzyme 2; TMPRSS2:Transmembrane protease, serine 2

Target	Drug	Material	Method	IC_{50} (-log mol·L^{-1})
ACE2	Captopril	Human enzyme	Angiotensin I as substrate	<3.0
ACE2	MLN-4760	Heart, mouse	2,4-Dinitrophenyl-7-methoxycoumarin as substrate	4.7
ACE2	MLN-4760	Mononuclear cells (bone marrow), mouse	2,4-Dinitrophenyl-7-methoxycoumarin as substrate	6.9
ACE2	MLN-4760	Recombinant human enzyme	2,4-Dinitrophenyl-7-methoxycoumarin as substrate	8.5
ACE2	*N*-(2-Aminoethyl)-1-aziridineethanamine	Recombinant human enzyme	Peptide 7Mca-Y-V-A-D-A-PK(Knp)-OH as substrate	4.2
ACE2	*N*-(2-Aminoethyl)-1-aziridineethanamine	SARS S1-expressing cells	Beta-galactosidase activity	<4
ACE2	Peptide 4	HeLa cells with ACE2	SARS pseudovirus infection	4.3
ACE2	Peptide 5	HeLa cells with ACE2	SARS pseudovirus infection	5.2
ACE2	Propofol	Artery (pulmonary), human	RNA assay	5
ACE2	Tiliquinol	Recombinant human enzyme	Peptide 7Mca-Y-V-A-D-A-PK(Knp)-OH as substrate	2.7
Furin	Agmatine	Recombinant human enzyme	*L*-pyroGlu-Arg-Thr-Lys-Arg-7-amino-4-methylcoumarin as substrate	<7.1
Furin	Andrographolide	Human enzyme	Fluorescent assay	3.7
TMPRSS2	ABBV-075	LNCaP clone FGC human prostate cancer cells	RNA assay	8.7
TMPRSS2	Apalutamide	LNCaP human prostate carcinoma cells (androgen-dependent)	RNA assay	6.3
TMPRSS2	Bicalutamide	LNCaP human prostate carcinoma cells (androgen-dependent)	RNA assay	6.1
TMPRSS2	Camostat	Human enzyme, trypsin		7.3
TMPRSS2	Enzalutamide	LNCaP clone FGC human prostate cancer cells	RNA assay	6.7

Table 3 Detailed analysis of identified marketed drugs, using enzalutamide as an example

Drug	Enzalutamide
Target	TMPRSS2
Mechanism of action	Molecular: androgen receptor antagonists; Cellular: signal transduction modulators
Condition	Active: cancer [bladder, endometrium, prostate metastatic, salivary glands, liver (hepatocellular carcinoma), ovary (epithelial), pancreas (adenocarcinoma), prostate (castration-resistant)]; Inactive: cancer [breast, prostate, triple negative breast cancer, peritoneum, fallopian tube, kidney (renal cell carcinoma, clear cell)]
Administration route	Oral
Organizations	Original: Astellas Pharma, Medivation; Active: Astellas Pharma, Medivation; Inactive: Shanghai Fosun Pharmaceutical (Group), State University of New Jersey (Rutgers), University of California, Oakland
Calculated properties	CLOG P = 2.13; PSA = 108.53; FRB =5.00; MW = 464.44; H DON = 1.00; H_ACC = 6.00; SOL pH 7.4 = 0.00; LOGD pH 7.4 =2.13
Therapeutic groups	Renal Cancer Therapy, Prostate Cancer Therapy, Oncolytic Drugs, Bladder Cancer Therapy , Ovarian Cancer Therapy, Pancreatic Cancer Therapy, Liver Cancer Therapy, Breast Cancer Therapy, Female Reproductive System Cancer Therapy, Head and Neck Cancer Therapy
Name brand	Xtandi

要建立和确证; ⑤ 临床试验环节鉴于伦理考虑, 对于重症新型冠状病毒肺炎患者如何开展有安慰剂组的对照试验问题; ⑥ 新型病毒突发来得快, 去得也快, 但新药研发是一个漫长的过程, 研发出来的针对特定病毒亚型的特效药, 治疗性抗体和疫苗不一定能赶得上使用, 从商业角度来讲开发抗病毒药不一定能盈利, 这也会挫伤医药行业开发新药的积极性。

解决问题的办法, 除了疾病爆发后的早筛查早隔离早治疗外, 可以效仿美国的生物盾计划 (Project Bioshield Act)[32], 或由政府、非盈利机构和药企三方联手, 在临床前阶段开发一批疫苗和强效的广谱抗病毒药, 以备突发的新型病毒爆发。临床前研发相对于临床研发所需的费用要少很多, 而且也不会有在健康人身上试验的医学伦理顾虑。一旦有新型病毒爆发, 可迅速启动这批疫苗和药物的临床试验和患者救治。由北京市政府、比尔盖茨基金会和清华大学三方联手的非盈利机构"全球健康药物研发中心", 就是为研发不盈利但在发展中国家有巨大市场需求的"穷人药", 如寄生虫感染而设立, 可为借鉴。

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