# TILs细胞的高效扩增策略及其在临床治疗中的应用

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**摘要** 肿瘤浸润淋巴细胞(tumor-infiltrating lymphocytes, TILs)疗法可以在多数转移性癌症患者中诱导有效且持久的临床反应, 且这推动着过继性细胞疗法(adoptive cell therapy, ACT)在实体肿瘤的治疗方面发生革命性的变化。与其他ACT相比, 多样化的T细胞受体(T cell receptor, TCR)克隆性、优越的肿瘤归巢能力以及低脱靶毒性等特点赋予了TILs在实体瘤治疗方面独特的优势。然而, 获取足够临床用量的TILs需要经过复杂的细胞处理和长时间的体外培养过程, 这给TILs的临床应用带来了技术和监管方面的难题。为了解决这一问题, 必须在细胞培养过程中维持TILs细胞高效扩增和活性的平衡, 以此来提高TILs在肿瘤患者体内的作用效果。近年来, 已有大量针对TILs各方面的研究, 尤其是T细胞增殖分化领域的研究。该文将重点探讨TILs的高效扩增策略以及近些年TILs在临床治疗中单用或者联合治疗的策略, 以期为TILs的高效扩增和临床应用提供新的见解。

**关键词** TILs免疫治疗; 工艺开发; 临床治疗

# Efficient Expansion Strategies of TILs Cells and Their Use in Clinical Treatment

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**Abstract** TILs (tumor-infiltrating lymphocytes) therapy can induce effective and durable clinical responses in most patients with metastatic cancer, revolutionizing ACT (adoptive cell therapy) in the treatment of solid tumors. The diverse TCR (T cell receptor) clonality, superior tumor homing ability, and low off-target toxicity give TILs a unique advantage over other ACTs in the treatment of solid tumors. However, obtaining sufficient clinical quantities of TILs requires complex cell processing and prolonged *in vitro* culture, which poses technical and regulatory challenges for the clinical application of TILs. To address this issue, the balance of efficient cell expansion and activity of TILs must be maintained during cell culture as a way to improve the effect of TILs in patients. In recent years, there have been numerous studies addressing various aspects of TILs, especially in the field of T cell proliferation and differentiation. This review will focus on the efficient expansion methods of TILs and the strategies of TILs in clinical treatment in recent years, either alone or in combination, in order to provide new insights into the efficient expansion and clinical application of TILs.

**Keywords** immunotherapy with TILs; process development; clinical treatment

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早在1980年, 淋巴因子激活的杀伤细胞(lymphokine activated killer cells, LAK)、细胞因子诱导的杀伤细胞(cytokine induced killer cell, CIK)等血液来源且未被改造的免疫细胞已被用来治疗晚期肿瘤, 然而疗效欠佳[1-2]。近年来, 以嵌合抗原受体T细胞(chimeric antigen receptor T-cell, CAR-T)疗法和肿瘤浸润淋巴细胞(tumor-infiltrating lymphocytes, TILs)疗法为主的细胞治疗分别在血液系统肿瘤和黑色素瘤等实体瘤的临床试验中取得了突破性进展[3-4]。

与其他过继性细胞疗法(adoptive cell therapy, ACT)相比, 多样化的T细胞受体(T cell receptor, TCR)克隆、优越的肿瘤归巢能力以及低脱靶毒性等特点使TILs在实体瘤的治疗中具有独特的优势。研究表明, 恶性肿瘤组织中的TILs细胞具有特异性的肿瘤细胞靶向能力, 经体外激活扩增后可以特异性杀伤肿瘤细胞, 其杀伤能力为LAK细胞的50~100倍[1]。早期动物实验表明, TILs和细胞因子白细胞介素-2(interleukin-2, IL-2)联用可以治愈100%的肝转移小鼠和50%的肺转移小鼠[1]。随后一项12名患者参与的针对多癌种的初期临床研究结果证实了TILs治疗的安全性及初步疗效[5]。近年来, 多项研究发现非小细胞肺癌、食管癌、胰腺癌等实体肿瘤中都存在TILs, 这些T细胞不仅具有抗肿瘤特性, 且其含量也与肿瘤预后密切相关[6-8]。

虽然已有基础研究和临床试验证实了TCR多样化的TILs能够特异地靶向实体瘤, 但TILs在实际临床应用过程中仍存在许多亟待攻克的障碍, 主要体现在以下几个方面: (1) TILs通常从肿瘤组织中获取, 一般只能通过手术获得, 然而并不是所有肿瘤患者都符合手术标准或者有手术意愿; (2) 肿瘤组织中直接获取的TILs细胞大多为耗竭性T细胞, 其增殖能力和杀伤肿瘤细胞的能力均较弱; (3) 实体肿瘤的物理屏障、血管异常、趋化因子分泌不足以及免疫抑制的肿瘤微环境等因素阻碍了TILs细胞归巢; (4) TILs制备过程繁琐, 大规模生产符合临床规范的TILs存在挑战。

因此, TILs的临床应用仍需大量基础研究的突破。其中, 首要问题是解决TILs的体外快速扩增。通常情况下, TILs治疗需要高达$1\times10^{9}$~$1\times10^{11}$的细胞数量, 而大多数患者接受TILs治疗时已是晚期, 缺乏有效的治疗窗口。因此, 优化TILs培养体系, 在保留TILs疗效的同时缩短TILs的培养时间, 具有重要的临床意义。本文将重点阐述TILs细胞快速增殖的方法及存在的问题, 为该领域的研究提供一定的参考依据。

## 1 TILs免疫治疗原理

肿瘤微环境组成复杂, 由肿瘤细胞、免疫细胞、基质细胞、内皮细胞等组成。其中, TILs受到肿瘤表面抗原的刺激而被激活, 是一类具有肿瘤抗原特异性的免疫细胞群。尽管已有研究报道可从肿瘤组织、转移的淋巴结、恶性胸腹水, 甚至从外周血中获取TILs, 但目前最广泛使用的方法依然是从肿瘤组织中分离TILs[9]。从肿瘤引流淋巴结、恶性胸腔积液及腹水中获取TILs相对容易, 但这些来源的TILs对肿瘤治疗的有效率远低于肿瘤组织来源的TILs[10]。抗程序性死亡分子1(programmed death-1, PD-1)抗体治疗后, 肿瘤特异性T细胞在患者外周血中迅速扩增[11-12]。然而, 对于不同患者, 外周血中的肿瘤特异性T细胞激活的时间点、具体数量都不相同。因此, 难以用相同的方案从药物治疗后的患者外周血中获取足够量的肿瘤特异性T细胞进行扩增和治疗。此外, 另一项研究同样表明, 从患者外周血$PD\text{-}1^{+}$ T细胞中虽然能够分离出肿瘤特异性T细胞[13], 但其数量较低, 体外扩增较困难。尽管也有研究报道, 深度TCR测序表明恶性黑色素瘤患者外周血$PD\text{-}1^{+}$ T细胞和肿瘤局部的TILs具有较高的吻合度, 但这仍需临床试验数据来检验[14]。综上, 这些研究进一步增强了使用肿瘤组织来源的淋巴细胞作为TILs来源的合理性。

TILs包含$CD3^{+}$ T淋巴细胞、$CD20^{+}$ B淋巴细胞、单核细胞、自然杀伤细胞(natural killer cell, NK)等多种免疫细胞[15]。实验证实, 通过体外IL-2长期诱导培养后的TILs以$CD3^{+}$ T淋巴细胞和NK细胞为主, 且TILs细胞杀伤肿瘤主要通过T淋巴细胞和NK细胞的协同作用, 进而造成肿瘤细胞免疫原性死亡, 其杀伤能力可在肿瘤局部实现免疫杀伤的自我放大[16]。此外, 研究表明TILs中T细胞表面高表达包括CD69、CCR5、CXCR3、CXCR6在内的关键因子受体, 这增强了TILs的肿瘤归巢能力[17-19]。总而言之, TILs杀伤肿瘤主要包括以下途径(图1): (1) TILs细胞中的T细胞与肿瘤细胞表面的抗原肽-MHC I类分子复合物结合, 通过穿孔素/颗粒酶途径、Fas/FasL途径和TNF-TNFR途径发挥特异性杀伤作用; (2) 肿瘤微环境中表达的趋化因子和黏附因子可以促进NK细胞向肿

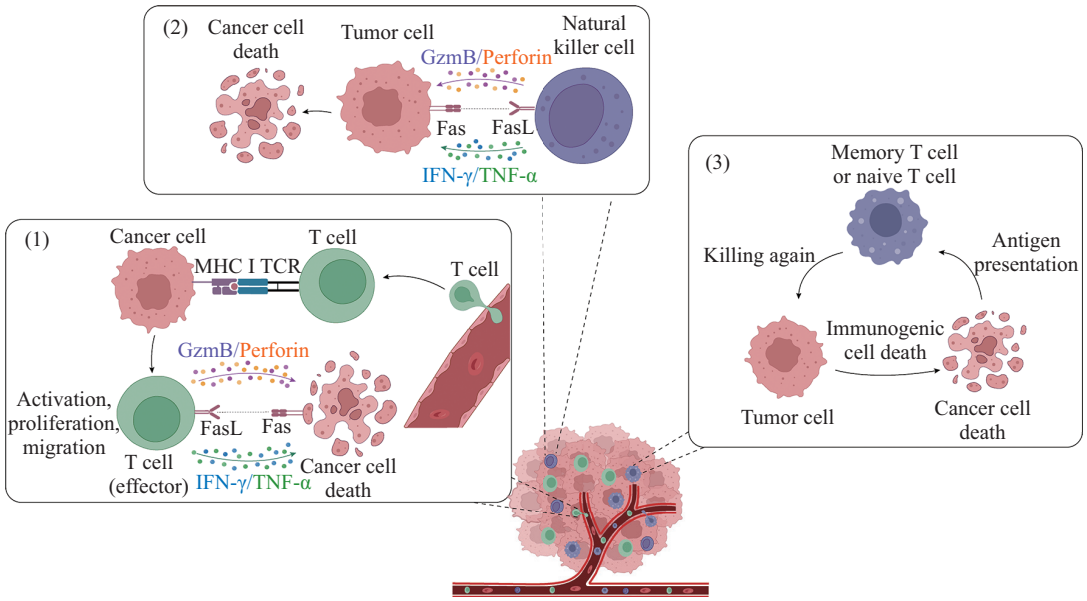


**图1 TILs杀伤肿瘤的机制**

**Fig.1 Mechanism of tumor killing by TILs**

瘤组织浸润，继而使得NK细胞表面活化受体识别肿瘤细胞表面相应的配体，释放穿孔素等杀伤介质，发挥抗肿瘤的细胞毒性作用[20]；(3) T淋巴细胞和NK细胞诱导肿瘤细胞发生免疫原性死亡，肿瘤细胞释放的肿瘤相关抗原可以通过抗原提呈细胞提呈给肿瘤引流淋巴结中的幼稚T细胞或记忆T细胞，形成从头免疫，增强TILs的杀伤效果[16]。

## 2 TILs的分离培养

TILs治疗通常是利用活检或手术切除的肿瘤组织，在体外分离出TILs，TILs经IL-2刺激并扩增至临床治疗所需的细胞量，然后被回输到患者体内。然而，由于肿瘤微环境中存在复杂的免疫抑制机制，造成肿瘤组织中存在的TILs数量少、活力低。因此，为了将TILs应用于肿瘤的临床治疗，需要寻找合适的方法，实现TILs在体外的快速大量扩增，并重新激活其肿瘤杀伤活性。

### 2.1 肿瘤组织中TILs的分离

TILs分离方法主要有组织块培养、酶消化、机械解离、细针抽吸等。目前常用的分离方法以组织块培养和酶消化为主。其中组织块培养方法是将肿瘤组织剪切成大小约为1 mm×1 mm×1 mm的小块后，置于含有IL-2(6 000 IU/mL)的培养基的小孔中培养，TILs将于3~10天内从肿瘤组织块中迁出[21]。酶消化分离TILs的方法是在胶原酶和DNA酶等消化酶的作用下将肿瘤碎片消化成单细胞悬液，之后使用100%和75%浓度的淋巴细胞分离液(Ficoll)进行密度梯度离心，进而得到三层细胞，其中100%淋巴细胞分离液上的白膜层为淋巴细胞，75%淋巴细胞分离液的上层为肿瘤细胞。值得注意的是，首次从肿瘤组织分离得到的TILs通常处于免疫抑制状态，且数量无法满足临床有效治疗所需的最低用量，因此，需要对其进行体外快速扩增和活化。

### 2.2 TILs扩增

传统的TILs扩增包括两个阶段(图2)，第一阶段为预扩增阶段，在此阶段，TILs从肿瘤碎片中分离或迁出，此时需要针对自体肿瘤或者人白细胞抗原配型的细胞系检测干扰素活性，从而筛选出肿瘤特异性的TILs细胞克隆。第二阶段为快速扩增阶段，在此阶段，一般将TILs浓度调整为$1\times10^6$个/mL，使用高浓度IL-2进行培养。随后观察细胞生长状况，可在加入IL-2的基础上添加CD3抗体、CD28抗体或饲养细胞，如辐射后的供体外周血单核细胞(peripheral blood mononuclear cells, PBMCs)，以此增强TILs的扩增能力[22]。在TILs治疗时，需向患者体内回输至少$1\times10^{10}$个TILs细胞，因此，TILs的分离培养过程通常需要6~8周[23]。然而，长时间的体外培养会造成TILs耗竭，且回输后TILs不能在患者体内长期存在[24-25]。此外，体外培养自体TILs的成功率较低，导致接受TILs治疗的患者退出率超过50%，这是TILs临床应用

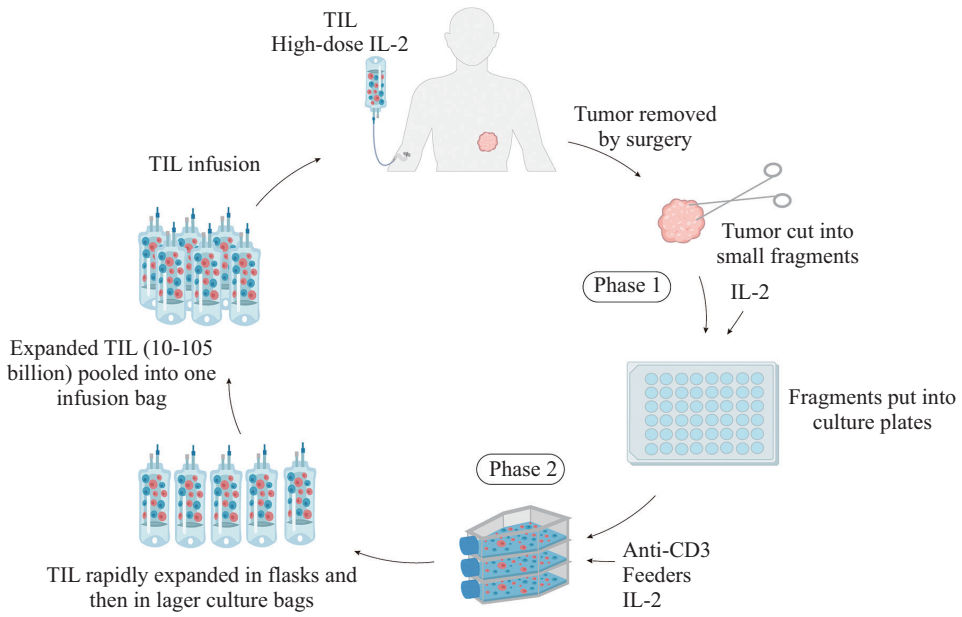


**图2 TILs的生产流程(根据参考文献[32]修改)**

**Fig.2 The process of TILs production (modified from the reference [32])**

面临的最大难题[26-27]。为此, ROSENBERG博士等[28]开发了“Young TIL”方法, 研究人员将10~18天后迁出的TILs进行汇集培养, TILs与自体肿瘤细胞共培养后, 其肿瘤的杀伤效果与传统TILs无异, 因此无需在体外对TILs的肿瘤反应性进行筛选, 即可实现其快速扩增, 此方法显著提高了TILs生产效率及其在体内的存活率和疗效。同时, “Young TIL”显示出与传统TILs治疗相似的临床结果[29], 且其治疗效果与输注TILs中的$CD8^+$ T细胞的数量成正比[30]。

## 3 改善TILs培养的方法

为了提高TILs对肿瘤患者的治疗效果, 在细胞培养过程中不仅要维持TILs细胞高效扩增, 还需要增强TILs的活性和归巢能力。目前为止, 已发现IL-2、IL-7、IL-12、IL-15和IL-21等多种细胞因子都对T细胞的生长分化有不同程度的影响。不仅如此, 研究还发现PD-1/PD-L1等免疫检查点的抗体及一些信号分子在促进T细胞增殖的同时还能增强TILs的活性。众所周知, TILs细胞有效归巢到肿瘤部位是发挥肿瘤治疗的关键因素, 因此, 在增强TILs活性和增加其数量的同时, 还需要改善TILs的归巢能力。接下来, 我们将对TILs的高效扩增和归巢进行重点讨论。

### 3.1 增强TILs扩增效率

3.1.1 利用细胞因子扩增TILs 早在1986年, IL-2就已应用于TILs培养[1]。后续研究表明, 添加CD3抗体、CD28抗体[31]、饲养层细胞能刺激TILs快速增长[32], 两周内, TILs细胞平均扩增1 392倍[33]。目前, TILs细胞培养中应用最多的细胞因子是IL-2, 它通过与白细胞介素-2受体(interleukin-2 receptor, IL-2R)结合, 进而促进T淋巴细胞和NK细胞等免疫细胞的增殖。然而, 研究显示IL-2不仅参与效应$CD8^+$ T细胞的增殖和分化, 也能促进具有免疫抑制能力的调节性T细胞(regulatory T cells, Treg)的分化[34-36]。由于TILs的体外培养时间较长, 因此, IL-2的使用势必会增加TILs中Treg的占比, 这不利于回输后的治疗。此外, 高剂量IL-2注射还可能造成肿瘤患者的生存质量下降等副作用。由此可见, 如何避免大剂量使用IL-2带来的副作用和免疫抑制细胞扩增是当前TILs药物发展中需要关注的重点之一, 而比较不同细胞因子组合对TILs增殖的影响, 或寻找IL2的替代品, 将可能成为解决这些难题的突破口。

目前, 已经有多种IL-2的突变体进入临床试验阶段。其中NKTR-214、THOR-707、Super-2、NEO-2/15和SHR-1916等突变体都更偏向于结合IL-2R的βγ链, 研究显示, 这些突变体保留了激活TILs大量扩增的能力, 又避免了Treg细胞的大量激活[37]。同时, 小鼠模型中NKTR-214作为单一疗法也表现出比IL-2更强的抗肿瘤活性, 且其诱导的肿瘤杀伤$CD8^+$ T细胞与Treg细胞的比例大于400, 而IL-2仅为18[38-39]。同样地, 在小

鼠实验中, THOR-707诱导$CD8^+$ T细胞和NK细胞的大规模活化和扩增, 但却不会诱导Treg的扩增[40]。NEO-2/15是BAKER团队[41]通过计算机拟合的一种人工蛋白, 其在人类和动物模型中都具有激活抗癌T细胞能力。在动物模型的研究中发现, NEO-2/15与天然的IL-2和IL-15具有相同的功能, 但其细胞毒性远低于后者, 可能成为一种优良的IL-2替代品[42]。此外, 也有研究开发出一种全新的IL-2突变体(又称OMCPmutIL-2), 其通过靶向NKG2D, 而不是高亲和力的IL-2R来进行TILs介导的免疫治疗。同样地, OMCPmutIL-2优先扩增TILs中的$CD8^+$ T细胞和NK细胞, 而非Treg细胞。此外, OMCPmutIL-2的TILs表达更高水平的肿瘤归巢受体, 如LFA-1、CD49a和CXCR3, 并具有更好的肿瘤抑制效果[43]。

IL-12是一种有效的促炎细胞因子, 可以直接增强T细胞的肿瘤溶解能力, 并增强T细胞的抗原呈递能力[44]。研究表明, IL-12不仅可促进幼稚T细胞的活化和增殖, 还能有效抑制T细胞的耗竭[45-46]。与未处理组相比, IL-2和IL-12处理的T细胞能有效促进小鼠黑色素瘤的清除和增强T细胞的抗肿瘤记忆能力[45,47]。此外, 在一项I期试验中, 使用经激活T细胞核因子(nuclear factor of activated T-cells, NFAT)诱导表达的IL-12转导TILs, 经过工程化TILs治疗的患者可达到63%的客观缓解率(objective response rate, ORR), 且所需细胞数量仅为传统TILs疗法的1%~10%[48]。

IL-15是一种常见的γ链细胞因子, 可促进T细胞增殖, 诱导细胞毒性淋巴细胞和记忆性$CD8^+$ T细胞的产生, 并刺激NK细胞的增殖和生长[49]。与IL-2不同, IL-15既不会诱导活化的$CD8^+$ T细胞的耗竭, 也不会持续激活Treg[49]。研究显示, 在体内, IL-15是维持记忆T细胞增殖和功能的关键因子[50-52]。但在体外, 与IL-2相比, IL-15的组合并不能更好地诱导效应T细胞产生[53]。因此, IL-15在培养过程中的作用还需要进一步证实。同样地, 尽管IL-7已经被部分研究者用于T细胞的扩增, 然而, 单独使用IL-7似乎也不能更好地促进T 细胞增殖[53]。此外, 与单独使用IL-2相比, IL-2、IL-15、IL-21组合已被证实可以增强肺癌和结直肠癌中的TILs扩增, 并能提高TILs中的$CD8^+$ T细胞百分比以及TCR克隆多样性[54]。因此, IL-2联合IL-15和IL-21可能是体外快速扩增TILs的一种新方法。

综上所述, IL-2依旧是目前应用最广泛的细胞因子, 除IL-12、IL-15外, 在培养过程中单独添加细胞因子似乎没有明显的效果, 仍需探索其他细胞因子对TILs扩增的影响。

3.1.2 *免疫检查点抗体增强TILs扩增能力* 研究发现, 免疫检查点抗体如PD-1、抗4-1BB、抗细胞毒性T淋巴细胞抗原4(cytotoxic T-lymphocyte antigen 4, CTLA-4)等都可以提高TILs的扩增能力[55-56]。有研究表明, 在胰腺癌TILs培养过程中进行PD-1阻断后, 3例患者中的TILs数量增加约2.5倍, 即抑制PD-1信号可促进胰腺肿瘤碎片中TILs的增殖, 增强胰腺肿瘤特异性T细胞的增殖能力。同样地, 与对照组相比, 添加抗4-1BB则导致$CD8^+$ T细胞数量变为原来的23倍, 这表明4-1BB拥有更强的诱导胰腺TILs扩增的能力[55]。此外, 添加抗4-1BB显著提升TILs的扩增这一现象也在原发性膀胱肿瘤[21]、肉瘤[57]、膀胱癌[58]、非小细胞肺癌[59]等肿瘤中得到证实。同样的研究显示, 在转移性卵巢癌初始TILs培养阶段添加抗CTLA-4抗体不仅可促进TIL的生长, 而且有利于$CD8^+$ T细胞的增殖, 从而增强TILs的抗肿瘤能力[56]。

3.1.3 *TILs的工程化改造增强其扩增能力* 此外, 利用CRISPR和TALEN等基因编辑技术, 或通过慢病毒感染的方式对TILs进行改造是增强其扩增能力和免疫活性的另一种方式。截至目前, 过表达IL-2和IL-12等细胞因子, 以及敲除*PD-1*分子的工程化TILs已经被应用于TILs治疗的研究[48,60]。同时, FORGET等[61]描述了一种通过逆转录病毒转导TILs的方法, 该方法对转移性黑色素瘤TILs的转导效率在31%至58%之间。然而, 在实际操作过程中, 由于TILs的细胞组成复杂, 各组分细胞生长速度不同, 因此, 对其进行基因编辑的难度较大。因此, 开发新的细胞转导方法是实现TILs工程化改造亟需解决的问题。

### 3.2 改善TILs的归巢能力

近年来, 过继性细胞疗法被成功应用于白血病、淋巴瘤等血液系统疾病的治疗, 然而其在实体肿瘤治疗应用中的进展缓慢。活化的T细胞不能有效靶向穿透到肿瘤实质, 成为限制过继性细胞治疗发挥有效抗肿瘤作用的关键因素。事实上, T细胞归巢包括滚动、黏附、外渗和趋化等一系列复杂的过程。研究发现, 在过继性细胞治疗过程中, 静脉注入的T细胞确实可以到达患者肿瘤部位, 但回输的T 细胞中仅有1%~2%真正浸润到肿瘤组织内部[62-64], 这可能是造成TILs在实体瘤治疗中疗效不佳的重要原

因。因此, 如何让更多回输的TILs细胞回到肿瘤内部是TILs应用于实体瘤治疗中必须解决的一个关键问题。

利用肿瘤细胞和肿瘤相关基质细胞产生的T细胞趋化因子是增强TILs向肿瘤部位迁移的重要方法之一。例如, 黑色素瘤产生大量趋化因子CXCL1, 但TILs细胞通常不表达其受体CXCR2[65]。在小鼠黑色素瘤肿瘤模型中, 过继转移的TCR转基因T细胞识别gp100, 在对表达CXCL1的gp100肿瘤细胞进行基因修饰并表达CXCR2后, 于体内外均表现出更高水平的肿瘤迁移性[66]。另一种方法是对TILs进行基因修饰, 使其表达更高水平的肿瘤组织特异性趋化因子受体, 增强其对趋化因子的亲和力, 促进TILs向肿瘤部位的迁移。研究发现, 肿瘤中活性氮物质大量积累, 诱导趋化因子CCL2高度硝基酪氨酸化, 降低了CCL2与T细胞上CCR2结合的亲和力[67]。因此, 使用基因转导增强T细胞中CCR2的表达也许可以克服这个问题。此外, *IL-17*、*CCL19*和*IL-12*等基因的过表达已被证实能够促进CAR-T细胞的归巢, 后期有望应用于TILs治疗领域, 提高TILs的归巢效应[48,68]。

除此之外, 增强TILs的靶向能力和联合治疗也可以显著提高TILs细胞的归巢能力。目前TILs联合PD-1/程序性死亡受体配体1(programmed cell death-ligand 1, PD-L1)免疫检查点抗体[69]、肿瘤疫苗[70]、溶瘤病毒[71]、化疗等治疗手段已经被用来提高TILs的归巢能力, 但仍需更多的临床试验加以验证。

## 4 TILs在临床治疗中的应用

截至目前, 针对TILs治疗开展的临床试验已超过70项, 涵盖包括宫颈癌、黑色素瘤、乳腺癌、非小细胞肺癌、结直肠癌等在内的恶性肿瘤。尤其在2018年, 两款基于TILs治疗的临床产品Iovance LN144[72] 和LN145[73]的II期试验取得成功, 为未来将TILs治疗应用于实体瘤的治疗奠定了基础。在TILs临床试验的相关报道中, 黑色素瘤的研究占比最多, 其次是非小细胞肺癌、卵巢癌和头颈癌(表1)。

### 4.1 TILs在临床治疗中的单用治疗策略

目前, TILs疗法单独使用的疗效已经在黑色素瘤、宫颈癌、非小细胞肺癌和乳腺癌中得到证实。其中, TILs疗法在转移性黑色素瘤患者中已达到36.0%~70.0%的ORR[22,85-86], 多名患者甚至达到完全缓解。TILs疗法在晚期宫颈癌[87]患者中的完全缓

**表1 TILs治疗的部分临床试验项目**

**Table 1 Selected clinical trial programs for TILs treatment**

| 肿瘤类型<br>Cancer type | 注册号<br>Registration number | 临床阶段<br>Clinical phase | 治疗方式<br>Treatment | 参考文献<br>References |
|---|---|---|---|---|
| Melanoma | NCT02621021 | II | TIL+Pembrolizumab | [74] |
| | NCT03638375 | I/II | TIL+Nivolumab/IFN-α | [75] |
| | NCT04924413 | II | TIL+Tislelizumab | [76] |
| Metastatic melanoma | NCT01236573 | I/II | TIL+IL-12 | [48] |
| | NCT01319565 | II | TIL+total body irradiation | [77] |
| | NCT01883323 | II | IL-2 | [78] |
| | NCT02354690 | I/II | TIL+Vemurafenib | [79] |
| | NCT02360579 | II | None | [80] |
| | NCT02379195 | I/II | TIL+peginterferon alfa-2b | [79] |
| Non-small cell lung cancer | NCT03215810 | I | TIL+Nivolumab | [81] |
| | NCT03645928 | II | None | [82] |
| | NCT03903887 | I/II | None | None |
| Metastatic NSCLC | NCT02133196 | II | TIL+Aldesleukin | [83] |
| Advanced ovarian cancer | NCT03158935 | I | TIL+Pembrolizumab | None |
| Metastatic ovarian cancer | NCT02482090 | I | None | [84] |
| | NCT03287674 | I/II | TIL+Ipilimumab/Nivolumab | None |
| Squamous cell carcinoma of the head and neck | NCT03083873 | II | None | None |
| Cervical carcinoma | NCT03108495 | II | TIL+Pembrolizumab | None |
| Colorectal cancer | NCT03904537 | I/II | TIL+anti-PD-1 antibody-activated TIL | None |

解率达到75.0%。更令人欣喜的是, 从免疫检查点抑制剂耐药的黑色素瘤患者肿瘤中分离的TILs细胞可以有效介导肿瘤消退[88]。多个临床试验同样显示, TILs疗法在抗PD-1耐药的黑色素瘤患者中显示较好的效果, 达到30%~40%的肿瘤消退[80,89-90], 而针对抗PD-1耐药的转移性肺癌患者, I期临床结果显示在13名可评估的患者中, 3名确认有反应, 11名患者肿瘤负荷减少[81]。其中两名患者在1.5年后取得了持续的完全缓解。此外, 仍有临床试验招募抗PD-1治疗失败的转移性黑色素瘤患者(NCT02278887)进行TILs治疗, 因此, TILs治疗可作为治疗免疫检查点耐药患者的选择之一。

此外, 还有许多正在进行中的TILs临床研究, 包括胆道癌(NCT03801083)、转移性葡萄膜黑色素瘤(NCT03467516)、妇科肿瘤(NCT04766320)、预处理的转移性三阴性乳腺癌(NCT04111510)、非小细胞肺癌 (NCT04614103)、结直肠癌(NCT03904537)、卵巢癌(NCT04072263)等。

### 4.2 TILs在临床治疗中的联合治疗策略

尽管TILs治疗已被证明是迄今为止转移性黑色素瘤最有效的治疗手段之一, 但在大多数恶性肿瘤中, 抑制性的肿瘤微环境及有限的T细胞归巢等因素降低了TILs杀伤肿瘤细胞的能力。因此, 仅单一使用TILs治疗肿瘤在临床上的效果并不稳定[91], 而通过其他癌症治疗手段先行改善肿瘤微环境中的免疫抑制状态, 再联合TILs治疗的策略, 就可能成为治疗晚期恶性肿瘤患者的可选方案。LU等[70]在小鼠乳腺癌模型中发现, 接受肿瘤疫苗治疗后的小鼠肿瘤组织中$CD8^+$ T细胞浸润明显增强。MA等[92]设计出一种肿瘤疫苗联合过继性细胞疗法, 此疗法可提高肿瘤局部的T细胞数量近200倍, 提升抗肿瘤功能5~10倍。这些研究表明, 肿瘤疫苗能显著提高外周T细胞的归巢能力。

近期披露的一些临床数据表明, TILs治疗与抗PD-1/PD-L1抗体治疗的联合治疗具有良好的治疗结果[69,81,93], 单独使用纳武利尤单抗治疗后, 患者的ORR仅有6.67%, 但TILs联合纳武利尤单抗治疗后患者的ORR提升至33.3%[69]。最近的研究发现, 树突状细胞(dendritic cell, DC)疫苗可以诱导免疫应答, 并激活TILs和增加其数量[94-95]。一项临床试验表明, TILs治疗与DC疫苗联合使用可达到50.0%的客观缓解率[96]。然而, PENG等[93]的研究发现DC表达高水平的PD-L1, 可削弱T细胞的活化并抑制抗肿瘤活性。因此, 之后的研究可能需要考虑利用TILs联合PD-L1抑制剂治疗解决这个问题。

此外, 研究表明TILs治疗联合鼠类肉瘤病毒癌基因同源物B1(V-raf murine sarcoma viral oncogene homolog B1, BRAF)抑制剂治疗[97]显示出较好的效果, 最近的一项实验表明BRAF/丝裂原细胞外激酶(mitogen-activated protein kinase, MEK)抑制剂可促进黑色素瘤患者黑色素瘤特异性T细胞的增殖[98], 且该研究显示TILs联合维莫非尼治疗后有64%患者的肿瘤消退, 其中18%患者的肿瘤完全消退。此外, 仍有许多针对TILs治疗与溶瘤病毒的联合治疗的相关研究正在进行[71,99]。因此, TILs联合多种肿瘤治疗方案有望成为一种更有效的策略。

## 5 展望

近年来, 肿瘤免疫治疗取得了巨大成功, 特别是以免疫检查点PD-1/PD-L1抗体为代表的生物药显著延长了晚期癌症患者的生存期[100]。然而, 目前的临床数据表明, 在PD-1/PD-L1疗法中, 平均只有25.0%的实体瘤患者有响应, 而针对微卫星稳定型结肠癌和三阴性乳腺癌等癌症的治疗几乎没有效果, 且部分患者容易产生耐药[101-102]。

TILs疗法可以在多数转移性癌症患者中诱导有效且持久的临床反应, 这使ACT在治疗实体肿瘤方面产生了革命性变化。然而, TILs的获取和标准化生产过程中仍存在许多亟待解决的问题。根据不同肿瘤类型的特点, 建立一个标准化、稳定的TILs生产流程至关重要。其中, 在较短的培养时间内丰富肿瘤特异性TILs是首要考虑的问题。目前, 虽然TILs联合低或中剂量IL-2对肿瘤的治疗可达到超过30%的客观缓解率[103-104], 但大多数TILs试验仍采用高剂量IL-2输注。然而, 高剂量的IL-2通常会引起全身毒性, 需要严密的监测和护理, 这增加了TILs治疗的复杂性。因此, IL-2是否是TILs体外回输最理想的细胞因子尚存争议。目前情况下, 亟需比较不同的细胞因子或者组合对TILs增殖的影响, 寻找IL-2的替代品, 以免TILs治疗在临床中产生强烈的副作用。与此同时, 大量基础研究正在积极探索增强TILs的体外培养和体内杀伤活性的方法。其中, 利用CRISPR全基因敲除、干扰或激活文库技术寻找T细胞或者TILs细胞增殖分化新靶

点是一种很有希望的方法[105-106]。

最后值得注意的是，大多数晚期癌症患者已经失去了手术机会，无法获得术后肿瘤组织。因此，从外周血或胸腹腔内积液内寻找具有肿瘤特异性的TILs并进行体外大规模培养，是该领域的重点研发方向之一。尽管从外周血中分离肿瘤特异性T细胞有很多困难，但是现在已经被证实的是，多种药物治疗后肿瘤患者的外周血中都能检测肿瘤特异性T细胞[11-12]，目前亟需探究药物治疗后肿瘤患者外周血中的肿瘤特异性T细胞激活的时间点，成功分离并在体外大量培养这些细胞进行回输，可能是解决目前大多晚期肿瘤患者无法获取TILs这一困境的最佳方案。因此，充分理解PBMC和TILs在表型和功能上的区别，并利用这些不同点开发新的TILs治疗策略，在不久的将来应该能有效治疗更大比例的人类癌症。

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