DOI:10.3724/cbls.2026085 **CSTR**:32203.14.cbls.2026001
文章编号:1004-0374(2026)04-0708-13

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基于肺癌免疫治疗的三特异性抗体的研究进展

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摘 要:肺癌是全球癌症死亡的首要病因,尽管肺癌免疫疗法已取得进展,但其临床响应率低、耐药性高及免疫逃逸等问题仍亟待解决。三特异性抗体(trispecific antibodies,TsAbs)通过三重靶点协同作用进一步提升了抗体疗效和安全性,在肺癌治疗中展现出突破现有瓶颈的巨大潜能。本文综述了TsAbs的构建模块及其在肺癌中的最新研究进展,重点探讨了其功能分类和临床转化动态。然而,TsAbs正面临免疫原性、免疫抑制性肿瘤微环境及精准靶向优化等方面的挑战。未来的研究方向应聚焦于抗体结构创新、条件激活性设计及四特异性抗体的探索,以推动TsAbs在肺癌治疗中的临床转化,为实现高效低毒的精准免疫治疗提供新策略。

关键词:三特异性抗体;肺癌;免疫治疗;T细胞接合器;NK细胞接合器;免疫检查点
中图分类号:Q511;R734.2 **文献标识码**:A

Research progress of tri-specific antibodies based on immunotherapy for lung cancer

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Abstract: Lung cancer remains the leading cause of cancer-related mortality globally. Although immunotherapy has brought revolutionary advances to the treatment of lung cancer, significant challenges—such as low clinical response rates, high rates

收稿日期:2025-11-25; **修回日期**:2026-01-13
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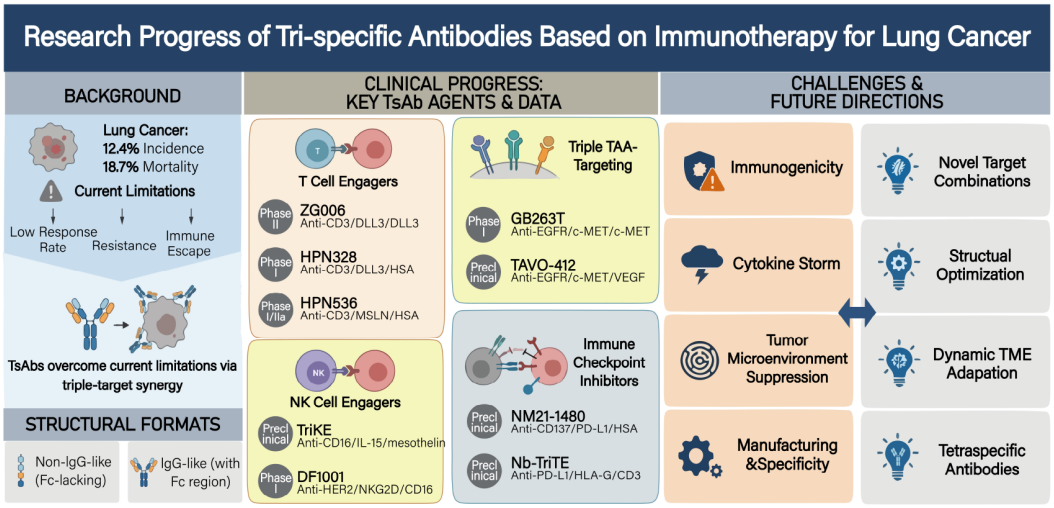
of primary and acquired resistance, and immune evasion—persist and underscore the urgent need for next-generation therapeutic strategies. In recent years, trispecific antibodies (TsAbs), which leverage synergistic targeting of three distinct antigens, have demonstrated breakthrough potential in achieving more precise immune activation while enhancing both efficacy and safety. This article systematically reviews the development and current landscape of TsAbs in lung cancer immunotherapy, highlighting their capacity to overcome limitations inherent to conventional monospecific and bispecific antibody approaches. The review begins by introducing the structural modules of TsAbs, which are broadly categorized into two formats: non-IgG-like formats designed to improve tumor penetration and reduce Fc-mediated toxicity, and IgG-like formats that retain Fc-dependent effector functions and exhibit extended serum halflife. Then, it provides a detailed overview of the functional classifications and clinical translation progress of TsAbs under investigation for lung cancer. These include T cell engagers, NK cell engagers, TsAbs targeting three tumor-associated antigens, and immune checkpoint inhibitors. Preclinical and earlyphase clinical data indicated that TsAbs could mediate potent tumor killing, mitigate common escape mechanisms, and remodel the immunosuppressive tumor microenvironment. Several candidates have already shown encouraging safety and efficacy profiles in Phase I/II trials. Despite these promising advances, TsAbs still face considerable challenges in clinical translation. Key hurdles include immunogenicity risks, manufacturing complexity, ontarget offtumor toxicities, and the highly heterogeneous and adaptive nature of the tumor microenvironment. Looking forward, we propose that future efforts should focus on innovative target combinations, structural optimization of antibody architectures, dynamic adaptation to the tumor microenvironment, and the exploration of tetraspecific antibody platforms. Through these strateges, TsAbs are poised to advance clinical translation in lung cancer therapy, offering new avenues for achieving highly effective, low toxicity, and precision immunotherapy.



Key words: trispecific antibody; lung cancer; immunotherapy; T cell engagers; NK cell engagers; immune checkpoint

2022年全球癌症统计数据显示,肺癌新发病例占全部癌症的12.4%,居恶性肿瘤之首,其死亡病例占全部癌症死亡的18.7%[1]。尽管诊疗手段的进步使肺癌患者5年相对生存率提升至27%,但肺癌仍属预后较差的癌症类型[2]。针对非小细胞肺癌(non-small-cell carcinoma,NSCLC;占比85%)和小细胞肺癌(small-cell carcinoma,SCLC;占比10%~15%),手术、化疗、放疗及靶向治疗虽取得进展,但晚期患者仍面临治疗瓶颈。在此背景下,免疫疗法的突破为肺癌治疗带来了革命性进展[3-5]。

目前肺癌免疫疗法包括免疫检查点抑制剂、细胞疗法及肿瘤靶向抗体等[6]。抗体药物作为其关键分支,已衍生出单特异性抗体、抗体-药物偶联物(ADC)及双/多特异性抗体三大类别[7]。然而传统单/双特异性抗体的疗效往往受到肺癌异质性和免疫微环境的限制,面临免疫逃逸、脱靶毒性和耐药性等挑战[8,9]。因此,具备多靶点协同作用的多特异性抗体成为突破瓶颈的关键。

三特异性抗体(trispecific antibodies,TsAbs)是一种可以同时靶向肿瘤细胞或免疫效应细胞三种不同抗原表位的创新疗法,通过多重机制协同实现精准免疫调控:(1)肿瘤抗原多靶点识别,降低单靶点逃逸风险;(2)免疫效应细胞双信号激活,克服免疫耗竭;(3)免疫抑制微环境干预。相较于单/双特异性抗体,三特异性抗体不仅能提升抗原结合特异性、降低脱靶效应,还能构建多重免疫调控网络,在肿瘤免疫治疗领域具有更广阔的应用前景[10,11]。本文讨论了三特异性抗体的构建模块及其在肺癌治疗中

的最新进展、当前挑战与未来方向。

1 三特异性抗体的构建模块

与双特异性抗体类似,TsAbs的构建模块可分为两大类:非IgG样(Fc缺失型)与IgG样(含Fc区域)[7]。

1.1 非IgG样(Fc缺失型)

非IgG样TsAbs的抗体衍生片段通过柔性连接体(如$(GGGGS)_3$)串联,形成“串珠样”(beads-on-string)结构。常用片段包括:(1)单链可变片段(single-chain variable fragment,scFv;25~30 kDa),由重链可变区(variable region of heavy chain,VH)和轻链可变区(variable region of light chain,VL)通过短肽连接,适合多价串联;(2)单域抗体(single-domain antibody,sdAb;12~15 kDa),源自骆驼科动物或鲨鱼,体积小、穿透力强,但需人源化降低免疫原性;(3)抗原结合片段(antigen-binding fragment,Fab;50 kDa),含完整轻链及重链VH-CH1,稳定性较高[12]。

非IgG样TsAbs分子量小(<100 kDa),易于渗透实体瘤致密微环境,同时避免Fc相关的抗体依赖性细胞吞噬或细胞因子释放综合征(cytokine release syndrome,CRS)等风险。因此,非IgG样TsAbs具有肿瘤穿透性强、灵活性高、安全性好的优势;但其小分子特性也会导致半衰期较短,需通过PEG(polyethylene glycol,聚乙二醇)化或与白蛋白结合延长半衰期;此外,连接体过长可能引发分子间聚集,需优化连接序列(如调节刚性/柔性比例)。

1.2 IgG样(含Fc区域)

IgG样TsAbs以IgG骨架为基础,保留了天然IgG的CH2-CH3结构,支持长效半衰期(约21 d);可通过异源二聚化技术(knob-into-hole,KIH)改造CH3结构域,强制异源重链配对,减少同源二聚体污染,或引入互补电荷促进异源链结合;其附加模块是在重链或轻链的N/C端融合scFv或sdAb,从而引入第三特异性[12]。因此,基于Fc介导的pH依赖性回收(FcRn结合),IgG样TsAbs可显著延长血清半衰期。同时,天然的IgG结构和Fc区域具有蛋白质降解风险低、保留效应功能的优势,适用于需要免疫激活的肺癌亚型。但分子量较大(>150 kDa)导致其难以渗透深层肿瘤组织;链配对也具有一定的复杂性,轻链错配可能产生非功能分子,需引入共同轻链或双抗平台(如Duobody[13])。图1展示了本文主要讨论的几种TsAbs的结构。

2 三特异性抗体在肺癌领域的研究进展

三特异性抗体按照作用机制的不同主要分为:T细胞接合器(T-cell engager,TCE)、NK细胞接合器(NK-cell engager,NKCE)、靶向三个肿瘤相关抗原(tumor associated antigen,TAA)、免疫检查点抑制剂等(表1)。

2.1 三特异性T细胞接合器

免疫细胞接合器是一类能够桥接免疫效应细胞(如T细胞或NK细胞)与肿瘤细胞的分子工具,通过重定向效应细胞的杀伤活性清除肿瘤。经典的免疫细胞接合器如卡妥索单抗(Catumaxomab)和贝林妥欧单抗(Blinatumomab)虽已获批用于临床,但在实体瘤治疗中面临特异性不足和脱靶毒性等挑战[14,15]。为克服这些局限,三特异性T细胞接合器(trispecific T-cell engager,TriTCE)通过引入第三功能域,进一步增强了肿瘤靶向性、免疫细胞活化效率及药代动力学特性[12]。

2.1.1 抗CD3/DLL3/DLL3

δ样蛋白3(delta-like ligand 3,DLL3)是Notch信号通路的非经典抑制性配体,在超过75%的SCLC中高表达,且与肿瘤增殖、转移及化疗耐药密切相关。其正常组织低表达的特性使其成为SCLC治疗的理想靶点[16,17]。早期靶向DLL3的抗体-药物偶联物Rova-T因疗效不足终止开发,而新型的多特异性T细胞接合器(如Tarlatamab)在临床试验中显示出显著临床获益,目前正在进一步开发中[18]。

ZG006是中国泽璟制药公司研发的抗CD3/DLL3/DLL3的三特异性T细胞接合器,可同时结合细胞毒性T淋巴细胞(cytotoxic T lymphocyte,CTLs)的CD3及肿瘤细胞双DLL3表位,激活并重定向CTLs特异性杀伤DLL3阳性肿瘤[19]。临床前研究表明,ZG006在小鼠模型中显著抑制肿瘤生长,并在非人灵长类动物中表现出良好安全性。根据泽璟制药公司口头报告,10 mg及以上剂量组在经多线治疗失败的晚期SCLC患者中疗效显著。目前两项Ⅱ期扩展研究正在推进中。

2.1.2 与人血清白蛋白结合的T细胞接合器

传统双特异性T细胞接合器缺乏Fc结构域、分子量小(约55 kDa)的特性使其易被肾脏快速清除(半衰期仅2~4 h),导致需提高给药频率或剂量以维

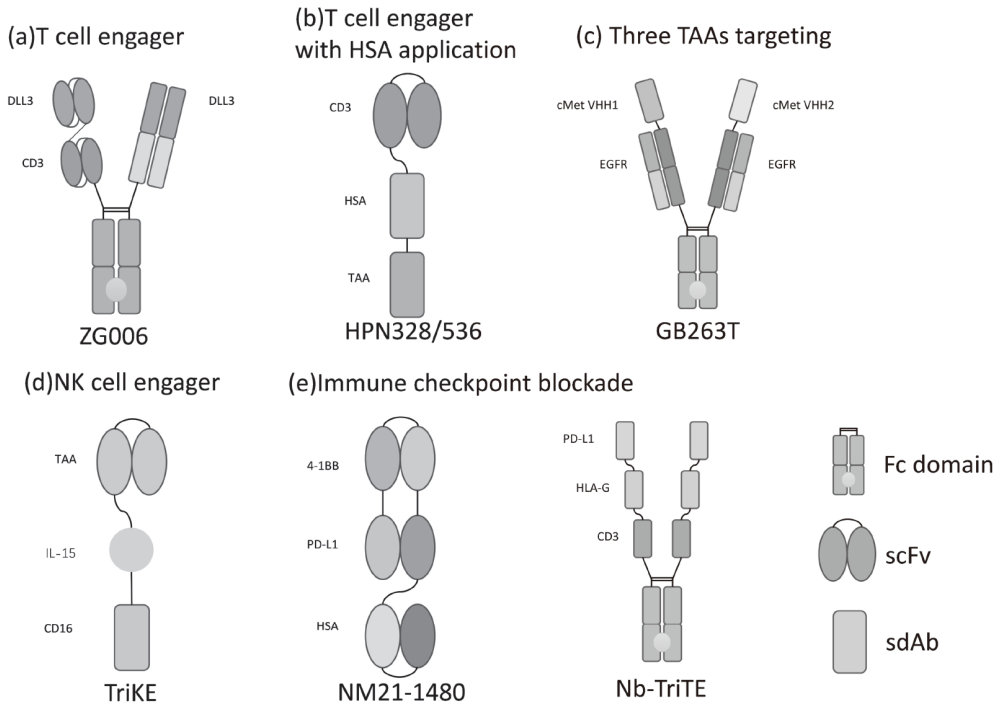


图1 几种TsAbs的结构示意图

(a)ZG006:包含一个抗CD3域和靶向两个不同的DLL3表位的抗DLL3域;(b)与HSA结合的TCE:包含靶向TAA的sdAb、抗HSA的sdAb和抗CD3的scFV;(c)GB263T:采用双人源化VHH结构设计,可同时识别EGFR及c-MET的两个不同表位;(d)TriKE:包含抗TAA的scFv,抗CD16的纳米抗体和IL-15。(e)NM21-1480:将分别靶向PD-L1、HLA-G和CD3的三个可变片段组装成三特异性单链双体;Nb-TriTE:包含抗 PD-L1、HLA-G、CD3的三种VHH。DLL3:δ样蛋白3;HSA:人血清白蛋白;cMet:细胞间充质-上皮转化因子;EGFR:表皮生长因子受体;TAA:肿瘤相关抗原;scFv:单链可变片段;sdAb:单域抗体。

Figure 1 Structural diagrams of several TsAbs

(a) ZG006: It contains an anti-CD3 domain and an anti-DLL3 domain targeting two different DLL3 epitopes; (b) TCE conjugated to HSA: It consists of an sdAb targeting TAA, an sdAb against HSA, and a scFv against CD3; (c) GB263T: It is designed with a dual humanized VHH structure capable of simultaneously recognizing two distinct epitopes of EGFR and c-MET; (d) TriKE: It includes an scFv against TAA, a nanobody against CD16, and IL-15. (e) NM21-1480: It assembles three variable fragments targeting PD-L1, HLA-G and CD3 into a trispecific single-chain dimer (scDb-scFv, also known as scMATCH3™ in the original text); Nb-TriTE: It contains three VHHs against PD-L1, HLA-G and CD3. DLL3: delta-like protein 3; HSA: human serum albumin; c-Met: mesenchymal-epithelial transition factor; EGFR: epidermal growth factor receptor; TAA: tumor-associated antigen; scFv: single-chain variable fragment; sdAb: single-domain antibody.

持疗效。为延长药物循环时间,基于人血清白蛋白(human serum albumin,HSA)的工程化策略被广泛应用于三特异性T细胞接合器的设计中。HSA不仅可通过FcRn介导的再循环延长药物半衰期(约19 d),还可通过高通透性和滞留效应(enhanced permeability and retention effect,EPR效应)被动富集于肿瘤组织,其小分子量特性亦有助于穿透实体瘤致密基质[20]。

2.1.2.1 抗CD3/DLL3/HSA

HPN328是一种抗CD3/DLL3/HSA的三特异性T细胞激活构建体,由靶向DLL3的sdAb、CD3ε单链可变片段及对HSA具有特异性的sdAb组成,其适应证为DLL3$^+$ SCLC和其他神经内分泌恶性肿瘤。HPN328在体外显著上调CD69/CD25的表达(5%~25%的T细胞活化),并诱导TNF-α、IFN-γ释放,从而高效杀伤SCLC细胞,EC50值为16~286 pmol/L[21]。

在免疫缺陷型NSG小鼠移植瘤模型中,HPN328(100 μg/kg)能抑制96%的肿瘤生长。在免疫健全的hCD3ε小鼠模型中,HPN328(200 μg/kg)可完全清除MC38-hDLL3肿瘤(9/9治愈)并诱导长期免疫记忆。此外,HPN328在食蟹猴中血清半衰期达78~187 h,

表1 治疗肺癌的三特异性抗体

Table 1 Tri-specific antibodies for lung cancer

药物名称	研发机构	作用靶点	适应证	NCT	研发阶段	分子量	半衰期
T细胞接合器							
ZG006	Suzhou Zelgen BioPharmaceuticals. Co.,Ltd.	Anti-CD3/DLL3/DLL3	SCLC and NET	NCT05978284	Ⅰ		
HPN328	Harpoon Therapeutics	Anti-CD3/DLL3/HSA	SCLC and NET	NCT04471727	Ⅰ/Ⅱ	~50 kDa	78~187 h
HPN536	Harpoon Therapeutics	Anti-CD3/MSLN/HSA	MSLN阳性肿瘤	NCT03872206	Ⅰ/Ⅱ	~53 kDa	49~113 h
靶向3个肿瘤相关抗原							
GB263T	Genor Biopharma Co. Ltd.	Anti-EGFR/c-MET/c-MET	NSCLC	NCT05332574	Ⅰ/Ⅱ		
TAVO-412	Tavotek Biotherapeutics Co. Ltd.	Anti-EGFR/c-MET/VEGF	NSCLC、GC、PDAC and TNBC	NCT06761651	Ⅰ		
NK细胞接合器							
TriKE	Philippa R Kennedy et al.	Anti-CD16/IL-15/Mesothelin	NSCLC		临床前	60 kDa	
CAM1615TEM8	Kaminski et al.	Anti-CD16/IL-15/STEM8	NSCLC		临床前		
DF1001	Dragonfly Therapeutics	Anti-HER2/CD16/NKG2D	HER2阳性实体瘤	NCT04143711	Ⅰ/Ⅱ		
免疫检查点抑制剂							
KB-436	Shugang Yao et al.	Anti-DRD2/PD-1/CD47	SCLC		临床前		~120 h
NM21-1480	Numab Therapeutics	PD-L1/CD137/HSA	NSCLC	NCT04442126	Ⅰ/Ⅱ		~14 d
Nb-TriTE	Yu-Chuan Lin et al.	antiPD-L1/HLA-G/CD3ε	NSCLC		临床前	~80 kDa	

注:SCLC,小细胞肺癌;NET,神经内分泌肿瘤;NSCLC,非小细胞肺癌;GC,胃癌;PDAC,胰腺导管癌;TNBC,三阴性乳腺癌

Note: SCLC, small cell lung cancer; NET, neuroendocrine tumor; NSCLC, non-small cell lung cancer; GC, gastric cancer; PDAC, pancreatic ductal adenocarcinoma; TNBC, triple negative breast cancer.

10 mg/kg重复给药(4周)耐受性良好,且无靶向毒性[21]。HPN328的Ⅰ/Ⅱ期研究中期结果显示其耐受性良好且具有临床活性,主要不良反应包括淋巴细胞减少以及CRS(IL-6、IFN-γ等;呈"首剂效应",后续给药未再现),最大耐受剂量测定、剂量递增和剂量优化正在进行中[22]。

2.1.2.2 抗CD3/MSLN/HSA

间皮素(mesothelin,MSLN)是多种实体瘤(如肺癌、卵巢癌、胰腺癌)高表达的糖蛋白,肿瘤特异性显著,是免疫治疗的理想靶点[23,24]。但当前MSLN靶向治疗存在反应率低、治疗窗窄的挑战。为此,Molloy等[25]设计了HPN536。

HPN536是一种抗CD3/MSLN/HSA的TriTAC,其小分子量设计(约53 kDa)兼顾了肿瘤穿透性与长效性(食蟹猴半衰期约63 h)。临床前研究显示,在卵巢癌、胰腺癌和NSCLC模型中,HPN536显著诱导T细胞活化和肿瘤细胞裂解,CD69/CD25表达上调(EC50=0.14~9.0 pmol/L),对MSLN阳性肺癌细胞的EC50低至3.8 pmol/L。在食蟹猴模型中,HPN536显示出良好的耐受性,单剂量10 mg/kg,无剂量限制毒性,可逆性不良反应包括短暂的淋巴细胞减少(24~72 h恢复)、IL-6瞬时升高等[25]。上述数据展示了HPN536在肺癌治疗中的潜力。当前Ⅰ/Ⅱa期研究(NCT03872206)正在评估其在MSLN阳性晚期癌症患者中的安全性、耐受性和药代动力学。

2.1.3 TriTCE与溶瘤病毒结合

溶瘤病毒(oncolytic virus,OV)是一类经基因工程改造的病毒,通过靶向感染及裂解肿瘤细胞、诱导免疫原性细胞死亡、激活肿瘤微环境中的免疫细胞等机制发挥抗肿瘤作用[26]。在临床前研究中,单纯疱疹病毒-1(HSV-1)、柯萨奇病毒B3(CVB3)、溶瘤痘苗病毒(OVV)、腺病毒(rAd-p53)、塞内卡谷病毒(SVV-001)等均显示出对肺癌的治疗潜力[27]。但OV单药疗法面临多种挑战,包括细胞外基质阻碍病

毒扩散、缺氧环境抑制病毒复制、抗病毒免疫记忆限制重复给药效果等[6]。

将三特异性T细胞接合器武装至OV载体的策略可提升免疫疗效,二者具有互补优势:OV选择性感染肿瘤细胞后,在肿瘤局部表达T细胞接合器分子,既突破肿瘤微环境对抗体传递的限制,又避免了脱靶毒性;OV诱导免疫原性细胞死亡释放的肿瘤抗原,与T细胞接合器介导的T细胞重定向可协同放大抗肿瘤免疫应答。同时,三特异性T细胞接合器诱导的T细胞活化不仅能清除邻近未感染的肿瘤细胞,还能增强与PD-L1抑制剂、CAR-T等联合治疗的协同效应[28]。这种联合精准的空间局部表达与系统性免疫激活的范式,为突破肺癌免疫治疗瓶颈提供了新方向。

2.2 靶向三个TAA

2.2.1 抗EGFR/c-MET/c-MET

表皮生长因子受体(epidermal growth factor receptor,EGFR)是一种可介导实体瘤生长信号的跨膜酪氨酸激酶受体,在超过60%的NSCLC患者中存在过表达或突变,是重要的治疗靶点[29]。第三代EGFR酪氨酸激酶抑制剂(tyrosine kinase inhibitor,TKI)虽已成为晚期EGFR突变NSCLC的一线治疗方案,但耐药问题仍亟待解决[30]。研究发现,细胞间质-上皮转化因子(cellular-mesenchymal epithelial transition factor,c-MET)信号通路的异常激活是EGFR-TKI耐药的主要机制之一[31]。c-MET作为一种关键受体酪氨酸激酶,其异常激活形式(METex14跳跃突变、MET-amp)在NSCLC中驱动肿瘤侵袭转移[32,33]。

GB263T采用双人源化VHH结构设计,可同时识别EGFR及c-MET两个不同表位[34],这种双重阻断机制显著抑制肿瘤细胞增殖并诱导细胞凋亡[31]。临床前研究显示,GB263T不仅抑制EGFR和c-MET受体的磷酸化及下游MAPK/PI3K通路的激活(优于同类药物JNJ-372),还能促进受体-抗体复合物的内化以降低膜表达水平,削弱信号转导能力[35]。在Ⅰ期临床试验(NCT05332574)中,GB263T在晚期EGFR突变NSCLC患者中安全性良好,且在1 260~1 680 mg剂量范围内表现出显著抗肿瘤活性,常见的治疗相关不良事件均为轻度,包括皮疹、疲劳、甲沟炎和输液相关反应等,验证了其临床转化潜能。

2.2.2 抗EGFR/c-MET/VEGF

实体瘤的进展不仅依赖EGFR和c-MET介导的增殖与转移,还与血管内皮生长因子(vascular endothelial growth factor,VEGF)驱动的血管生成有关。三者形成交互调控网络,导致治疗耐药及肿瘤复发[36]。因此,同时阻断EGFR、c-MET及VEGF的三靶点策略成为突破现有治疗瓶颈的新方向。

TAVO-412是全球首个抗EGFR/c-MET/VEGF的TsAb,其设计融合了抗血管生成与直接抗肿瘤的双重机制:(1)通过结合EGFR和c-MET受体,抑制其磷酸化及下游促存活信号(如STAT3和NF-KB);(2)阻断VEGF与VEGFR的结合,抑制内皮细胞增殖及肿瘤血管生成;(3)通过Fc段介导的抗体依赖性细胞毒性、抗体依赖性细胞吞噬作用和补体依赖性细胞毒性,进一步增强对NSCLC细胞系(如H1975)的杀伤效力(EC50≈0.003 nmol/L)[37]。临床前研究表明,TAVO-412在23种CDX及9种肺癌PDX模型中均显著抑制肿瘤生长,10 mg/kg剂量可实现肿瘤部分/完全消退,联合EGFR-TKIs(拉泽替尼、奥希替尼)更能增强疗效[36,37]。TAVO-412独特的多靶点协同作用可有效逆转由单一通路抑制引发的代偿性激活,为克服耐药提供了新思路。

2.3 NK细胞接合器

当前三特异性NK细胞接合器(trispecific NK-cell engager,TriKE)的优化聚焦于NK细胞受体的选择(如CD16a、NKG2D、NKP30)、免疫检查点的加入(PD-1和CTLA4)、细胞因子的组合(如加入IL-15和IL-2可增强NK细胞增殖和细胞毒性,同时还能增强$CD8^+$ T记忆细胞的扩增)以及肿瘤微环境的调控(如抑制血管生成)[38,39]。

2.3.1 抗CD16/IL-15/间皮素

间皮素作为NSCLC中过表达的侵袭性标志物,其靶向治疗常受限于NK细胞失活和髓源性抑制细胞浸润的免疫抑制性肿瘤微环境[23]。Kennedy等[40]据此设计了一种抗CD16/IL-15/间皮素的TriKE,包括三个结构域:抗间皮素scFv,特异性结合NSCLC细胞表面间皮素;骆驼源抗CD16纳米抗体,通过FcγRIIIa受体直接激活NK细胞脱颗粒;IL-15,通过IL-15R信号通路促进NK细胞增殖、存活及活化。体外研究显示,其显著提升NSCLC患者NK细胞增殖能力(约2.5倍)及细胞毒性,且未引起脱靶效应。在NSG小鼠模型中,TriKE联合NK细胞使肿瘤体积缩小60%,并显著降低$CD31^+$血管密度。值得注意的是,TriKE可克服晚期患者$CD16^+$ NK比例下降及MDSC浸润的微环境障碍,在低CD16表达的

NK细胞中仍保持激活效力。临床前数据证实了其低毒高效特性,尤其适用于晚期免疫抑制性肿瘤微环境,且与PD-1抑制剂联用潜力显著。

2.3.2 抗CD16/IL-15/STEM8

肿瘤内皮标志物8(tumor endothelial marker 8,TEM8)是一种整合素样受体,特异性高表达于肺癌等实体瘤的血管内皮细胞及成纤维细胞,参与血管生成与基质重塑,是理想的治疗靶点[41]。CAM1615TEM8是一种抗CD16/IL-15/STEM8的TriKE,通过抗TEM8的单链抗体特异性结合肿瘤细胞和基质细胞表面的TEM8,引导NK细胞靶向杀伤肿瘤细胞。CAM1615TEM8的设计是对多靶点免疫治疗策略的创新:通过同时攻击肿瘤细胞(如肺癌A549细胞)和肿瘤基质(表达TEM8的肺微血管内皮细胞和正常肺成纤维细胞),有效破坏TAA的支持网络。三维肿瘤球体模型显示其清除效率显著优于IL-15单药。体内数据表明,CAM1615TEM8可使A549移植瘤体积缩小为原来的1/3,并使$CD31^+$血管密度降低约70%[42]。CAM1615TEM8在肺癌模型中的显著疗效和低毒性特征,为NSCLC的免疫治疗及联合治疗提供了新的方向。

2.3.3 抗HER2/NKG2D/CD16

人表皮生长因子受体2(human epidermal growth factor receptor 2,HER2)是EGFR家族成员,其激活机制包括蛋白质过表达、基因扩增和突变(如外显子20插入突变)[43],在约3%的肺腺癌中为驱动突变[44]。

DF1001是一种抗HER2/NKG2D/CD16的TriKE,靶向HER2的同时激活NK细胞和$CD8^+$ T细胞,将“冷肿瘤”转化为“热肿瘤”,增强免疫细胞浸润,重塑肿瘤免疫微环境。其适应证覆盖NSCLC等HER2低表达(IHC 1+)至高表达(IHC 3+)的实体瘤。在Ⅰ期临床试验中,DF1001表现出良好的安全性和耐受性,临床受益率为39.7%。其中79%的患者出现治疗相关不良事件,大多为低级别(86%为1~2级),包括输液相关反应、虚弱和疲劳[45]。不管是单药治疗还是联合用药,DF1001都为肺癌免疫治疗提供了新的方向。

2.4 免疫检查点阻断

免疫检查点阻断疗法(immune checkpoint blockade therapy,ICB)已被广泛应用于晚期 NSCLC的一线治疗,显著改善了患者的生存结局,但单独使用免疫检查点抑制剂(immune checkpoint inhibitor,ICI)疗效有限且具有耐药性[46]。因此,ICI正在往多特异性抗体的方向发展,通过双重阻断免疫抑制受体或联合激活免疫细胞发挥协同互补作用[11]。

2.4.1 抗DRD2/PD-1/CD47

多巴胺受体D2(dopamine receptor D2,DRD2)是一种在肺癌中高表达的G 蛋白偶联受体[47],DRD2拮抗剂可通过调控STAT、Wnt及PI3K/Akt通路抑制肿瘤生长,并诱导自噬和凋亡[48]。类似于PD-1,该受体也成为多特异性治疗中有吸引力的靶点。CD47是一种跨膜蛋白,在84%的肺癌患者中过表达,通过与SIRPα结合对巨噬细胞传递“别吃我”的信号从而逃逸免疫监视,有望成为NSCLC免疫检查点阻断疗法的新靶点[49]。

KB-436由三个单域VHH构成:抗DRD2域干扰肿瘤细胞内信号,抗PD-1域恢复T细胞功能,抗CD47域阻断吞噬抑制信号并招募T细胞。在DRD2阳性SCLC的SCID小鼠模型中,KB-436单药显著抑制肿瘤生长,与顺铂联用可克服化疗耐药并阻断转移,显著延长生存期[50]。这些数据凸显了KB-436针对晚期转移性实体癌适应证(包括 SCLC)的治疗潜力,为晚期肺癌治疗提供了新的转化方向。

2.4.2 抗CD137/PDL-1/HSA

共刺激受体4-1BB(CD137)是TNF受体超家族成员,其激活可增强T细胞存活、增殖,恢复肿瘤微环境中$CD8^+$ T细胞的抗肿瘤功能[51]。但早期4-1BB激动剂(如乌瑞芦单抗Urelumab)因肝脏毒性限制了其临床应用[52]。因此,开发兼具肿瘤靶向性、高效共刺激且无系统毒性的新型免疫疗法成为研究热点。

NM21-1480是基于scMATCH3技术构建的抗CD137/PDL-1/HSA三特异性融合蛋白,通过PD-L1靶向定位实现CD137特异性激活,避免系统性T细胞活化;同时,无Fc结构域设计消除了Fc介导的肝脏毒性风险,GLP猴毒理实验(140 mg/kg,每周给药一次,共5周)显示无肝损伤标志物升高。在HCC827(NSCLC)人源化小鼠模型中,NM21-1480使相对肿瘤体积(RTV)降低超过80%,并促进肿瘤内$CD8^+$ T细胞浸润,展现出广谱抗肿瘤潜力[53]。

2.4.3 抗PD-L1/HLA-G/CD3ε

在NSCLC免疫治疗中,HLA-G作为其中一种TAA和免疫检查点,通过结合抑制性受体(如LILRB1/KIR2DL4)抑制T/NK细胞功能,与PD-L1协

同促进免疫逃逸[54]。

新型三特异性T细胞衔接器(Nb-TriTE)通过抗PD-L1/HLA-G/CD3ε结构实现了双检查点阻断,克服了传统ICI靶点单一的局限性。实验显示,Nb-TriTE可同时靶向肿瘤细胞表面PD-L1及HLA-G,阻断其与PD-1/LILRB1的相互作用。在外周血单核细胞(PBMC)与肿瘤细胞共培养时,Nb-TriTE显著提升细胞杀伤效率且呈剂量与时间依赖性(PBMC与肿瘤细胞比值为6:1时,100 μg/mL Nb-TriTE 72 h杀伤率达80%左右),并促进穿孔素、颗粒酶B及促炎因子(TNF-α、IFN-γ)释放;在人源化原位肺癌小鼠模型中,Nb-TriTE使肿瘤生长抑制率提高40%,中位生存期显著延长。在安全性方面,Nb-TriTE不会导致肝肾毒性或CRS,且组织交叉反应低,仅与胎盘、淋巴结、胸腺等免疫相关组织微弱结合[55]。这样的双靶点设计有效应对了NSCLC 中PD-L1/HLA-G代偿激活的问题,具有更显著的临床转化潜力。

3 挑战与展望

3.1 挑战

三特异性抗体虽已取得重大进展,但仍面临以下关键挑战。(1)现有的临床前模型难以模拟人类免疫系统,存在肿瘤异质性不足、成本高昂等问题,亟需开发可靠模型以解析免疫调控机制及治疗毒性[6]。(2)肿瘤基因型和免疫表型的个体差异要求对患者实施个性化治疗,但目前临床上缺乏高效的预测标志物和动态监控体系。肿瘤微环境的时空异质性显著,具体表现为抑制性Treg细胞、M2型巨噬细胞等细胞亚群的动态分布以及免疫因子、免疫分子标志物的区域差异性[56,57],这使得精准靶向和持久应答难以实现。(3)TsAbs的免疫原性可能诱导抗药物抗体生成,降低疗效或引发超敏反应[58,59]。肺癌患者的慢性炎症状态、TsAbs的复杂结构(如异源序列、非经典IgG构型)和协同作用机制(如免疫细胞激活、双检查点阻断)均可能导致免疫原性风险升高,因此需在研发早期采用免疫球蛋白释放分析(immunoglobulin release assay,IgRA)策略进行风险预测,结合结构优化与动态临床监测降低风险[60]。为了避免脱靶毒性,未来对于新靶点的选择也应更加严格[61]。(4)TsAbs的多结合域和异源结构易在生产过程中导致蛋白质聚集、水解和产率低等问题[62]。肿瘤的靶点异质性导致TsAbs的特异性设计要求严苛,进一步增加了生产工艺难度,需开发新的表达系统和质控技术以突破生产瓶颈。

此外,不同类型的TsAbs在疗效上各有利弊。TriTCEs能够精准激活T细胞并增强抗肿瘤活性,解决了免疫逃逸的难题,克服了肿瘤因MHC分子缺失或抗原丢失导致的耐药性。相较于CAR-T疗法,TCE无需个体化制备,成本更低且可快速应用,但TCE仍存在一些局限。(1)TCE激活大量T细胞释放炎症因子(如IL-6、IFN-γ),可能导致CRS;在肺癌等实体瘤治疗过程中,其高剂量需求会进一步加剧CRS风险[63]。目前已开发出剂量递增方案、皮质类固醇预处理、皮下给药等措施来应对CRS[64]。(2)实体瘤的肿瘤微环境中PD-L1、TGF-β等免疫抑制性分子会削弱TCE的疗效[65]。TriTCEs武装的OV实现了局部精准递送与协同效应,在一定程度上突破了上述局限,但此策略仍处于早期研究阶段,面临着许多挑战:(1)OV的肿瘤选择性依赖特定受体(如CD46),因此肺癌中靶受体的异质性会限制其疗效[66];(2)病毒载体可能引发抗病毒免疫反应,加速载体的清除;(3)基因工程OV需复杂的设计与质控,工业化生产难度大;(4)OV与TriTCE的协同机制尚未完全阐明,且目前在肺癌领域的应用尚不成熟,有待进一步发掘。

NKCE激活的是NK细胞而非T细胞,因此降低了CRS和神经毒性风险,具有更好的安全性,但其临床应用仍受限于以下难题:(1)肿瘤微环境中NKG2A/HLA-E轴、TGF-β等抑制性信号会限制NK细胞活性[38];(2)NKCE诱导的IFN-γ水平需控制在生理范围内以避免过度炎症反应,但可能限制抗肿瘤效果,需平衡疗效与活化强度。

ICB疗法已彻底改变癌症治疗格局,但其毒性、响应率限制及耐药问题仍需突破。(1)ICIs引发的免疫相关不良事件(immune-related adverse events,irAEs)包括皮肤、消化道、肝等多器官毒性以及肺炎、心血管毒性等严重反应。其中,CTLA-4抑制剂的毒性高于PD-1/PD-L1抑制剂,且联合治疗的毒性风险会进一步增加[67-69],给TsAbs的靶点选择带来了挑战。对于毒性管理,早期识别症状、糖皮质激素干预及个体化剂量调整是关键,但仍需平衡免疫抑制与抗肿瘤效果。(2)目前肺癌患者对ICIs的长期响应率较低,仅约30%的NSCLC患者对PD-1/PD-L1抑制剂长期响应[70]。此外,ICB还面临耐药性的挑

战,其耐药机制包括原发性耐药(IFN-γ信号通路缺失或抗原呈递机制APM失活)和获得性耐药(PD-1启动子去甲基化等)[67]。(3)PD-L1的检测受不同检测方法(如22C3、SP263抗体)及肿瘤内/间异质性的影响,ICIs理想生物标志物的开发仍具挑战[71]。

3.2 未来研究方向

3.2.1 靶点组合的创新

肺癌免疫抑制微环境的异质性和多重逃逸机制要求TsAbs的靶点选择必须具备更高的精准性与协同性。通过同步靶向免疫细胞(如CD3)、共刺激分子(如CD28)、TAA或免疫检查点,TsAbs可在解除抑制信号的同时,增强T细胞或NK细胞活性,突破传统单靶点疗法的局限性。例如,IL-15受体激动剂与ICB联用已在对ICB无效的NSCLC患者中显示出协同作用[72]。基于此,Li等[73]开发的抗PD-L1-CD16a-IL15三功能抗体融合蛋白在体内、体外实验中均对PD-L1阳性的肿瘤细胞表现出较强的抗肿瘤活性,进一步验证了靶点灵活组合和多维度协同为TsAbs带来的巨大潜能。

未来新的肺癌生物标志物及靶点的纳入为TsAbs的模块设计指明了方向,CKAP4、CLIP1-LTK融合、NCAPG等都是NSCLC的潜在生物标志物和新靶点[74-76]。利用单细胞测序、TCR测序等方法可筛选患者特异性新抗原,从而针对不同的肿瘤异质性定制TsAbs。CAM1615TEM8也为未来设计提供了新方向:可纳入肿瘤微环境相关成纤维细胞或血管内皮细胞靶点,重塑TAA的同时增强免疫细胞浸润,实现对基质细胞-免疫细胞-肿瘤细胞的三方调控[42]。

3.2.2 抗体结构的优化

抗体的长期稳定性是其临床转化的重要前提。TsAbs的分子构型直接影响其功能与安全性[12],因此需在蛋白质工程领域持续突破:比如通过Fc域改造、scFv串联或纳米抗体融合等手段,平衡三价结合亲和力与分子尺寸,减少脱靶毒性;通过优化抗体结构的二硫键网络和电荷分布,减少聚集倾向,提升体内药代动力学特性等[77]。

3.2.3 肿瘤微环境的动态适应

肿瘤微环境的动态变化要求TsAbs具备动态响应的能力。可根据肿瘤微环境设计条件性激活抗体,开发pH敏感型或蛋白酶依赖性TsAbs,仅在肿瘤局部微环境中释放活性,降低全身性毒性[78]。比如,PROBODY®可激活治疗分子(Pb-Tx)是一种能被肿瘤微环境中局部表达的蛋白酶水解激活的PD-L1抑制剂,在一项针对NSCLC的模拟临床试验中展现出治疗潜力[79]。还有一种策略是利用纳米材料逆转肿瘤微环境的免疫抑制。通过添加靶向肿瘤相关巨噬细胞(TAMs)(如CD86促进M1巨噬细胞极化)或腺苷通路(如CD73)等的靶点,逆转微环境的代谢抑制状态,增强效应细胞功能[80,81]。此外,通过多重免疫荧光(MIF)和成像质谱流式(IMC)等技术可实时监测患者微环境变化从而优化治疗时机[6]。

3.2.4 四特异性抗体的探索

在TsAbs基础上增加第四靶点,可协同激活多种效应细胞并提供增殖信号。目前多种新型的多功能平台正崭露头角,CrossMab平台是靶向EGFR/HER2/HER3/VEGF的四抗,在肺癌模型中显示出高亲和力与低浓度下的强效抗肿瘤活性[82];TriTECM平台融合了抗IL6R的scFv,能够在减轻CRS风险的同时维持抗肿瘤活性[83]。此外,Demaria等[84]设计了一种抗NKp46、CD16a、白细胞介素2受体(IL-2R)β链和TAA的四特异性NK细胞接合器ANKET。NKp46和CD16a的共结合通过ITAM信号通路激活NK细胞,而IL-2通过JAK/STAT通路促进NK细胞增殖与存活。两者的协同作用不仅增强了NK细胞功能,其精准靶向作用还克服了传统IL-2疗法因激活Treg和内皮细胞导致的严重毒性,为肺癌等实体瘤的免疫治疗提供了新思路。

3.3 总结

三特异性抗体通过多重机制协同作用,有望成为未来肺癌免疫治疗的重要策略。未来研究需聚焦于联合疗法优化、抗体结构革新、个性化设计及耐药性挑战,同时解决CRS管理、生产复杂性及临床转化相关难题。随着三特异性抗体的不断发展和四特异性抗体平台技术的不断成熟,多特异性抗体将进一步拓展肺癌的治疗边界,为晚期患者提供更高效、更安全、更精准的治疗方案。

参考文献

[1] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2024, 74: 229–63.

[2] Siegel RL, Kratzer TB, Giaquinto AN, et al. Cancer statistics, 2025. CA Cancer J Clin, 2025, 75: 10–45.
[3] 揭光灵, 杨莹, 虞永峰, 等. 非小细胞肺癌治疗年度进展2023. 中华医学杂志, 2024, 104: 3446–54.
Jie GL, Yang Y, Yu YF, et al. Annual progress in treatment of non-small cell lung cancer. Chin Med J, 2024, 104: 3446–54.
[4] Reck M, Renom J, Hellmann MD. First-line immunotherapy for non-small-cell lung cancer. J Clin Oncol, 2022, DOI:10.1200/JCO.21.01497.
[5] 曾怡, 黄昱, 董晓荣. 小细胞肺癌免疫治疗进展. 中华医学杂志, 2024, 104: 3081–6.
Zeng Y, Huang Y, Dong XR. Advances in immunotherapy for small cell lung cancer. Chin Med J, 2024, 104: 3081–6.
[6] Lahiri A, Maji A, Potdar PD, et al. Lung cancer immunotherapy: progress, pitfalls, and promises. Mol Cancer, 2023, 22: 40.
[7] Paul S, Konig MF, Pardoll DM, et al. Cancer therapy with antibodies. Nat Rev Cancer, 2024, 24: 399–426.
[8] Meyer ML, Fitzgerald BG, Paz-Ares L, et al. New promises and challenges in the treatment of advanced non-small-cell lung cancer. Lancet, 2024, 404: 803–22.
[9] Rajagopal D, Macleod E, Corogeanu D, et al. Immune-related adverse events of antibody-based biological medicines in cancer therapy. J Cell Mol Med, 2024, 28: e18470.
[10] 许林, 宁文静, 刘雪, 等. 三特异性抗体在肿瘤免疫治疗中的研究进展. 生命科学, 2024, 36: 433–47.
Xu L, Ning WJ, Liu X, et al. Research progress of tri-specific antibodies in tumor immunotherapy. Chin Bull Life Sci, 2024, 36: 433–47.
[11] Yao Y, Hu Y, Wang F. Trispecific antibodies for cancer immunotherapy. Immunology, 2023, 169: 389–99.
[12] Tapia-Galisteo A, Compte M, Alvarez-Vallina L, et al. When three is not a crowd: Trispecific antibodies for enhanced cancer immunotherapy. Theranostics, 2023, 13: 1028–41.
[13] Hofmann T, Krah S, Sellmann C, et al. Greatest hits-innovative technologies for high throughput identification of bispecific antibodies. Int J Mol Sci, 2020, 21: 6551.
[14] Fenis A, Demaria O, Gauthier L, et al. New immune cell engagers for cancer immunotherapy. Nat Rev Immunol, 2024, 24: 471–86.
[15] Ojemolon PE, Kalidindi S, Ahlborn TA, et al. Cytokine release syndrome following blinatumomab therapy. Cureus, 2022, 14: e21583.
[16] Zhang H, Yang Y, Li X, et al. Targeting the notch signaling pathway and the notch ligand, DLL3, in small cell lung cancer. Biomed Pharmacother, 2023, 159: 114248.
[17] Rudin CM, Reck M, Johnson ML, et al. Emerging therapies targeting the delta-like ligand 3 (DLL3) in small cell lung cancer. J Hematol Oncol, 2023, 16: 66.
[18] Su PL, Chakravarthy K, Furuya N, et al. DLL3-guided therapies in small-cell lung cancer: From antibody-drug conjugate to precision immunotherapy and radioimmunotherapy. Mol Cancer, 2024, 23: 97.
[19] Wang Q, Chai X, Lin R, et al. 686TiP preliminary phase I results from a first-in-human study of ZG006, a trispecific anti-T cell engager targeting CD3/DLL3/DLL3, as monotherapy in patients with advanced small cell lung cancer or neuroendocrine carcinoma. Ann Oncol, 2024, 35: S532–3.
[20] Murphy G, Brayden DJ, Cheung DL, et al. Albumin-based delivery systems: Recent advances, challenges, and opportunities. J Control Release, 2025, 380: 375–95.
[21] Molloy ME, Aaron WH, Barath M, et al. HPN328, a trispecific T cell-activating protein construct targeting DLL3-expressing solid tumors. Mol Cancer Ther, 2024, 23: 1294–304.
[22] Beltran H, Dowlati A, Jain P, et al. Interim results from a phase 1/2 study of HPN328, a tri-specific, half-life (T1/2) extended DLL3-targeting T-cell engager, in patients (pts) with neuroendocrine prostate cancer (NEPC) and other neuroendocrine neoplasms (NEN). Ann Oncol, 2023, 34: S486.
[23] Ho M, Bera TK, Willingham MC, et al. Mesothelin expression in human lung cancer. Clin Cancer Res, 2007, 13: 1571–5.
[24] Faust JR, Hamill D, Kolb EA, et al. Mesothelin: An immunotherapeutic target beyond solid tumors. Cancers, 2022, 14: 1550.
[25] Molloy ME, Austin RJ, Lemon BD, et al. Preclinical characterization of HPN536, a trispecific, T-cell-activating protein construct for the treatment of mesothelin-expressing solid tumors. Clin Cancer Res, 2021, 27: 1452–62.
[26] Ma R, Li Z, Chiocca EA, et al. The emerging field of oncolytic virus-based cancer immunotherapy. Trends Cancer, 2023, 9: 122–39.
[27] Santos Apolonio J, Lima de Souza Goncalves V, Cordeiro Santos ML, et al. Oncolytic virus therapy in cancer: A current review. World J Virol, 2021, 10: 229–55.
[28] Mehrabadi AZ, Tat M, Alvanegh AG, et al. Revolutionizing cancer treatment: The power of bi- and tri-specific T-cell engagers in oncolytic virotherapy. Front Immunol, 2024, 15: 1343378.

[29] Da Cunha Santos G, Shepherd FA, Tsao MS. EGFR mutations and lung cancer . Annu Rev Pathol, 2011, 6: 49–69.

[30] Zhou F, Guo H, Xia Y, et al. The changing treatment landscape of EGFR-mutant non-small-cell lung cancer. Nat Rev Clin Oncol, 2025, 22: 95–116.

[31] Zaman M, Huang C, Kankanamalage SG, et al. Abstract LB069: Development of cMET/cMET/EGFR trispecific antibody as therapeutic modality for non-small cell lung cancer. Cancer Res, 2021, 81: LB069.

[32] Socinski MA, Pennell NA, Davies KD. MET exon 14 skipping mutations in non-small-cell lung cancer: An overview of biology, clinical outcomes, and testing considerations. JCO Precis Oncol, 2021, 5: PO.20.00516.

[33] Jabbarzadeh Kaboli P, Roozitalab G, Farghadani R, et al. c-MET and the immunological landscape of cancer: Novel therapeutic strategies for enhanced anti-tumor immunity. Front Immunol, 2024, 15: 1498391.

[34] Yang JJ, Pavlakis N, Xie F, et al. 1272P updated results from a first-in-human, phase I/II study of GB263T, a novel EGFR/cMET/cMET tri-specific antibody, in patients with advanced EGFR-mutated (EGFRm) non-small cell lung cancer (NSCLC). Ann Oncol, 2024, 35: S813.

[35] Yang JJ, Pavlakis N, Xie F, et al. Preliminary dose escalation results from a first-in-human, phase I/II study of GB263T, a novel EGFR/cMET/cMET trispecific antibody, in patients with advanced EGFR-mutated (EGFRm) non-small cell lung cancer (NSCLC). J Clin Oncol, 2023, 41: e21085.

[36] Jin Y, Sun P, Chen P, et al. A trispecific antibody targeting EGFR/cMET/VEGF-a demonstrates multiple mechanisms of action to inhibit wild-type and mutant NSCLC animal models. Front Oncol, 2025, 15: 1533059.

[37] Jin Y, Chen P, Zhou H, et al. Developing transcriptomic biomarkers for TAVO412 utilizing next generation sequencing analyses of preclinical tumor models. Front Immunol, 2025, 16: 1505868.

[38] Zhu A, Bai Y, Nan Y, et al. Natural killer cell engagers: From bi-specific to tri-specific and tetra-specific engagers for enhanced cancer immunotherapy. Clin Transl Med, 2024, 14: e70046.

[39] Felices M, Lenvik T, Davis Z, et al. Generation of BiKEs and TriKEs to improve NK cell-mediated targeting of tumor cells. Methods Mol Cell Biol, 2016, 1441: 333–46.

[40] Kennedy PR, Vallera DA, Ettestad B, et al. A tri-specific killer engager against mesothelin targets NK cells towards lung cancer. Front Immunol, 2023, 14: 1060905.

[41] Kareff SA, Corbett V, Hallenbeck P, et al. TEM8 in oncogenesis: Protein biology, pre-clinical agents, and clinical rationale. Cells, 2023, 12: 2623.

[42] Kaminski MF, Bendzick L, Hopps R, et al. TEM8 tri-specific killer engager binds both tumor and tumor stroma to specifically engage natural killer cell anti-tumor activity. J Immunother Cancer, 2022, 10: e004725.

[43] Nutzinger J, Bum Lee J, Li Low J, et al. Management of HER2 alterations in non-small cell lung cancer–The past, present, and future. Lung Cancer, 2023, 186: 107385.

[44] Li BT, Smit EF, Goto Y, et al. Trastuzumab deruxtecan in HER2-mutant non-small-cell lung cancer. N Engl J Med, 2022, 386: 241–51.

[45] Cassier PA, Vicier C, Forget F, et al. Phase 1/2 study of DF1001, a novel tri-specific, NK cell engager therapy targeting HER2, in patients with advanced solid tumors: Phase 1 DF1001 monotherapy dose-escalation results. J Clin Oncol, 2023, 41: 2508.

[46] Konen JM, Wu H, Gibbons DL. Immune checkpoint blockade resistance in lung cancer: Emerging mechanisms and therapeutic opportunities. Trends Pharmacol Sci, 2024, 45: 520–36.

[47] Kanakis G, Grimelius L, Spathis A, et al. Expression of somatostatin receptors 1-5 and dopamine receptor 2 in lung carcinoids: Implications for a therapeutic role. Neuroendocrinology, 2015, 101: 211–22.

[48] Hoeppner LH, Wang Y, Sharma A, et al. Dopamine D2 receptor agonists inhibit lung cancer progression by reducing angiogenesis and tumor infiltrating myeloid derived suppressor cells. Mol Oncol, 2015, 9: 270–81.

[49] Lau APY, Khavkine Binstock SS, Thu KL. CD47: The next frontier in immune checkpoint blockade for non-small cell lung cancer. Cancers, 2023, 15: 5229.

[50] Yao S, Cui Y, Kazanats A, et al. Pre-clinical development of a dopamine receptor 2, PD-1 and CD47 trispecific antibody for treatment of small cell lung cancer. Mol Cancer Ther, 2021, 20: 200.

[51] Ye L, Jia K, Wang L, et al. CD137, an attractive candidate for the immunotherapy of lung cancer. Cancer Sci, 2020, 111: 1461–7.

[52] Claus C, Ferrara-Koller C, Klein C. The emerging landscape of novel 4-1BB (CD137) agonistic drugs for cancer immunotherapy. MAbs, 2023, 15: 2167189.

[53] Warmuth S, Gunde T, Snell D, et al. Engineering of a

trispecific tumor-targeted immunotherapy incorporating 4-1BB co-stimulation and PD-L1 blockade. Oncoimmunology, 2021, 10: 2004661.

[54] Krijgsman D, Roelands J, Hendrickx W, et al. HLA-G: A new immune checkpoint in cancer? Int J Mol Sci, 2020, 21: 4528

[55] Lin YC, Chen MC, Huang SW, et al. Targeting dual immune checkpoints PD-L1 and HLA-G by trispecific T cell engager for treating heterogeneous lung cancer. Adv Sci, 2024, 11: 2309697.

[56] Genova C, Dellepiane C, Carrega P, et al. Therapeutic implications of tumor microenvironment in lung cancer: Focus on immune checkpoint blockade. Front Immunol, 2022, 12: 799455.

[57] Wang L, Zhu B, Zhang M, et al. Roles of immune microenvironment heterogeneity in therapy-associated biomarkers in lung cancer. Semin Cell Dev Biol, 2017, 64: 90–7.

[58] Borregon M, Martinez K, Ramos A, et al. Anti-drug antibodies in the current management of cancer. Cancer Chemother Pharmacol, 2022, 89: 577–84.

[59] Vultaggio A, Perlato M, Nencini F, et al. How to prevent and mitigate hypersensitivity reactions to biologicals induced by anti-drug antibodies? Front Immunol, 2021, 12: 765747

[60] Zhou Y, Penny HL, Kroenke MA, et al. Immunogenicity assessment of bispecific antibody-based immunotherapy in oncology. J Immunother Cancer, 2022, 10: e004225.

[61] Li H, Er Saw P, Song E. Challenges and strategies for next-generation bispecific antibody-based antitumor therapeutics. Cell Mol Immunol, 2020, 17: 451–61.

[62] Ma H, O′fagain C, O′kennedy R. Antibody stability: A key to performance - analysis, influences and improvement. Biochimie, 2020, 177: 213–25.

[63] Morris EC, Neelapu SS, Giavridis T, et al. Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy. Nat Rev Immunol, 2022, 22: 85–96.

[64] Shanshal M, Caimi PF, Adjei AA, et al. T-cell engagers in solid cancers—Current landscape and future directions. Cancers, 2023, 15: 2824.

[65] van de Donk NWCJ, Zweegman S. T-cell-engaging bispecific antibodies in cancer. Lancet, 2023, 402: 142–58.

[66] Guo ZS, Lotze MT, Zhu Z, et al. Bi- and tri-specific T cell engager-armed oncolytic viruses: Next-generation cancer immunotherapy. Biomedicines, 2020, 8: 204.

[67] Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy for cancer: An overview of FDA-approved immune checkpoint inhibitors. Int Immunopharmacol, 2018, 62: 29–39.

[68] El Osta B, Hu F, Sadek R, et al. Not all immune-checkpoint inhibitors are created equal: Meta-analysis and systematic review of immune-related adverse events in cancer trials. Crit Rev Oncol Hematol, 2017, 119: 1–12.

[69] Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Eng J Med, 2015, 373: 23–34.

[70] Reck M, Rodriguez-Abreu D, Robinson AG, et al. Five-year outcomes with pembrolizumab versus chemotherapy for metastatic non-small-cell lung cancer with PD-L1 tumor proportion score >/= 50. J Clin Oncol, 2021, 39: 2339–49.

[71] Ilie M, Long-Mira E, Bence C, et al. Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: A potential issue for anti-PD-L1 therapeutic strategies. Ann Oncol, 2016, 27: 147–53.

[72] Del Bano J, Flores-Flores R, Josselin E, et al. A bispecific antibody-based approach for targeting mesothelin in triple negative breast cancer. Front Immunol, 2019, 10: 1593.

[73] Li Y, Wu L, Liu Y, et al. A novel multifunctional anti-PD-L1-CD16a-IL15 induces potent cancer cell killing in PD-L1-positive tumour cells. Transl Oncol, 2022, 21: 101424.

[74] Cooper AJ, Sequist LV, Johnson TW, et al. LTK fusions: A new target emerges in non-small cell lung cancer. Cancer Cell, 2022, 40: 23–5.

[75] Nagoya A, Sada R, Kimura H, et al. CKAP4 is a potential exosomal biomarker and therapeutic target for lung cancer. Transl Lung Cancer Res, 2023, 12: 408–26.

[76] Sun H, Zhang H, Yan Y, et al. Correction: NCAPG promotes the oncogenesis and progression of non-small cell lung cancer cells through upregulating LGALS1 expression. Mol Cancer, 2022, 21: 221.

[77] Goulet DR, Atkins WM. Considerations for the design of antibody-based therapeutics. J Pharm Sci, 2020, 109: 74–103.

[78] McCue AC, Demarest SJ, Froning KJ, et al. Engineering a tumor-selective prodrug T-cell engager bispecific antibody for safer immunotherapy. mAbs, 2024, 16: 2373325.

[79] Ippolito A, Wang H, Zhang Y, et al. Virtual clinical trials via a QSP immuno-oncology model to simulate the response to a conditionally activated PD-L1 targeting antibody in NSCLC. J Pharmacokinet Pharmacodyn,

2024, 51: 747–57.

［80］ Gao F, Dong J, Xue C, et al. Nanomaterials-involved strategies for reversing the immunosuppressive factors and improving antitumor immunotherapy. Nano Today, 2023, 50: 101831.

［81］ Kowash RR, Akbay EA. Tumor intrinsic and extrinsic functions of CD73 and the adenosine pathway in lung cancer. Front Immunol, 2023, 14: 1130358.

［82］ Hu S, Fu W, Xu W, et al. Four-in-one antibodies have superior cancer inhibitory activity against EGFR, HER2, HER3, and VEGF through disruption of HER/MET crosstalk. Cancer Res, 2015, 75: 159-70.

［83］ Carrara SC, Harwardt J, Grzeschnik J, et al. TriTECM: A tetrafunctional T-cell engaging antibody with built-in risk mitigation of cytokine release syndrome. Front Immunol, 2022, 13: 1051875.

［84］ Demaria O, Gauthier L, Vetizou M, et al. Antitumor immunity induced by antibody-based natural killer cell engager therapeutics armed with not-alpha IL-2 variant. Cell Rep Med, 2022, 3: 100783.