中国细胞生物学学报 Chinese Journal of Cell Biology 2026, 48(1): 129–145
DOI: 10.11844/cjcb.2026.01.0009
CSTR: 32200.14.cjcb.2026.01.0009

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细胞外囊泡作为治疗药物: 从基础研究到临床转化的研究进展及挑战

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摘要 细胞外囊泡(EVs)作为一种具有良好的生物相容性与一定的靶向特性的天然纳米级生物活性载体, 能够有效递送生物活性物质至受体细胞并调控其功能, 这一特性使其在创新药物研发中显示出广阔前景。该综述系统回顾了EVs药物的发展历程与研究进展, 涵盖其基础药理机制、临床应用潜力及产业化关键挑战。EVs在神经系统疾病、肿瘤免疫治疗与组织再生等领域具有独特治疗优势, 部分候选药物已进入临床研究阶段。然而, 该类药物的产业化仍面临诸多瓶颈, 包括

收稿日期: 2025-09-16 接受日期: 2025-12-16
中国医学科学院医学与健康创新工程基金(批准号: 2021-I2M-1-005、2021-I2M-1-035、2023-I2M-2-005)和国家自然科学基金青年科学基金(批准号: 82401712、82402139)资助的课题
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Received: September 16, 2025 Accepted: December 16, 2025
This work was supported by the CAMS Innovation Fund for Medical Sciences (CIFMS) (Grant No.2021-I2M-1-005, 2021-I2M-1-035, 2023-I2M-2-005), and the Young Scientists Fund of the National Natural Science Foundation of China (Grant No.82401712, 82402139)
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生产工艺体系和质量控制体系的标准化困难, 以及全球范围内监管指南尚不完善等问题。该文系统梳理了EVs药物的研发路径与转化现状, 提出了基于“质量源于设计”(QbD)理念的开发策略, 并探讨了符合监管要求的非临床研究中关键问题的考量方向。最后, 该文呼吁科学家、企业家和监管部门通力合作, 共同推动EVs创新药物的研发、提高药物评审和产品转化, 从而开辟药物研发的全新赛道。

关键词 细胞外囊泡; 临床转化; 质量控制; 监管科学

Extracellular Vesicles as Therapeutic Agents: Progress and Challenges in Translational Research

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Abstract EVs (extracellular vesicles), as natural nanoscale bioactive carriers with favorable biocompatibility and certain targeting properties, can effectively deliver bioactive cargo to recipient cells and regulate their functions. These biological features of EVs make them promising candidates for innovative drug development. This review provides a comprehensive overview of the historical development and contemporary advancements in EV-based therapeutics, encompassing fundamental pharmacological mechanisms, clinical application potential, and key challenges in industrial-scale translation. Accumulating evidence indicates that EVs possess unique therapeutic advantages in neurological disorders, cancer immunotherapy, and tissue regeneration, with several candidate products having already advanced to clinical trial stages. However, the industrial translation of such drugs still faces multiple bottlenecks, including difficulties in standardizing the production process and quality control systems, as well as the lack of comprehensive regulatory guidelines globally. This article systematically reviews the development pathway and current translation status of EV-based drugs, proposes a development strategy based on the QbD (quality by design) concept, and discusses key considerations in non-clinical research to align with regulatory requirements. Finally, it calls for collaborative efforts among scientists, entrepreneurs, and regulatory authorities to jointly advance the development of innovative EV-based therapeutics, enhance the drug evaluation process, and promote product translation, thereby pioneering a new frontier in drug development.

Keywords extracellular vesicles; clinical translation; quality control; regulatory science

细胞外囊泡(extracellular vesicles, EVs)是由细胞分泌的、具有脂质双层膜结构的纳米颗粒, 其内部包裹蛋白质、核糖核酸(ribonucleic acid, RNA)、脂质及代谢物等多种生物活性成分。EVs不具备自我复制能力, 其主要功能是介导细胞、组织、器官乃至整个生物体之间的信息交流[1]。根据EVs的生成机制不同, EVs可分为两类: 一类通过质膜出芽形成的微囊泡、微颗粒和大囊泡(粒径约50 nm~1 μm); 另一类由质膜内陷形成多泡体(multivesicular body, MVB), MVB与质膜融合后释放的粒径为30 nm~150 nm的外泌体。值得注意的是, MVB可与其他细胞器或囊泡融合, 进一步丰富外泌体的组成成分[2]。EVs具有与亲本细胞相似的生物学特性及天然靶向能力, 不仅能有效穿越包括血脑屏障在内的多种生物屏障, 还因其低免疫原性、低致瘤性以及富含多种生物活性分子的特点, 成为极具治疗潜力的创新药物载体[3-4]。

EVs携带亲本细胞的生物学信息, 将蛋白质、脂质、代谢物和核酸传递到受体细胞中, 有效调控受体细胞的生物学反应[2-5]。这种调控作用具有双向性:

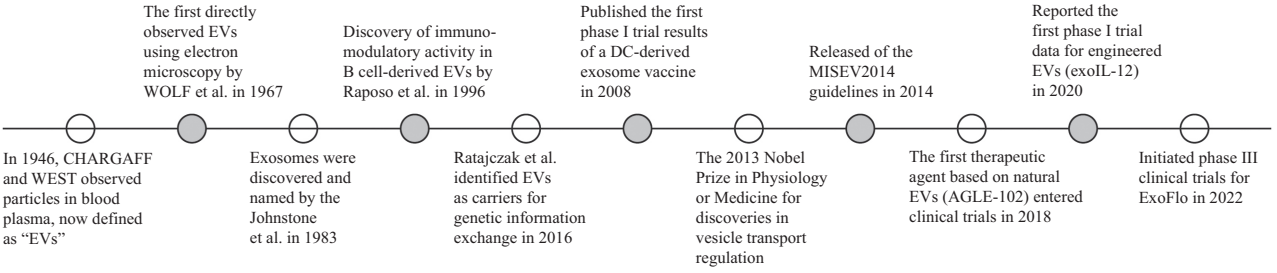


图1 EVs药物发展历史

Fig.1 The development history of EVs-based therapeutics

既可参与疾病发展进程, 也可能对病理发展产生抑制作用[6-9]。EVs在复杂细胞内信号通路调节中的固有特性, 推动其在神经退行性疾病、癌症及其他难治性疾病中的应用。EVs可通过递送自身活性成分发挥治疗作用, 同时也可负载外源性或内源性治疗分子, 包括蛋白质[10-11]、核酸[12-13]、小分子化合物[14-15], 或通过表面蛋白修饰提高血液循环时间、组织靶向性及细胞摄取率, 实现疾病的精准治疗[4,16-18]。

尽管EVs具有独特优势, 但其药物制备与表征尚缺乏基于关键质量属性(critical quality attributes, CQAs)的标准化方法, 同时在药品监管方面面临巨大挑战, 包括产品定义和分类不明确, 以及缺乏与研发相关的官方技术指南。本文系统回顾了EVs药物的发展历程与基础研究进展, 总结其生产工艺与质量控制技术, 并分析面临的挑战与可能的解决策略, 为EVs临床转化提供参考。

1 EVs药物发展历史

EVs的发现可追溯至1946年, CHARGAFF与WEST[19]在血浆中观察到颗粒状结构物质, 后被确认为EVs, 但因技术限制, 其生物学意义未获重视。1967年, WOLF团队[20]利用电子显微镜首次直接观察到EVs的形态, 为后续研究提供了超微结构基础。至1983年, JOHNSTONE团队[21-22]在网织红细胞成熟过程中发现并命名了"外泌体", 标志着EVs一类重要亚群的身份确立。

随后近二十年, EVs一度被误认为是细胞代谢副产物或"细胞垃圾"[21]。RAPOSO团队[23]首次从功能层面揭示EVs在免疫系统中的潜在作用, 为其在细胞信号传递及调控网络中的研究开辟了新方向。2006年, RATAJCZAK团队[24]发现外泌体可在细胞间转移信使RNA(messenger RNA, mRNA)与microRNA, 并且这些核酸分子在受体细胞中发挥重要功能, 确认EVs是功能性遗传信息载体, 而非单纯信号分子。此发现引发了全球研究热潮。2008年, 首个基于树突细胞外泌体的癌症疫苗I期临床试验结果发表, 标志外泌体研究从基础向临床迈进[25]。2013年, 研究囊泡运输调控机制的科学家(James E. ROTHMAN、Randy W. SCHEKMAN、Thomas C. SUDHOF)获得诺贝尔生理学或医学奖, 为EVs的生物发生、分泌及靶向机制提供理论基础, 推动该领域进入分子机制研究快车道。随着研究深入, EVs异质性及研究可重复性问题逐渐显现。2014年, 国际细胞外囊泡协会(International Society for Extracellular Vesicles, ISEV)发布《最小实验要求指南》(Minimal information for studies of extracellular vesicles 2014, MISEV2014), 为EVs的分离、鉴定及功能研究设立全球共识标准, 显著提升了研究规范性与可比性[26]。2022年, 间充质干细胞来源EVs产品ExoFlo率先进入III期临床试验(NCT05354141), 用于急性呼吸窘迫综合征(acute respiratory distress syndrome, ARDS)治疗, 成为全球首个进入关键试验阶段的EVs疗法(图1)。最近几年, EVs在基础研究和创新药物研发领域成为热点之一, 本文主要总结了EVs药物在基础研究、临床应用的进展以及产业化研究进展, 并对其成药性及未来研究方向进行了探讨。

2 EVs药物基础研究进展

EVs凭借优异的生物相容性、低免疫原性及较高的结构稳定性, 在神经系统疾病、炎症性疾病、癌症及再生医学等多个领域展现出独特的应用潜力。下文主要围绕干细胞和免疫细胞来源EVs进行阐述。

2.1 神经系统疾病

WIKLANDER等[27]研究表明，静脉注射的EVs中约有总剂量的1%进入大脑，显示EVs具有优异的穿过血脑屏障的能力。基于该特性，多项研究致力于探索EVs在神经系统疾病的治疗潜力，包括脑卒中、创伤性脑损伤、阿尔茨海默病(Alzheimer's disease, AD)、帕金森病等神经系统的创伤性和退行性疾病。这些疾病通常涉及神经元死亡和神经系统炎症因子风暴等为关键致病机制。研究表明，EVs可通过抑制神经元凋亡[28-29]，减轻神经炎症反应[30-31]，促进神经元或血管再生[32]等多重机制发挥治疗作用。此外，通过对EVs进行工程化改造，可进一步增强EVs的靶向性和治疗效果。例如，负载脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)[33]或miR-210[34]等活性分子的EVs，能够显著促进缺血性脑卒中动物的神经功能恢复并改善预后；经狂犬病毒糖蛋白(rabies virus glycoprotein, RVG)修饰的间充质干细胞(mesenchymal stem cells, MSCs)来源的EVs，显著增强了EVs对AD模型小鼠的脑靶向性[35]。除上述疾病外，近期研究还报道EVs在人类免疫缺陷病毒(human immunodeficiency virus, HIV)相关的神经认知疾病[36]、痴呆[37]、抑郁[38]、癫痫[39]等多种中枢神经系统疾病中也展现出显著的治疗潜力。

2.2 癌症

EVs在肿瘤免疫治疗中显示广阔前景。基于免疫或肿瘤细胞来源的EVs具有天然免疫调节功能，可直接激活抗肿瘤免疫反应，或作为药物载体递送抗癌药物。树突细胞来源外泌体(dendritic cell-derived exosomes, DEX)富含主要组织相容性复合体I/II类(major histocompatibility complex class I/II, MHC I/II)、白细胞分化抗原63(cluster of differentiation 63, CD63)、白细胞分化抗原81(cluster of differentiation 81, CD81)及整合素等膜蛋白，具有显著免疫激活能力[40-43]。经过Toll样受体3(Toll-like receptor 3, TLR-3)配体修饰的DEX，明显激活黑色素瘤特异性$CD8^+$ T淋巴细胞，并招募细胞毒性$CD8^+$细胞、自然杀伤细胞(natural killer cell, NK)和NK-T细胞至肿瘤病灶区，从而发挥肿瘤杀伤作用，并40天生存率从0%提高至60%[44]。此外，ZHU等[45]研究表明，NK细胞来源的外泌体能特异性杀伤黑色素瘤细胞，且对正常细胞无明显毒副作用。为了进一步增强NK细胞来源EVs(natural killer cell-derived extracellular vesicles, NEO)的免疫调节功能，ZHANG等[46]利用亲水性小干扰RNA(short interfering RNA, siRNA)和疏水性光敏剂Ce6对NEO进行工程化修饰，获得光激活沉默NK衍生外泌体(light-activatable silencing NK-derived exosome, LASNEO)。LASNEO表现出明显的肿瘤杀伤能力，并恢复T淋巴细胞在肿瘤微环境中的免疫监视功能。同时光照后产生的活性氧促进M1型巨噬细胞激化、树突状细胞(dendritic cell, DC)成熟和加速siRNA在肿瘤微环境中的细胞进入和体内逃逸，实现免疫治疗。此外，肿瘤来源EVs激活DC，实现抗肿瘤免疫激活[47]。肿瘤来源EVs携带内源性肿瘤抗原和CpG DNA(cytosine-phosphate-guanine DNA)，显著激活DC2.4细胞，增强肿瘤抗原提呈能力及抗肿瘤效果[48]。

2.3 再生医学

干细胞疗法在再生医学领域具有广泛应用，例如在伤口修复与组织再生方面展现出巨大潜力。然而，干细胞疗法存在免疫排斥、致瘤和病毒干扰等安全性风险。并且，近年来研究表明，干细胞疗法的主要作用机制可能更倾向于旁分泌途径，特别是通过EVs介导的再生、促增殖以及免疫调节等功能[49]。因此基于EVs的治疗策略逐渐成为替代活细胞疗法、降低相关风险的新方向[50-51]。

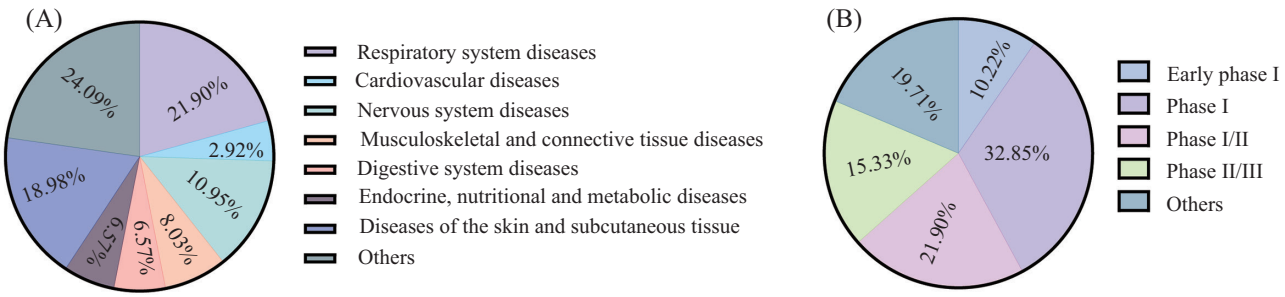
目前，EVs 已被应用于骨、心脏、肝、肾、皮肤和毛发等多种组织的再生。例如，骨髓来源的间充质干细胞来源的EVs富含miR-25，通过保护Runx2免受泛素化降解，促进小鼠骨折愈合[51]，也可以通过激活PI3K/AKT和MAPK信号通路促进骨形成[52]。MSC-EVs亦通过免疫调节、抑制上皮细胞凋亡、促进血管再生和抗炎等多重作用机制促进皮肤修复[53-56]。负载血管内皮生长因子A(vascular endothelial growth factor A, VEGF-A)全长mRNA的EVs增加缺血性损失小鼠动脉灌注，促进血管新生和改善左心室功能[57]。巨噬细胞来源的EV通过激活Wnt/β-catenin通路，有效促进毛囊生长并增加毛囊的毛干尺寸[58]。此外，TAN等[59]研究表明，MSC-EVs激活静息态肝细胞，抑制炎症因子表达，上调再生基因，减少肝损伤，促进肝脏再生。KO等[60]也证实MSC-EVs具有改善功能性肾组织的再生和修复的作用。

3 EVs药物临床试验进展

为了全面检索与EVs相关的临床研究, 我们以“exosome”或“extracellular vesicles”为关键词, 在ClinicalTrials.gov数据库进行系统性检索。截至2025年6月25日, 共识别出相关临床研究710项, 其中137项旨在探索EVs用于疾病治疗的潜力, 涵盖呼吸系统疾病(如COVID-19)、心血管疾病(如扩张型心肌病)、神经系统疾病(如脑卒中、癫痫)、肌肉骨骼系统相关疾病(如膝关节炎)、消化系统疾病(如结肠炎)、内分泌/营养/代谢病(如糖尿病足)、皮肤与皮下组织疾病(如皮肤损伤, 脱发)等多个领域。呼吸系统疾病研究占比最高, 达21.90%, 其次为皮肤与皮下组织疾病(18.98%)及神经系统疾病(10.95%)(图2A)。在研究阶段方面, 27项研究未明确研究阶段, 43.1%的研究处于早期I期或I期研究阶段, 15.3%的研究已经进入II期或更高阶段临床试验(图2B), 其中有7项研究进入III期临床试验(表1)。其中, 在中国, 共开展43项研究(表2)。

值得注意的是, 共有16项临床研究专注于探索EVs作为药物递送载体的应用。其中, 2项由美国路易斯维尔大学发起的研究, 通过利用植物来源EVs负载姜黄素分别用于治疗结肠癌(NCT01294072)和炎症性肠病(NCT04879810)。其余14项研究则是基于基因工程化EVs展开的试验(表3)。例如, 以色列研究者及其合作团队开发了一款基于CD24蛋白工程化过表达的293细胞来源EVs药物(EXO-CD24), 并在急性呼吸窘迫综合征患者中对其安全性和有效性进行了系统评估[61](NCT04969172、NCT05947747、NCT04902183、NCT04747574)。其他代表性策略包括利用EVs通过递送*Ldlr* mRNA治疗家族性高胆固醇血症(NCT05043181)、递送KRAS G12D siRNA基因治疗KRAS G12D突变型转移性胰腺癌(NCT03608631), 递送miR-124治疗急性缺血性脑卒中(NCT03384433), 以及使用同时表达CD3和CD19抗体的MSC-EVs治疗复发/难治性急性B细胞白血病(NCT06890494)。

从细胞来源角度, 在当前临床研究中, MSCs来源外泌体的临床研究占主导地位, 超过全部研究数量的70%, 其中以骨髓和脂肪来源的间充质干细胞为主。除间充质干细胞来源EVs外, 其他类型干细胞衍生的EVs也展现出令人瞩目的治疗潜力。例如, 同济医院发起的一项临床研究正在评估人诱导神经干细胞来源的EVs(NouvSoma001)治疗视神经脊髓炎谱系疾病的安全性、耐受性和初步有效性的探索性临床研究。值得注意的是, 人诱导多能干细胞来源EVs(human induced pluripotent stem cell-derived extracellular vesicles, hiPSC-EVs)在规模化生产和质量控制体系等药学研发方面显示出巨大优势, 同时在多种适应症上也表现出良好的安全性和有效性。北京协和医院药理研究中心和神经内科癫痫中心开展了一项针对成人难治性局灶性癫痫的安全性、难治性及初步有效性的探索性临床研究(NCT05886205)。结果显示, 未报告任何药物相关的不良事件或严重不良事件, 并观察到初步有效性。另外, hiPSC-EVs在多个适应症上也开展了临床研究, 包括特应性皮炎(NCT05969717)、儿童癫痫性脑病(ChiCTR2400091107)、急性缺血性脑卒中(NCT06138210)、孤独症(ChiCTR2500102199)、局限性白癜风、中重度宫腔粘连(NCT06810869)等。在这些试验中, 均未发生药物相关的不良事件或严重不良事件。此外, 一款源于神经干细胞的EVs创新药AB126, 已于2024年获得美国食品药品



A: EVs药物临床适应症分类统计图; B: EVs药物临床研究阶段统计图。

A: statistical classification of clinical indications for EV-based therapeutics; B: distribution of clinical trial phases for EV-based therapeutics.

图2 基于ClinicalTrial.gov (截至2025-06-25)统计的EVs药物临床结果

Fig.2 Clinical outcomes of EVs-based therapeutics based on ClinicalTrials.gov statistics (data cutoff: June 25, 2025)

表1 EVs/外泌体临床试验进入II/III期和III期研究信息表

Table 1 Clinical trial information for extracellular vesicle/exosome-based therapeutics in phase II/III and phase III studies

NCT号 NCT number	研究题目 Study title	适应症 Conditions	项目发起者 Sponsor	研究阶段 Phases	开始日期 Study start	截至日期 Study completion	状态 Study status
NCT05354141	Extracellular vesicle treatment for ARDS (acute respiratory distress syndrome) (extinguish ARDS)	Acute respiratory distress syndrome	Direct Biologics, LLC	Phase III	2022-07-01	2025-08-31	Recruiting
NCT04761562	Use of autologous plasma rich in platelets and extracellular vesicles in the surgical treatment of chronic middle ear infections	Otitis media chronic, tympanic membrane perforation	University Medical Centre Ljubljana	Phase II/III	2021-02-14	2023-09-30	Unknown status
NCT06539273	Exosome treatment in androgenetic alopecia	Androgenetic, alopecia	Yeditepe University Hospital	Phase III	2024-01-01	2024-05-05	Completed
NCT02138331	Effect of microvesicles and exosomes therapy on β-cell mass in T1DM (type I diabetes mellitus)	Diabetes mellitus type 1	General Committee of Teaching Hospitals and Institutes, Egypt	Phase II/III	2014-04	2014-09	Unknown status
NCT05216562	Efficacy and safety of exosome-MSC therapy to reduce hyper-inflammation in moderate COVID-19 patients	SARS-CoV2 infection	Dermama Bioteknologi Laboratorium	Phase II/III	2021-07-01	2022-12-30	Unknown status
NCT05413148	The effect of stem cells and stem cell exosomes on visual functions in patients with retinitis pigmentosa	Retinitis pigmentosa	TC Erciyes University	Phase II/III	2022-08-05	2023-12-15	Unknown status
NCT05126758	A study of deramiocel (CAP-1002) in ambulatory and non-ambulatory patients with Duchenne muscular dystrophy	Myocardial infarction	Capricor Inc.	Phase III	2022-06-22	2026-12	Active, not recuriting

表2 中国地区EVs/外泌体的临床试验表

Table 2 Clinical trial list of EVs/exosomes in China

NCT号 NCT number	EVs来源 Source	适应症 Conditions	项目发起者 Sponsor	截至日期 Study completion
NCT06598202	MSCs	Amyotrophic Lateral sclerosis	Xuanwu Hospital, Beijing	2026-05-30
NCT05787288	MSCs	COVID-19 pneumonia	First Affiliated Hospital of Wenzhou Medical University	2025-01-23
NCT06825572	MSCs	Liver failure	Third Affiliated Hospital, Sun Yat-Sen University	2026-09-30
NCT06612710	Human-induced neural stem cells	Ischemic stroke	Tongji Hospital	2027-11-30
NCT06620809	Human-induced neural stem cells	Neuromyelitis Optica spectrum disorders	Tongji Hospital	2027-11-30

续表2

NCT号 NCT number	EVs来源 Source	适应症 Conditions	项目发起者 Sponsor	截至日期 Study completion
NCT05881668	MSCs	Liver failure, acute on chronic	Third Affiliated Hospital, Sun Yat-Sen University	2025-04-30
NCT05940610	MSCs	Acute-on-chronic liver failure, acute liver failure	Third Affiliated Hospital, Sun Yat-Sen University	2025-10-01
NCT06607900	Umbilical cord mesenchymal stem cells	Alzheimer' disease, Parkinson disease, Lewy body dementia, multiple system atrophy, Fronto-temporal dementia	Xuanwu Hospital, Beijing	2028-08-31
NCT06890494	Engineered mesenchymal stem cell line stably co-expressing surface CD3 and CD19 antibodies	Leukemia	Beijing GoBroad Hospital	2026-12-31
NCT05808400	Umbilical cord mesenchymal stem cells	Long COVID-19 syndrome	Huazhong University of Science and Technology	2025-02-15
NCT06253975	Human adipose tissue	Wound heal	Shanghai Ninth People's Hospital Affiliated to Shanghai Jiao Tong University	2025-12-31
NCT05326724	Acupuncture-induced Exosome	Post-stroke dementia, acupuncture	China Medical University Hospital	2025-07-31
NCT05475418	Human adipose tissue	Wounds and injuries	Shanghai Ninth People's Hospital Affiliated to Shanghai Jiao Tong University	2023-10-15
NCT05043181	LDLR mRNA-overexpressing bone marrow MSCs	Familial hypercholesterolemia	Tang-Du Hospital	2026-12-01
NCT06932393	Unknown	Hair loss	Guangzhou Bio-Gene Technology Co., Ltd	2028-04-30
NCT06853522	Umbilical cord mesenchymal stem cells	Ulcerative colitis	Shanghai East Hospital	2028-04-04
NCT05871463	MSCs	Decompensated liver cirrhosis	RIGLD (Research Institute for Gastroenterology and Liver Diseases)	2023-12-11
NCT04544215	MSCs	Drug-resistant	Ruijin Hospital	2025-03-01
NCT04276987	MSCs	Coronavirus	Ruijin Hospital	2020-07-31
NCT05158101	Allogeneic adult umbilical cord derived MSCs	Stroke	The Foundation for Orthopaedics and Regenerative Medicine	2026-02-01
NCT06810869	hiPSCs	Stable vitiligo	Second Affiliated Hospital of Wannan Medical College	2026-11-17
NCT06138210	hiPSCs	Acute ischemic stroke	Xuanwu Hospital, Beijing	2025-08-30
NCT05152394	Allogeneic adult umbilical cord derived MSC	Parkinson disease	The Foundation for Orthopaedics and Regenerative Medicine	2027-01-01
NCT06896747	Mechanically engineered stem cells	Thin endometrial lining, female infertility, intrauterine adhesions	Tang-Du Hospital	2027-03-31
NCT04213248	Umbilical cord mesenchymal stem cells	Dry eye disease	Zhongshan Ophthalmic Center, Sun Yat-sen University	2023-12-01
NCT05738629	PSC-derived MSCs	Dry eye disease	Second Affiliated Hospital, School of Medicine, Zhejiang University	2025-02-01
NCT05559177	APC-tumor chimeric cells	Recurrent or metastatic bladder cancer	Shanghai Pudong Hospital	2023-09-01
NCT04313647	MSCs	Healthy	Ruijin Hospital	2020-07-31

续表2

NCT号 NCT number	EVs来源 Source	适应症 Conditions	项目发起者 Sponsor	截至日期 Study completion
NCT03437759	MSCs	Macular holes	Tianjin Medical University	2021-12-30
NCT04602104	MSCs	Acute respiratory distress syndrome	Ruijin Hospital	2023-01-11
NCT05152368	Allogeneic adult umbilical cord stem cells	Peripheral neuropathy, trigeminal neuralgia	The Foundation for Orthopaedics and Regenerative Medicine	2026-01-01
NCT05969717	hiPSCs	Atopic dermatitis	Peking Union Medical College Hospital	2025-06-30
NCT05886205	hiPSCs	Refractory focal epilepsy	Peking Union Medical College Hospital	2025-11-13
NCT04388982	Allogenic adipose MSC	Alzheimer disease	Ruijin Hospital	2022-08-01
NCT06919380	MSCs	anti-MDA5 positive dermatomyositis-associated RP-ILD, rapidly progressive interstitial lung disease	Guangzhou Medical University	2027-05-31
NCT06245746	Umbilical cord mesenchymal stem cells	Acute myeloid leukemia, neutropenia, anemia, thrombocytopenia, infections, bleeding	Wuhan Union Hospital, China	2027-02-01
NCT04356300	MSCs	Multiple organ failure	Fujian Medical University	2030-09-01
NCT02957279	Dendritic cells	Sepsis	Jinling Hospital, China	2017-10-01
NCT06221787	Umbilical cord mesenchymal stem cells	Melasma	Fujian Medical University Union Hospital	2026-07-01
NCT06677931	Umbilical cord mesenchymal stem cells	Melasma	Fujian Medical University Union Hospital	2026-08-01
NCT04850469	MSCs	Sepsis, critical illness	Children's Hospital of Fudan University	2024-12-31
NCT06697080	Umbilical cord-derived mesenchymal stem cells	Androgenic alopecia	Nanfang Hospital, Southern Medical University	2024-12-28
NCT06599346	MSCs	Mucositis, chemotherapy-induced mucositis, radiation-induced mucositis	The General Hospital of Western Theater Command	2025-09-30

监督管理局(Food and Drug Administration, FDA)批准，将开展用于缺血性脑卒中治疗的Ib/IIa期临床试验，标志着干细胞来源EVs药物在神经疾病治疗领域迈向新的临床阶段。在137项研究中，45项研究是激活和/或招募状态，27项试验是完成或终止状态，剩余的试验属于未激活状态(包括未招募、暂停、退出或状态未知)。在已完成的研究中，仅有7项研究报告了研究结果。NCT05523011研究评估了间充质干细胞外泌体软膏的安全性与耐受性，共招募了10例患者(女性6例，男性4例)。所有患者每天用药3次，连续用药20天，未观察到不良事件。NCT04491240研究共招募了30例患者，其中20例接受EVs吸入气雾剂治疗，10例接受安慰剂治疗。患者出院30天后，未观察到不良事件发生，疗效指标保持稳定。由上海交通大学医学院附属瑞金医院发起的NCT04313647研究评估了间充质干细胞EVs吸入雾化剂的安全性，共纳入24例患者，分别接受5个剂量组单次给药，观察7天。仅在最低和最高剂量组中各报告1例患者窦性心动过缓。NCT03857841研究旨在评估静脉注射干细胞来源EVs(UNEX-42)对高风险早产儿的安全性，因商业决策提前终止。在纳入的3例患者中，2例患者接受20 pmol磷脂/kg治疗，1例患者接受安慰剂治疗。治疗组中1例患者出现坏死性结肠炎(严重不良事件)，1例患者出现非严重不良事件。NCT04493242研究为ExoFlo产品用于治疗COVID-19的I期临床试验。结果显示，ExoFlo在所有受试者(n=68)中耐受性良好，未发生任何严重不良事件。与安慰剂治疗组(n=34)相比，Exo-Flo

降低患者死亡风险, 目前该产品已进入III期临床研究。NCT03406780试验评价了来源于心肌球细胞的EVs(CAP-1002)用于治疗杜氏肌营养不良症的有效性和安全性。治疗组(n=8)较安慰剂组(n=11)症状得到显著改善。在治疗过程中, 有三名患者出现了输注相关的过敏反应(其中1例患者因严重过敏反应中止治疗), 无长期后遗症或其他重大不良事件, 无死亡报告[62]。

基于已报道的临床研究结果, 相比于细胞治疗, EVs在临床应用中展现出优异的安全性和耐受性特征, 并且在多种类型疾病中表现出显著的治疗潜力。随着技术的飞跃与对EVs生物学机制的深度破译, EVs作为一种具有良好临床转化前景的新型治疗药物平台, 正在不断突破传统治疗的局限性, 拓展其应用疆界。目前其在恶性肿瘤、免疫相关疾病等重大疾病领域中的应用范围正在迅速扩展, 预示着其在再生医学与精准医疗中将发挥日益重要的推动作用。

4 EVs药物的产业化进展

为推动EVs疗法的临床转化, 目前仍面临诸多挑战。近年来, 多项研究围绕EVs来源获取、纯化工艺优化及产率提升展开探索(表3)。

4.1 上游

细胞来源EVs的上游工艺包括细胞培养与培养上清收集。EVs的生物学活性与供体细胞密切相关, 因此选择合适细胞来源并确定最佳培养条件至关重要。原代细胞(如MSCs和其他干细胞)的生物学特性可能存在供体个体间差异, 即使来自同一供体, 不同批次细胞之间的特性亦可能存在差异。相比之下, hiPSCs作为EVs的供体细胞展现出多方面的优势(表4)。首先, hiPSCs可从自体或异体来源的1个体细胞经重编程获得, 便于建立遗传背景一致的单克隆细胞株, 从源头上降低了细胞异质性; 其次, hiPSCs具有强大的自我更新能力, 可在体外长期培养并维持未分化状态和多向分化潜能; 再次, hiPSCs更易于实现质量可控和规模化培养, 为标准化生产EVs提供了稳定可控的细胞来源; 此外, hiPSCs来源的EVs富含多种细胞因子, 如转化生长因子(transforming growth factor-beta, TGF-β)、干细胞因子(stem cell factor, SCF)、血管内皮生长因子(vascular endothelial growth factor, VEGF)等。hiPSC-EVs能够介导广泛的细胞间通讯, 靶向多种细胞类型, 发挥抗炎、再

表3 基因工程化EVs/外泌体的临床试验表

Table 3 Clinical trials of genetically engineered extracellular vesicles/exosomes

NCT号 NCT number	研究题目 Study title	适应症 Conditions	工程化靶点 Engineered	项目发起者 Sponsor	研究阶段 Phases
NCT05043181	Exosome-based nanoplatform for *Ldlr* mRNA delivery in FH	Familial hypercholesterolemia	*Ldlr* mRNA	Tang-Du Hospital	Phase I
NCT04969172	A phase II randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of exosomes overexpressing CD24 to prevent clinical deterioration in patients with moderate or severe COVID-19 infection	COVID-19 disease	CD24	Eli Sprecher, MD	Phase II
NCT05947747	Safety and efficacy of Exo-CD24 in preventing clinical deterioration in patients with mild-moderate ARDS	ARDS	CD24	Nano24med	Phase II
NCT04902183	Safety and efficacy of exosomes overexpressing CD24 in two doses for patients with moderate or severe COVID-19	COVID19	CD24	Athens Medical Society	Phase II
NCT04747574	Evaluation of the safety of CD24-exosomes in patients with COVID-19 infection	SARS-CoV-2	CD24	Tel-Aviv Sourasky Medical Center	Phase I

续表3

NCT号 NCT number	研究题目 Study title	适应症 Conditions	工程化靶点 Engineered	项目发起者 Sponsor	研究阶段 Phases
NCT03608631	iExosomes in treating participants with metastatic pancreas cancer with KrasG12D mutation	KRAS NP_004976.2:p.G12D|Metastatic pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma, stage IV pancreatic cancer AJCC v8	*KRAS G12D* siRNA	M.D. Anderson Cancer Center	Phase I
NCT04173650	MSC EVs in dystrophic epidermolysis bullosa	Dystrophic epidermolysis bullosa	COL7COL7A1	Aegle Therapeutics	Phase I/II
NCT05843799	A study to evaluate the safety and tolerability of ILB-202	Healthy	superrepressor IκBα	ILIAS Biologics Inc.	Phase I
NCT05375604	A study of exoASO-STAT6 (CDK-004) in patients with advanced HCC (hepatocellular carcinoma) and patients with liver metastases from either primary gastric cancer or CRC (colorectal cancer)	Advanced HCC (hepatocellular carcinoma), gastric cancer metastatic to liver, colorectal cancer metastatic to liver	STAT6	Codiak BioSciences	Phase I
NCT05078385	Safety of extracellular vesicles for burn wounds	Burns	COL7COL7A1	Aegle Therapeutics	Phase I
NCT03384433	Allogenic mesenchymal stem cell derived exosome in patients with acute ischemic stroke	Cerebrovascular disorders	miR124	Isfahan University of Medical Sciences	Phase I/II
NCT01159288	Trial of a vaccination with tumor antigen-loaded dendritic cell-derived exosomes	Non-small cell lung cancer	MAGE-A1, MAGE-A3, NY-ESO, Melan-A/MART1 (all MHC-I restricted peptides); MAGE-A3, MAGE-A1, MAGE-A3, NY-ESO, Melan-A/MART1 (all MHC-I restricted peptides); MAGE-A3, MAGE-A1, MAGE-A3, NY-ESO, Melan-A/MART1 (all MHC-I restricted peptides); MAGE-A3, EBV (MHC-II–restricted peptides)	Gustave Roussy, Cancer Campus, Grand Paris	Phase II
NCT04592484	A first-in-human study of CDK-002 (exoSTING) in subjects with advanced/metastatic, recurrent, injectable solid tumors	Advanced solid tumor	STING	Codiak BioSciences	Phase I/II
NCT06890494	Clinical study on the safety and efficacy of BiTE-EV in relapsed/refractory acute B-cell leukemia	Leukemia	CD3, CD19	Beijing GoBroad Hospital	Early phase I

生和促进血管生成等重要作用, 为hiPSC-EVs应用于多种疾病的治疗提供了坚实的理论基础。

为确保EVs的质量一致性, 建立符合规范的细胞库至关重要。除使用自体细胞外, 应根据《国际人用药品注册技术协调会(International Council for Harmonisation, ICH) Q5D指南》[63]或其他相关指导原则建立细胞库。对于基因修饰的细胞, 还需参照ICH Q5B指南对基因表达构建体进行分析, 并对细胞库实施严格管控[64], 以确保生产所用细胞的身份明确、质量一致。DAVID等[65]提出了细胞培养的规范化管理参考标准, 为获得可重复的高质量的细胞提供了指导文件。

细胞培养条件和培养基组成与EVs的质量和产量呈直接相关性。近年来, 多种扩大细胞培养规模的技术和工艺不断发展, 例如3D培养技术[66]和生物反应器系统[67]的开发应用, 这些方法的目的不仅实现获得最大数量的细胞, 并通过减少操作时间、培养时间和消耗品(如培养基)的用量来降低总体成本。另一个策略是通过改变细胞生长环境, 如血清饥饿、缺氧或采用物理/化学刺激等方式刺激细胞, 促进细胞对EVs的释放[68-69]。尽管这种方式能够显著提高EVs的产量, 但是可能会产生具有其他表型的EVs[70-71], 因此, 在工艺开发中需要充分评估其对EVs质量的影响, 特别关注生物学活性和杂质组成(如非EVs颗粒的占比)等方面的变化。

为了解决EVs产量和内容物有限等问题, HU等[72]开创了一种自上而下的方法制备细胞来源的新型仿生纳米颗粒, 为EVs替代物的制备提供了一个高产量低成本的替代方案。采用挤出、超声处理、低渗处理、碱处理、反复冻融循环、细胞匀浆器处理或这些方法的组合来处理一种或多种细胞[73-77], 使细胞分解成携带亲本细胞的内容物, 并具备与EVs类似的功能的小囊泡。但是, 目前此类纳米颗粒仍处于探索阶段。并且, 物理处理手段对蛋白质结构造成不可控影响, 多组分膜融合增加产品复杂性的同时也为产品的质控带来巨大的挑战。

4.2 下游

无论EVs来源于细胞培养上清、生物体液或植物组织裂解液, 均需下游纯化去除杂质。EVs的最终产率、性质与纯度取决于纯化和富集策略。目前常用方法包括超速离心、密度梯度离心、离子交换色谱、亲和层析等。2020年, ISEV严谨性与标准化分会委员会公布的一项针对EVs分离和表征方法的调查报告, 结果显示, 截至2019年, 超速离心法的使用率仍超60%, 而自2016年以来, 离子交换色谱法的使用率显著增加[78]。在工业规模化生产中, 通常联合使用多种纯化策略以提高EVs的生产和纯度。例如, 在EVs下游纯化生产中, 使用过滤的方式进行样品澄清, 随后采用切向流超滤(tangential flow filtration, TFF)结合多种色谱纯化技术(阳离子交换色谱、阴离子交换色谱或混合模式色谱)进一步纯化样品。该类工艺的产能约为传统离心法的2 000倍[79]。过滤与色谱联用的纯化策略也已被应用于工程化EVs的纯化生产中, 例如通过亲和层析实现高效、特异性分离目标EVs[80]。此外, 有报道使用聚乙二醇(polyethylene glycol, PEG)沉淀法结合超速离心法, 用于制备异体脂肪干细胞来源的EVs雾化剂, 治疗急性肺损伤/ARDS[81]。另一种工艺则采用0.22 μm聚醚砜滤器进行初步澄清, 随后采用500 kDa截留分子量的切向流超滤膜对脂肪干细胞来源的EVs进行纯化和浓缩, 用于治疗急性肾损伤[82]。

目前, 难以有单一方法同时实现高纯度与高得率。超速离心虽是小剂量、实验室研发阶段的“金标准”, 但存在EVs破裂/聚集、杂质共分离、批间差异大及放大困难等问题[83], 不适用于工业级规模化生产。因此, 选择纯化方法时需兼顾重复性、经济性与通量, 在保证产品纯度和活性的同时, 满足商业化需求。

5 EVs药物临床转化面临的挑战

5.1 监管政策有待完善

尽管EVs在临床转化中展现出较好的前景, 但其开发仍面临多重挑战。根据ISEV发表的专家共识, 基于EVs的治疗产品分为4类: 未经基因修饰细胞来源的天然EVs、不含转基因产物的基因改造细胞来源的天然EVs、来自转基因细胞的含有转基因产物的EVs和作为药物递送系统递送化合物或重组分子的EVs[84]。然而, 不同国家和地区官方监管机构对EVs的分类标准并不统一。对于天然EVs, 含有丰富的蛋白质、核酸、脂质等成分。如果EVs作为治疗药物, 且治疗作用无法归因于一个或多个特定分子, 那么EVs本身将被视为活性物质。欧洲药品管理局(European Medicines Agency, EMA)将天然EVs作为活性物质来源于生物提取物, 因而将其划分为生物

表4 hiPSCs和MSCs作为EVs供体细胞比较优势表

Table 4 The key advantages and challenges of using hiPSCs *vs* MSCs for EV production

对比维度 Aspects for comparison	MSCs	hiPSCs
Source of the donor	Placenta, umbilical cord, etc.	A single cell
Method of donor acquisition	Overwhelmingly allogeneic	Autologous/allogeneic
homogeneity and stability	MSCs exhibit high heterogeneity The efficacy of exosomes derived from MSCs beyond passage 6 declines significantly and may even produce opposite effects	Single source, stable passaging, scalable production, and homogeneous characteristics
Manufacturing complexity	Lack of standardized processes Challenges in CMC	Standardized and scalable manufacturing process
Safety	Clinical validation	Clinical validation
Regeneration potential	Limited	Strong

制品。对于通过工程化手段，使EVs携带“重组”核酸时，该EVs药品符合EMA对基因治疗的定义，可将其划分为先进治疗药品(advanced therapy medicinal products, ATMPs)。如果递送物质不属于重组核酸，如合成的siRNA，那么该EVs则不属于ATMPs。FDA及其生物制品评价与研究中心(center for biologics evaluation and research, CBER)已确定，天然的、未经修饰的外泌体产品将被视为生物制品[85]。基于日本药品和医疗器械管理局(Pharmaceuticals and Medical Device Agency, PMDA)的科学委员会对EVs的评估报告，未经基因修饰细胞来源的天然EVs和作为药物递送系统递送化合物或重组分子的EVs视为生物制品，来自转基因细胞的含有转基因产物的EVs归类为基因治疗产品[86]。此外，2025年6月10日，中国国家药品监督管理局药品评审中心(Center for Drug Evaluation, CDE)发布的公开征求意见稿中拟将EVs纳入ATMPs监管体系[87]。

鉴于EVs药物领域较新且潜在产品种类广泛，在产品开发的早期阶段，建议开发者向相关监管机构咨询产品的分类问题，以明确产品开发计划的相关预期。

5.2 缺乏标准化的生产和质控指南

目前，EVs的生产、质量研究和质量控制缺少官方的技术指导原则，可参考的标准主要是由各组织发表的团体标准[1]。符合GMP(Good Manufacturing Practice)标准的规模化生产是EVs商业化的基础。然而，由于EVs来源的多样性，包括来源种类、培养环境、培养基、细胞库等因素的差异，导致了其异质性较高。对于细胞培养上清液来源的EVs，通过建立质量可控的细胞库可以在一定程度上降低EVs的异质性。相较之下，植物或牛奶等非细胞培养来源的EVs，其来源质量控制仍未有很好解决办法。此外，下游纯化工艺直接影响EVs的纯度与有效性。过度纯化虽然能够获得高纯度EVs，但可能降低产品的治疗效果，因为共分离(如可溶性蛋白)可能参与组成EV表面的蛋白质冠[88]，不应简单地被视为是杂质。因此，选择合适的纯化工艺需要充分结合产品适应症，安全性和有效性进行考量，保证去除安全性风险杂质同时保证产品的生物学活性。

MISEV2023(Minimal Information for Studies of Extracellular Vesicles 2023)为EVs表征提供了最低标准[1]。虽然目前有多种EVs的表征方法和设备，但是各种方法手段各自存在一定的局限性。例如，EVs浓度和粒径指标的检测，不同类型的设备对相同样品的检测结果差异明显，即使是相同原理的设备，对同一种样品的检测同样存在统计学差异[89-90]。并且目前检测颗粒浓度的设备仍无法区分EVs和非EVs颗粒。因此，需要结合其他方法评估产品的纯度。例如通过使用表面活性剂破坏EVs膜结构，通过检测破坏前后颗粒数的变化，推测样品中具有膜结构的颗粒占比[91-92]。但是，非EVs颗粒中可能包含同样具有脂膜结构的成分，如脂蛋白、凋亡小体、病毒颗粒[93]。因而在将该方法纳入质控体系时，需要严格遵循ICH Q2指导原则，系统验证其专属性。此外，酶活性分析法用于检测EVs膜完整颗粒的含量可能并不完全准确[94]，该种方法不能很好的将膜完整的EVs从膜破损或破裂的EVs中区分开。另

一方面, 由于EVs作用机制复杂, 目前没有通用的EVs活性评价方法, 开发者需要根据适应症和药物作用机制(mechanism of action, MOA)开发合适的EVs活性检测方法。同时, 最好能够确定EVs的可能活性成分, 并建立该成分含量检测方法。如若明确EVs是通过多种活性成分发挥治疗效果, 那么应针对不同成分建立能够反映其作用机制的活性评价方法。

6 EVs药物临床转化策略

EVs药物的临床转化亟需多学科协同创新和监管科学的共同发展。相较于传统生物制品, EVs药物在组成与功效上更为复杂, 其质量属性与疗效机制尚不完全明确, 生产和质控方面面临着多重挑战。因此, 在药物开发早期阶段, 引入“QbD“理念, 建立从CQAs到临床性能的连贯证据链, 将有助于加速药品申报进程。在产品开发初期, 明确适应症、给药方式、剂型等临床预期用途, 有助于指导非临床研究阶段的方案设计, 如选择与适应症相关的疾病动物模型及合适的给药方式。同时, 采用风险评估工具识别产品的CQAs, 并开发建立相应的质量评价方法, 有助于后续物料属性(如细胞类型、传代次数、培养条件)和工艺参数的筛选和优化。根据MISEV2023指南, EVs的质量特征包括形态、粒径、颗粒浓度、蛋白质含量、脂质含量、特定表面标志物、功能性分子(如蛋白质、miRNA)等。其中, 部分指标的检测方法可参考现行针对细胞/蛋白/核酸类药物的相关指导原则; 而对于EVs特有的属性, 如颗粒浓度、纯度、生物活性, 则需要结合产品特性选择适当的方法和检测设备, 实现对其特征的多维度表征, 并在符合ICH Q2(R1)对分析方法验证要求的前提下, 确保检测体系的可靠性; 另外, 监管机构也应加快出台对EVs的质量评价指南, 为企业明确的技术依据。在产品生产过程中, 产品的有效性和安全性需要权衡考虑。高纯度产品通常意味着杂质少, 安全性高。但是, 某些共分离物(如“蛋白质冠”)可能对EVs功能具有重要影响, 过度纯化反而可能削弱其生物活性。此外, 当前分离技术尚难以实现EVs和非EVs组分的完全分离。研究人员需要深入探究“杂质”的种类和安全限度, 监管机构需明确相关杂质的可接受最低标准, 以保障临床用药安全。

对于EVs药物的非临床研究, 动物种属的选择是首要考虑的问题。在传统药物非临床安全性评价中, 通常选择至少2种以上动物进行安全性评估。而针对EVs药物, 监管机构通常建议申办方依据其与临床使用的相关性充分论证所用动物模型的合理性。PMDA科学委员会已明确, 若EVs制剂来源于已用于人体且安全性风险较低的细胞, 其脱靶毒性风险较低, 不要求开展毒理的脱靶毒性研究, 并且可只在一种动物种属(包括啮齿动物)中进行毒性研究。如果天然EVs在临床使用小于6个月, 则无需开展致癌性试验研究。此外, 由于目前EVs制剂在体内的浓度难以准确定量, 一般不要求系统完成吸收、分布、代谢、排泄/药代动力学/药效学(absorption, distribution, metabolism, excretion/pharmacokinetics/pharmacodynamics, ADME/PK/PD)的研究[86]。特别是人源天然EVs, 其由人源成分构成, 评估其代谢和排泄的必要性较小。对于天然EVs体内分布试验, 不论采用何种方法对EVs进行标记, 都可能无法反映其生理学动态变化, 故而推荐考虑多种方法交叉验证。在实践中, 申报方可基于“风险–获益”分析策略, 结合具体问题具体分析原则, 制定非临床研究方案。同时鼓励申办方积极主动与监管机构保持沟通, 在满足申报需求的前提下尽可能控制成本、缩短研发周期。

目前, 在ClinicalTrail.gov网站上注册的EVs相关临床试验超过100项, 但仅约5%的临床研究公布了试验结果。积极推动临床数据的公开和共享, 对促进EVs药物的临床应用具有重要意义。另外, 在制定临床研究方案时, 特别是首次人体试验(first-in-human, FIH), 必须基于所有可用信息, 审慎设计试验方案, 包括初始剂量、给药间隔及风险管理措施, 以保障受试者安全并科学评估其初步疗效和安全性。

7 结论与展望

本综述系统回顾了EVs作为治疗性药物从基础研究到临床探索阶段所取得的重要进展, 充分揭示了EVs在临床应用领域的广阔前景。文章还探讨了EVs规模化生产的技术发展现状, 并剖析了当前转化过程中面临的关键瓶颈。目前, 该领域仍缺乏完善的监管框架与审评指南, 此类制度性缺失显著制约了EVs药物的规范开发与产业化进程, 同时导致开发者缺乏明确的技术和法规依据。

展望未来, 人工智能(artificial intelligence, AI)与机器学习(machine learning, ML)技术的深度融合,

将为EVs药物的研发带来革命性的推动力，特别是对于加速精准医疗进程至关重要。AI能够处理EVs复杂且高维的组学数据(如蛋白质组、脂质组和核酸组)，快速识别与特定疾病疗效或毒性相关的关键质量属性和生物标志物。例如，通过ML模型预测不同亲本细胞、培养条件下的EVs的生物活性和靶向性，从而指导“质量源于设计”理念下的上游工艺优化，实现特定功能EVs的精准筛选和规模化生产。然而，这一结合也面临挑战：首先是数据标准化和互操作性的难题，EVs的天然异质性要求建立大规模、高质量、符合MISEV标准的数据库；其次，需要开发可解释的AI模型(explainable AI, XAI)，以满足监管机构对药物作用机制和质量控制的透明化要求，确保AI辅助下的EVs创新药物的安全性和有效性。

我们倡议产学研各方与监管机构协同努力，共同推进EVs生产工艺的标准化、表征方法的创新及监管共识的形成，以期加速EVs药物的临床转化与上市应用，为多种疾病治疗提供新的解决方案与希望。

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