Advances in Clinical Medicine 临床医学进展, 2024, 14(2), 4445-4452
Published Online February 2024 in Hans. https://www.hanspub.org/journal/acm
https://doi.org/10.12677/acm.2024.142617

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外周血生物标志物在非小细胞肺癌免疫治疗疗效预测研究进展

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收稿日期：2024年1月29日；录用日期：2024年2月23日；发布日期：2024年2月29日

摘　要

近年来，以免疫检查点抑制剂(ICIs)为代表的免疫治疗在非小细胞肺癌(NSCLC)综合治疗中已经取得重大突破，彻底更改了不同期别NSCLC的整体治疗策略。虽然肿瘤组织相关标记物如：细胞程序性死亡－配体1 (PD-L1)、肿瘤突变负荷(TMB)等能在一定程度上很好的预测应答反应；但受制于肿瘤标本获取、检测技术、经济负担等因素，限制其在临床大规模应用，如何早期、高效、无创的识别免疫治疗的潜在获益人群是当务之急。外周血成为理想的样本来源，也显示了作为免疫治疗效果预测因子的潜力。本文旨在综述外周血生物标志物在NSCLC免疫治疗疗效预测研究进展，为后续临床治疗提供参考依据。

关键词

免疫检查点抑制剂，外周血，非小细胞肺癌，生物标志物

Progress of Peripheral Blood Biomarkers in Predicting the Efficacy of Immunotherapy for Non-Small Cell Lung Cancer

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Received: Jan. 29th, 2024; accepted: Feb. 23rd, 2024; published: Feb. 29th, 2024

Abstract

In recent years, immunotherapy represented by immune checkpoint inhibitors (ICIs) has made significant breakthroughs in the comprehensive treatment of non-small cell lung cancer (NSCLC),

文章引用: 孙维蔚, 贾敬好. 外周血生物标志物在非小细胞肺癌免疫治疗疗效预测研究进展[J]. 临床医学进展, 2024, 14(2): 4445-4452. DOI: 10.12677/acm.2024.142617

completely changing the overall treatment strategy of NSCLC of different stages. Although tumor tissue-associated markers such as programmed cell death-ligand 1 (PD-L1) and tumor mutational load (TMB) can predict response well to a certain extent; however, factors such as tumor specimen acquisition, detection technology, and economic burden limit their large-scale clinical application, and it is imperative to identify the potential beneficiary populations of immunotherapy in an early, efficient, and non-invasive manner. Peripheral blood has become an ideal sample source and has also shown potential as a predictor of immunotherapy efficacy. The aim of this article is to review the progress of peripheral blood biomarkers in NSCLC immunotherapy efficacy prediction research, and provide a reference basis for subsequent clinical treatment.

Keywords

Immune Checkpoint Inhibitors, Peripheral Blood, Non-Small Cell Lung Cancer, Biomarkers

1. 前言

肺癌具有发现晚、预后差的临床特点，其发病率和死亡率均居我国首位[1]。近年来，以程序性死亡蛋白及配体 1 (programmed death-1 and ligand-1, PD-1/PD-L1)抑制剂为代表的免疫治疗彻底改变了非小细胞肺癌(non-small cell lung cancer, NSCLC)的治疗格局，免疫单药或联合化疗已经成为转移性 NSCLC 一线或二线的标准治疗，但免疫治疗客观缓解率仍然较低[2] [3]。以肿瘤组织表达的 PD-L1 和 TMB 为预测因子相关研究中，低表达者同样能观察到临床获益，而部分高表达者依然存在低应答反应，提示 PD-L1 和肿瘤突变负荷(tumor mutation burden, TMB)作为预测因子具有一定局限性[4] [5]，受制于标本获取难易程度、肿瘤异质性、检测平台检测标准差异，结果并不精确[6] [7]。而液体活检技术的优势在于侵入性小，允许重复、多次取样，能够追踪治疗期间的动态变化过程[8]，外周血标志物变化有可能更早的反映肿瘤和宿主微环境变化[9]，弥补活检或穿刺样本无法获取肿瘤全貌的局限性。随着高通量测序和人工智能技术的快速发展，基于外周血生物标志物检测预测免疫治疗疗效的研究受到了越来越多的关注。

2. PDL1

PD-1 是表达在多种活化 T 细胞表面的一种受体，与肿瘤细胞表达的 PD-L1 配体结合能增强肿瘤细胞的免疫耐受性，介导免疫逃避机制[10]。研究表明：PD-L1 不仅表达在肿瘤细胞、免疫细胞和肿瘤微环境的其他细胞表面，还可能以细胞外形式存在于肿瘤患者外周血的血浆或血清中，且已被证明在肿瘤免疫抑制中发挥作用[11]。

可溶性 PDL1 (soluble PD-L1, sPD-L1)在肿瘤细胞和成熟树突细胞中产生和释放，通过血液和淋巴循环播散至全身，与细胞表面受体结合抑制 T 细胞的活化和增殖，抑制抗肿瘤免疫[12]。在接受 ICIs 治疗的 NSCLC 患者中，高水平 sPD-L1 与较短的无进展生存期(progression-free survival, PFS)和总生存期(overall survival, OS)显著相关，且高 sPD-L1 组(≥90 pg/mL)的疾病控制率(objective response rate, ORR)明显低于低 sPD-L1 组(<90 pg/mL) (37% vs 57%, P = 0.0158) [13]。Fabio 等[14] meta 分析结果同样显示：高水平的 sPD-L1 与较差的 PFS 和 OS 相关。外泌体 PD-L1 是另外一种重要形式，可以与 T 细胞表面 PD-1 或髓样细胞表面的 toll 样受体结合，直接和间接地抑制抗肿瘤免疫反应[15] [16]。2019 年 Li 等[17]发现，

较高的外泌体 PD-L1 与 NSCLC 疾病进展相关。2021 年 Yang 等[18]对转移性 NSCLC 患者免疫治疗 2 个月后血液的 PD-L1 表达进行了动态分析，提示血液 PD-L1 mRNA 和外泌体 PD-L1 表达上调预示着良好疗效和生存率，且两种生物标志物的组合可以更好地筛选获益亚群。目前，支持 sPD-L1 和外泌体 PD-L1 作为免疫治疗响应的预测标志物的数据大多以回顾性研究为主，尚缺乏大型前瞻性研究的有力支持。

循环肿瘤细胞(circulating tumor cell, CTC)在肿瘤形成早期，从肿瘤病灶脱落并存在于外周血中，能够较好反映肿瘤的异质性。2015 年 Mazel 等[19]首次发现 PD-L1 在转移性乳腺癌患者外周血 CTC 上表达，并验证了 CTC PD-L1 检测的可行性。Guibert 等[20]前瞻性分析了 96 例晚期 NSCLC 患者在纳武利尤单抗治疗前和疾病进展时 CTC PD-L1 的表达，发现 CTC PD-L1 表达率高于组织样本(83% vs 41%)。Nicolazzo 等[21]纳入了 24 例接受免疫治疗的晚期 NSCLC 患者，分别对基线期、治疗后 3 个月、6 个月外周血中 CTC 和 CTC 的 PD-L1 表达进行了评估。发现基线期和治疗后 3 个月的 CTC 及其表面 PD-L1 的表达与预后不佳相关；在治疗 6 个月后所有患者均检测到了 CTC，CTC PD-L1 阴性患者均出现临床获益，而 CTC PD-L1 阳性患者均出现病情进展，表明 CTC PDL1 阳性可能是早期耐药的预测性标志物。多项研究显示了 CTC 上 PD-L1 表达的预后价值，如何量化 PD-L1 在 CTC 上的表达成为临床进一步应用的关键[22] [23]。此外，血小板 PD-L1 不仅能够反映肿瘤整体 PD-L1 表达情况，而且具有抑制 $CD4^+$和 $CD8^+$T 细胞的能力。开发的一种计算血小板 PD-L1 (pPD-L1)的算法，与基于免疫组化的肿瘤 PD-L1 定量相比，在预测 ICIs 反应方面更具有优势[24]。基于液体活检技术下的 PD-L1 检测在预测方面展现出了良好的应用前景，且获取的临床信息较为全面，能够满足治疗期间的动态监测需求。

3. 血液肿瘤突变负荷

外周血循环肿瘤 DNA (circulating tumor DNA, ctDNA)计算的血液肿瘤突变负荷(blood tumor burden, bTMB)定义为每百万碱基中检测出的体细胞基因编码错误、碱基替换、基因插入或确实错误的总数，作为一种新兴的免疫预测标志物可能比组织 TMB 更具优势[25] [26] [27]。Gandara 等[25]表示，bTMB ≥ 16 mut/Mb 的 NSCLC 患者接受阿替利珠单抗治疗的 PFS 和 OS 获益更好，首次证实了 bTMB 对于免疫治疗疗效的预测价值。MYSTIC [26]研究证实：bTMB ≥ 20 mut/Mb 的患者可观察到更高的 PFS 和 OS。FIRST [27]作为首个前瞻性评估 bTMB 的临床研究同样发现：bTMB 越高，越倾向于观察到更高的 ORR，更长的 OS 和 PFS；在长期随访中，bTMB ≥ 16 mut/Mb 的患者 OS 获益显著。前瞻性论证了 bTMB 对免疫治疗疗效的预测价值，为 bTMB 进入临床实践奠定了重要基础。然而，ctDNA 突变丰度和肿瘤异质性程度均会对 bTMB 的检测造成偏倚[28] [29]，影响检测结果准确性，多项 bTMB 的改进算法正在被提出并验证[30]。

4. 循环免疫细胞

免疫细胞是肿瘤微环境的重要组成部分，在机体的抗肿瘤免疫中发挥关键作用。识别和监测外周血中循环免疫细胞的数量和亚群变化，能够帮助预测机体免疫状态及 ICIs 的治疗反应。

1) 常规外周血标志物

中性粒细胞是肿瘤微环境中主要的免疫细胞类型，在细胞因子及表观遗传学信号诱导下可极化成不同的表型，发挥抗肿瘤或促肿瘤的双重作用，在一定程度上能够作为肿瘤免疫治疗疗效和预后评估的标志[31] [32]。Tanizaki 等[33]研究发现，低中性粒细胞绝对计数(absolute neutrophil count, ANC)、高淋巴细胞绝对计数和高嗜酸性粒细胞绝对计数(absolute eosinophil count, AEC)的基线特征与更好的 PFS 和 OS 显著相关。此外，肿瘤诱导的中性粒细胞通过产生诱导型一氧化氮合酶抑制细胞毒性 $CD8^+$T 淋巴细胞，一氧化氮合酶是一种主要的炎症介质，能够限制肿瘤扩散，以中性粒细胞和淋巴细胞计数衍生的免疫炎症

指数可能更具预测价值[34] [35]。中性淋巴细胞比值(neutrophil-to-lymphocyte ratio, NLR)与 NSCLC 患者预后相关性的 Meta 分析中[36]，基线期 NLR ≥ 5 的患者与纳武利尤治疗后较差的 PFS 和 OS 相关。治疗后 NLR 的变化对 PFS 和 OS 也有预测作用。Chen 等[37]研究发现，治疗后 6 周或治疗后 12 周 NLR 的降低与较高的 ORR 相关。研究表明：基线衍生的 NLR [dNLR，ANC/(白细胞 − ANC)]可能比 NLR 更与临床结果相关，因为还考虑了单核细胞和其他粒细胞亚群[38] [39]。多项研究表明：所选样本的差异，造成 NLR 或 dNLR 高低水平的判断略有不同，但治疗前两项的升高均提示提示预后不良[40] [41]。血小板淋巴比(platelet-to-lymphocyte ratio, PLR)、淋巴单核比(lymphocyte-to-monocyte ratio, LMR)同样反映机体炎症状态。研究表明：高 PLR、低 LMR 常常与更差的 OS 和 PFS 相关[42] [43]。最近的一项研究证实了血小板可促进抗肿瘤免疫[44]。这一发现，使得血小板这种特殊的免疫细胞受到了诸多关注，一系列关于血小板与免疫疗效相关性的研究正在积极展开[24] [45]。外周血标志物的预测作用尚有待大规模的前瞻性数据支持下确定最佳检测时机和各种免疫细胞的最佳截断值，如何确定最佳的联合预测模型仍是要解决的难题。

2) 淋巴细胞亚群

淋巴细胞亚群的数量和百分比反映机体当前的免疫功能状态，能够作为疾病进展、疗效预测和预后的潜在生物标志物[46] [47]。$CD8^+$ T 细胞在免疫系统对肿瘤细胞的破坏中起着重要作用，PD-1/L1 阻断可使表达 PD-1 的 $CD8^+$ T 细胞重新活化并诱导其增殖，与多种肿瘤类型对 ICIs 的反应相关[48]。研究表明[49]，ICI 治疗前较少的循环 $CD8^+$ T 细胞与持久临床获益(durable clinical benefit, DCB)相关，其准确率为 70%。Kim 等[50]对 T 细胞亚型进行更详细的前瞻性分析，发现 NSCLC 患者外周血 $Ki\text{-}67_{D7/D0}$ (给药后 7 天 Ki-67+细胞百分比的倍数变化) ≥ 2.8，与更好的 DCB、PFS 和 OS 相关，ICIs 治疗后循环 $PD\text{-}1^+$ $CD8^+$ T 细胞的早期增殖可能是预测实体瘤抗 PD-1 治疗反应和预后的有用替代生物标志物。另一项研究[51]提示，治疗前外周血 PD-1 + $CD8^+$ T 细胞的 T 细胞受体(T-cell receptor, TCR)多样性较高且治疗后 TCR 克隆性增加的患者，ICIs 反应和生存结局更好。总之，无论是监测 $CD8^+$ T 细胞及亚型的数量变化，还是 TCR 克隆增，都有助于预测 ICIs 益处，更好地指导临床治疗。$CD4^+$ T 细胞通过辅助细胞因子产生，调节免疫应答和记忆作用等方式增强抗肿瘤免疫[52] [53]。Miao 等[54]对 NSCLC 患者的外周血亚群及对应的预后数据进行探索，发现基线外周血 $CD4^+$ $CD45RA^-$ T 细胞与 NSCLC 患者应用 ICIs 的预后密切相关，能够作为预测 ICIs 疗效的潜在生物标志物。$CD45RA^-$是记忆 T 细胞的表面特征之一，表明记忆 $CD4^+$ T 细胞在 ICIs 的起效中同样发挥重要作用。此外，治疗前后淋巴细胞比值的变化与免疫治疗疗效相关[55]。

髓源抑制性细胞(myeloid-derived suppressor cells, MDSC)是一种在骨髓细胞分化过程中异常积累的异质细胞，通过肿瘤微环境(tumor microenvironment, TME)中的 T 细胞活性，促进肿瘤免疫逃逸[56]。Youna 等[57]评估了 62 例 NSCLC 患者治疗前后外周血样本中自然杀伤细胞(natural killer cell, NK)和 MDSC 的频率与免疫治疗反应发现：首次治疗后应答者的 NK 细胞百分比中位数明显高于非应答者，而 $Lox\text{-}1^+$多形核 MDSC 百分比中位数则呈现出相反趋势。在第一个治疗周期后，应答者的 NK 细胞与 $Lox\text{-}1^+$多形核 MDSC 比率(NMR)明显高于非应答者，且第一周期后 NMR ≥ 5.75 的患者 ORR 更高，PFS 和 OS 更长。Kim [58]等人同样观察到，对纳武利尤单抗应答的患者，外周血 $Lox\text{-}1^+$多形核 MDSC 的水平出现显著降低。综上，PD-1 抑制剂治疗期间多形核 MDSC 的相对变化有望成为抗 PD-1 治疗反应的早期预测指标，待进一步验证。

3) 细胞因子

细胞因子是由免疫细胞和某些非免疫细胞(内皮细胞、表皮细胞、纤维母细胞等)经刺激而合成、分泌的一类具有广泛生物学活性的小分子蛋白质。某些细胞因子，尤其是参与适应性免疫应答的细胞因子(如 IFNγ、IL-6 和 IL-8)，有助于抗肿瘤免疫[59] [60]。Sanmamed 等[59]表明，在接受抗 PD-1 治疗的黑色素

瘤和 NSCLC 患者中，血清 IL-8 水平的早期下降与黑色素瘤和 NSCLC 患者 OS 的延长相关。Kauffmann-Guerrero [60]则在二线接受抗 PD-1 治疗的 NSCLC 患者中观察到，低水平的 IL-6、IL-8 和高水平的 IFN-γ 是患者对 ICI 治疗长期反应的特征。上述研究均提示了外周血细胞因子数值变化与免疫治疗疗效密切相关。基于细胞因子抗体在肿瘤免疫调节其他疾病中的应用进展。在肿瘤免疫循环中，可借助细胞因子增强免疫应答或降低免疫治疗副作用。具有很大的应用前景。

5. 营养和炎症指标

C 反应蛋白(C reactive protein, CRP)是肝起源的急性期蛋白，反映组织损伤和全身性炎症。Riedl 等[61]进行的一项回顾性双中心研究表明：在接受 PD-1 或 PD-L1 抑制剂治疗的 NSCLC 患者中，基线期 CRP 水平升高与较差的 PFS 和 OS 相关。研究表明：CRP flare 反应(CRP 在最初治疗时急剧升高，随后降至基线以下)能够预测抗 PD-1 单药治疗的反应和生存率[62]。早期 CRP 动力学可能成为一种易于确定、具有成本效益且无创的生物标志物，用于预测 NSCLC 在第一个月内对检查点抑制剂的反应。

在恶性肿瘤的患者中经常看到乳酸脱氢酶(lactate dehydrogenase, LDH)的升高。LDH 水平是癌细胞有氧糖酵解效应重要标志，促进癌细胞生存；通过肿瘤代谢和肿瘤免疫影响治疗；LDH 和肿瘤本身侵袭性以及靶向、免疫治疗疗效可能有关；LDH 具有协调肿瘤细胞增殖功能，LDH 抑制剂可能作为抗肿瘤治疗的新靶点。Mezquita 等[63]一项回顾性研究中，治疗前 LDH 大于正常值上限且 dNLR > 3 制定了肺免疫预后指数(lung immune prognostic index, LIPI)，结果证实 LIPI 可以作为晚期 NSCLC 患者选择 ICI 治疗时的潜在有用工具。炎症和营养指标易于评估，能够帮助临床医生早期识别治疗效果较好的患者，为必要的治疗方案更改提供参考依据。

从临床实践来看，相较于单一标志物，基于外周血相关数据建立的预测模型，能够提高预测的特异性及有效性，利于指导个体化治疗方案的选择。Clelia [64]等证实，通过结合 NSCLC 患者恶病质状态及营养、代谢和炎症参数相关变化建立的预测模型，是预测 ICI 治疗生存率和临床反应的关键。在本综述背景下，筛选更多肿瘤免疫相关变量，进行预测模型的开发及验证，将会成为未来的研究方向之一。

6. 小结和展望

随着人工智能算法、机器学习、质谱流式技术的发展，能够对肿瘤进行更深入的信息挖掘，可获得多维度、多阶段的微环境的动态改变图景。尤其是肿瘤微环境刚度的异质性更能免疫治疗应答反应的生物学行为改变。外周血生物标志物仍面临一些挑战：首先，目前研究多为回顾性、探索性、小样本研究，缺乏大型随机前瞻性临床数据支持。其次，对于外周血标志物的检测缺乏标准化平台，且需要对采样时间点、特异度和敏感度进一步规范化，另外外周血标志物受机体循环系统多种因素调节影响较大。再者，影响 NSCLC 免疫预后因素众多，单一标志物存在一定的局限性，未来的应用模式势必会结合肿瘤、TME、治疗因素及宿主等多方面因素的联合预测模型将是未来研究的方向。

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