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T细胞耗竭与CAR-T细胞免疫疗法①

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［**摘　要**］　T细胞耗竭(Tex)即T细胞持续暴露于炎症或抗原信号下而处于功能疲惫的一种状态，增殖和记忆能力逐渐降低，表达多个抑制性受体。嵌合抗原受体T(CAR-T)细胞免疫疗法是目前最具前景的肿瘤免疫疗法之一，在恶性血液系统肿瘤治疗中已取得显著成绩，但CAR-T细胞中Tex形成，尤其是$CD8^+$T细胞耗竭严重影响CAR-T细胞抗肿瘤效应。本文主要对Tex与CAR-T细胞免疫疗法进行综述。

［**关键词**］　T细胞耗竭；嵌合抗原受体T细胞；肿瘤免疫疗法

T cell exhaustion and CAR-T cell immunotherapy

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［**Abstract**］　T cell exhaustion (Tex), namely T cells were in a state of functional exhaustion due to persistent exposed to inflammatory or antigen signals. Characterised by progressive loss of proliferation and memory abilities, while expression of multiple inhibitory receptors. Chimeric antigen receptor T(CAR-T) cell immunotherapy was one of the most promising Immunotherapy for tumor, who has achieved remarkable results in treatment of malignant hematologic system tumors. However, formation of Tex in CAR-T cells, especially exhaustion of $CD8^+$T cells, seriously affected anti-tumor effect of CAR-T cells. This paper aimed to make a clarify of Tex and CAR-T cell immunotherapy.

［**Key words**］　T cell exhaustion; Chimeric antigen receptor T cell; Tumor immunotherapy

CAR-T细胞免疫疗法是以T细胞为来源，进行体外基因修饰后回输治疗的一种过继性细胞疗法，其在识别肿瘤细胞时由于不受MHC限制，可有效避免肿瘤逃逸[1]。但长期肿瘤抗原及其他免疫抑制信号刺激导致CAR-T细胞功能逐渐衰减，无法有效杀伤肿瘤细胞。因此，阐明Tex形成的具体分子机制有利于寻找逆转CAR-T细胞功能的最佳策略，此外，联合其他肿瘤免疫治疗方法有望进一步延伸CAR-T细胞疗法在肿瘤治疗中的应用。

1　T细胞耗竭

T细胞耗竭(T cell exhaustion，Tex)最初用于描述慢性病毒感染时T细胞在持续抗原刺激下的一种功能异常状态，最近报道，参与肿瘤免疫的T细胞也存在相似现象[2-3]。T细胞发生耗竭时，其生物学功能主要表现为：抑制性受体PD-1、CTLA-4、TIM-3、LAG-3等表达持续增加或同时共表达多个抑制性受体；效应功能渐进性降低：包括细胞因子释放情况(IFN-γ、IL-2)与穿孔素、颗粒酶产生情况；增殖能力及自我更新能力(依赖于IL-7、IL-15)降低；代谢改变，耗竭性T细胞线粒体活性降低，糖酵解受限，代谢储备能力降低。

研究表明，耗竭性T细胞不同于效应性T细胞(T effector，Teff)$KLRG1^+CD127^-$及记忆性T细胞(T memory，Tmem)KLRG1-$CD127^+$等，而是具有独特分子表型的一群异质性细胞[4-5]。$TCF\text{-}1^+PD\text{-}1^{int}TIM\text{-}3^-$为Tex祖细胞表型，$TCF\text{-}1^-PD\text{-}1^{hi}TIM\text{-}3^{hi}$为终末Tex表型，此外，CXCR5也可能作为祖细胞标志之一[6-7]。HUDSON团队[8]最新发现，$TCF\text{-}1^-PD\text{-}1^+TIM\text{-}3^+CX3CR1^+$可能为耗竭性T细胞中间态细胞群，这种中间态的$CX3CR1^+$细胞发挥效应的程度近似Teff。阻断PD-1后$TCF\text{-}1^-PD\text{-}1^+TIM\text{-}3^+CX3CR1^+$细胞群增加，暗示该中间态可能是PD-1营救后主要发挥抗肿瘤效应的细胞群体。目前，Tex来源尚未统一，可能为初始T细胞(naïve T cell，Tn)接受活化信号后先分化为耗竭性T细胞前体，前体细胞进一步分化为中间态，最终发育为$TCF\text{-}1^-PD\text{-}1^{hi}$末端状态[9]。

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2　Tex分子机制

急性感染时，Tn经抗原活化增殖发挥杀伤效应，约5%T细胞转化为记忆性细胞，以非抗原依赖方式进行自我更新，当再次接触相同抗原时，立即进行免疫应答。但当感染持续存在时，T细胞接受慢性持久刺激，活化与分化发生异常改变。导致T细胞处于耗竭状态的潜在分子机制主要为：①DNA甲基化或组蛋白乙酰化的改变，如编码抑制性受体PD-1，以及与记忆、效应分子等相关基因发生DNA甲基化或组蛋白乙酰化，从而影响基因表达[10-11]。GHONEIM等[10]发现DNA甲基化酶3a（De novo methyltransferase 3a，DNMT3a）介导的DNA从头甲基化促进耗竭性T细胞前体形成，给予去甲基化药物decitabine治疗后，$CD8^+$T细胞耗竭得到改善，联合PD-1单抗治疗时，小鼠肿瘤负荷降低，转录因子表达改变。CHEN等[4]采用淋巴细胞脉络丛脑膜炎病毒（LCMV）Arm与C113分别制备急性及慢性小鼠感染模型，采用单细胞测序技术结合模拟分化时间预测发现，T细胞在发展为Tex的早期阶段，TCF-1起决定性作用，其潜在机制可能是通过拮抗促进T细胞向Teff分化的转录因子，而正向调节Emoes、C-Myb等与记忆及存活相关的转录因子，从而促进T细胞向Tex方向发育。此外该研究发现，PD-1表达有助于耗竭性T细胞前体形成，保护其因过度活化而凋亡。除TCF-1作为促进Tex发展的转录因子外，2019年《Nature》3篇文章报道TOX同样促进Tex祖细胞形成[5, 12-13]。此外NR4A家族、BLIMP-1、NFAT、Emoes、T-bet等均参与耗竭T细胞生成[14-17]；②肿瘤微环境因素，包括调节性T细胞（regulatory cells，Tregs）、骨髓来源抑制性细胞（myeloid-derived suppressor cells，MDSCs）、M2型巨噬细胞等抑制性免疫细胞，IL-10、IL-6、TGF-β等抑制性细胞因子，以及肿瘤细胞周围纤维母细胞等均促进$CD8^+$T细胞耗竭形成[18-20]；③小分子RNA参与，如miRNA-155、miR-143、miR-146a等通过多种方式调节T细胞记忆、效应功能及代谢等相关基因表达[21-23]。

3　CAR-T细胞免疫疗法

CAR-T细胞疗法是指通过体外对T细胞进行基因修饰，赋予其靶向杀伤肿瘤细胞特性，经扩增培养后回输患者的一种细胞免疫疗法。目前该免疫疗法主要用于血液系统肿瘤治疗，靶点为CD19，此外，CD20、CD22等多靶点联合治疗也显现出较好效果[24-26]。实体瘤治疗方面，由于肿瘤特异性抗原缺乏及肿瘤微环境等因素，其应用受阻。在研靶点包括HER-2、GD2、Mesothelin、CEA、PSCA等，主要针对乳腺癌、肝癌、肺癌、结直肠癌等，针对CAR-T细胞在实体瘤治疗方面的应用，主要应对策略包括：①采用基因编辑技术（ZFN、TALEN、CRISPR/Cas9）改造CAR-T细胞。HU等[27]与GUO等[28]研究发现，敲除PD-1后，CAR-T细胞对肝癌细胞及Mesothelin阳性的三阴性乳腺癌细胞杀伤力明显增强。此外，EYQUEM等[29]利用CRISPR/Cas9技术将CAR基因“精准”递送至T细胞受体（T-cell receptor α constant，TRAC）基因座，避免常规慢病毒载体或其他递送方式将CAR基因随机插入T细胞基因组而引发潜在细胞癌变，这种编辑方式制备的CAR-T细胞在小鼠白血病模型中相比常规CAR-T细胞显现出更强的杀伤力；②设计多靶标CAR。BIELAMOWICZ等[30]将HER2、IL13Rα2、EphA构建到同一CAR框中研究其对恶性胶质瘤的杀伤作用，结果表明其杀伤效应优于单独或双靶点；③联合其他免疫疗法。MA等[31]开发一种可使CAR-T细胞在淋巴结提前进入激活状态的疫苗，结果显示，该联合治疗在胶质母细胞瘤、乳腺癌及黑色素瘤等多种小鼠肿瘤模型中均产生较好效果；④组成性表达IL-23、IL-7/CCL-19等细胞因子[32-33]；⑤胞内共刺激分子选择、制备通用CAR-T细胞、优化CAR-T细胞体外培养体系、制备TSCM、TCM表型CAR-T细胞、改善CAR-T细胞耗竭等对CAR-T细胞杀伤实体瘤均有一定促进作用[34-41]。

4　耗竭性CAR-T细胞

CHEN等[16]和SEO等[17]发现，与HBV/HCV感染时出现的Tex相似，肿瘤浸润性CAR-T细胞相较于体外制备的CAR-T细胞，可高表达NR4A、TOX等T细胞耗竭相关转录因子。当敲除或沉默TOX、TOX2，Nr4a1、Nr4a2、Nr4a3基因后，黑色素小鼠肿瘤负荷减小，生存时间延长，同时沉默TOX与NR4A效果更为显著。进一步研究发现，NR4A、TOX及NFAT 3个转录因子可能在pdcd-1启动子上游约23 kb共同形成转录增强子复合体，从而调节pdcd-1基因表达。WEI等[42]从细胞代谢角度出发，靶向代谢相关因子REGNASE-1，$CD8^+$T细胞发生重编程，扩增能力、体内存留时间、效应功能等都得到提升，对小鼠白血病与黑色素瘤均显示较好疗效，可能通过影响BAFT、PTPN2与SOCS1等基因表达发挥作用。ZOU等[43]采用CRISPR/cas9技术同时敲除

PD-1、TIM-3、LAG3免疫抑制性受体，表现出更佳肿瘤控制效果，具体分子机制可能为敲除抑制性受体后一方面阻断肿瘤细胞传导抑制信号，另一方面CAR-T细胞表面CD56表达上调，促进CAR-T细胞存活，改善耗竭状态从而增强功能。

除肿瘤微环境因素导致CAR-T细胞耗竭外，CAR-T细胞自身区别于天然T细胞，CAR结构引入，尤其胞内共刺激分子不同，使制备的CAR-T细胞因“噪声信号”引起非抗原依赖T细胞过度活化，导致Tex发生。LONG等[34]观察到胞内刺激分子为4-1BB时，相比CD28 CAR-T细胞存活时间更久；LYNN等[15]发现，相较于CD19-28z CAR T细胞，HA-28z CAR T细胞表现出更显著的Tex，进一步研究表明，AP-1家族转录因子c-jun表达降低，过表达c-jun后，CAR-T细胞增殖能力、效应分子分泌能力提升，而分化程度、抑制性受体表达降低，小鼠肿瘤负荷降低。此外，共表达IL-15、抗PD-1单链抗体，封闭TGF-β信号等方法在一定程度上可改善CAR-T细胞耗竭状态，加速肿瘤清除[41-47]。

5　T细胞耗竭与CAR-T细胞免疫疗法国内外研究现状与前景

基于耗竭性T细胞对抗肿瘤治疗效果的影响，国内外研究者开始关注Tex营救，即免疫检查点阻断剂（immune checkpoint inhibitors，ICI）的应用，如阻断PD-1/PD-L1信号传递，该方法一方面阻断肿瘤细胞诱导的抑制作用，另一方面可逆转PD-1lo的Tex祖细胞并促进其成熟分化，发挥杀伤功能[48]。目前，临床常采取的ICI靶点包括PD-1、CTLA-4，主要适应症为恶性黑色素瘤，此外也用于非霍基金淋巴瘤、非小细胞肺癌等研究[49]。2011年，CTLA-4特异性抗体Ipilimumab获FDA上市批准，临床数据分析显示，5年无复发生存率为40.8%[50]；2014年，PD-1特异性单克隆抗体Opdivo、Keytruda获批上市，最初用于恶性黑色素瘤治疗，Keytruda五年生存率达34%[51]。HUANG等[52]研究发现，PD-1疗效取决于T细胞活化效应与患者肿瘤负担。2016年，PD-L1单抗Tecentriq获批用于治疗膀胱癌、非小细胞肺癌治疗等；2018年，我国首个PD-1单抗Toripalimab获批上市，其治疗黑色素瘤、肺癌、淋巴瘤等肿瘤均取得较好反响[53-54]。随后Sintilimab、Tislelizumab、Camrelizumab也进入临床试验，拟用于非小细胞肺癌、肝癌、胃癌、霍基金淋巴瘤等肿瘤治疗。由于耗竭T细胞一般同时表达多个抑制性受体，临床上采取抑制剂联合治疗，常见组合为CTLA-4与PD-1，治疗恶性黑色素瘤时五年生存率达52%[55]。PD-1与其他抑制剂联用也在进一步研究中[56-58]。

CAR-T细胞疗法目前主要用于血液系统肿瘤治疗。2012年，EMILY WHITEHEAD在美国宾夕法尼亚大学医院接受肿瘤CAR-T细胞治疗，目前未复发，成为世界上第一个被CAR-T细胞疗法治愈的儿童，极大程度推进了细胞疗法在肿瘤免疫中的应用。2017年，FDA先后批准Kymriah、Yescarta上市，适应证为复发或难治性儿童急性淋巴细胞白血病和复发或难治性大B细胞淋巴瘤，治疗靶点为CD19，Kymriah 3个月响应率为81%，12个月生存率76%[59]。Yescarta完全缓解率可达51%[60]。此外，包括以BCMA、CD20、CD33、CD123为靶点用于多发性骨髓瘤、急性髓系白血病等研究，同种异体CAR-T细胞申报的临床试验也显示较好疗效[61-62]。国内相关研究积极跟进，2018年3月，我国第一个CAR-T细胞疗法临床申请获CFDA批准，为南京传奇生物申报，以BCMA为靶点，适应证为复发性或耐药性多发性骨髓瘤，总反应率为88.2%，一年总生存率为82.3%[63]。继南京传奇后，上海明聚生物、上海优卡迪生物、复星凯特、银河生物等公司相继开展临床试验。目前，全球登记的CAR-T细胞治疗临床研究约630项，美国约234项，我国达315项，在研靶点主要以CD19为主，BCMA次之。与血液肿瘤相比，CAR-T细胞疗法在实体瘤研究领域面临更大挑战，登记的在研项目约30多项，主要靶点为GD2、MUC1、EGFR、Her2等，此外也有联合ICI同时进行治疗的研究[62]。近年细胞免疫疗法在血液肿瘤与实体瘤治疗方面呈明显上升趋势，治疗效果可观，CAR-T细胞疗法更为显著，后续对CAR-T细胞治疗的整个流程不断优化，有望为肿瘤患者带来福音。

6　展望

CAR-T细胞疗法作为目前肿瘤治疗中最具前景的免疫疗法之一，改善其耗竭状态将增强CAR-T细胞功能，提升肿瘤免疫治疗效果。目前，联合治疗成为肿瘤治疗研究热点，在一定程度上呈现“协同效应”，如ICI与CAR-T，溶瘤病毒（OVs）等两两组合，或前期辅助放化疗、其他抗肿瘤药物。SAMSON等[64]发现，瘤内注射OVs进行肿瘤微环境预处理，促进胶质瘤周围T细胞浸润，此外，辅之PD-1封闭处理，相较于对照组，小鼠肿瘤得到明显改善；经改造后可分泌EGFR双特异性抗体的溶瘤病毒（OAd-

BiTE),与CAR-T联用促进T细胞体外活化、增殖及细胞毒性,且在小鼠肿瘤模型中展现较好效果[65]。GULATI等[66]表明,CAR-T细胞与PD-1抗体联合治疗可加速肿瘤清除,其在MPM患者体内存留时间长达21 d。最近,NAKAZAWA等[67]采用CRISPR/cas9技术敲除CAR-T细胞表面PD-1,其对恶性胶质瘤细胞具有一定抑制作用,而T细胞自身表型及抑制性受体表达未受影响,进一步体内实验尚待研究。可见,联合治疗的确增强了抗肿瘤效应,未来,CAR-T、ICI、溶瘤病毒三者联用可能成为肿瘤免疫治疗的发展趋势[68]。尽管耗竭性T细胞依然可分泌细胞毒性效应分子,在维持长期肿瘤或感染性疾病中发挥作用,但总的来说其抗肿瘤效应仍是局限的。因此,着力于Tex状态改善,更深入研究CAR-T细胞耗竭,以及与其他抗肿瘤疗法联合治疗将进一步拓展CAR-T细胞疗法的临床应用,尤其在肝癌、肺癌、乳腺癌等实体瘤治疗方面。

参考文献:

[1] KUWANA Y, ASAKURA Y, UTSUNOMIYA N, *et al*. Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions[J]. Biochem Biophys Res Commun, 1987, 149(3): 960-968. DOI: 10.1016/0006-291x(87)90502-x.

[2] PALEY M A, KROY D C, ODORIZZI P M, *et al*. Progenitor and terminal subsets of CD8$^+$T cells cooperate to contain chronic viral infection[J]. Science, 2012, 338(6111): 1220-1225. DOI: 10.1126/science.1229620.

[3] MCLANE L M, ABDEL-HAKEEM M S, WHERRY E J. CD8 T cell exhaustion during chronic viral infection and cancer[J]. Annu Rev Immunol, 2019, 37: 457-495. DOI: 10.1146/annurev-immunol-041015-055318.

[4] CHEN Z, JI Z, NGIOW S F, *et al*. TCF-1-centered transcriptional network drives an effector versus exhausted CD8 T cell-fate decision[J]. Immunity, 2019, 51(5): 840-855. e845. DOI: 10.1016/j.immuni.2019.09.013.

[5] KHAN O, GILES J R, MCDONALD S, *et al*. TOX transcriptionally and epigenetically programs CD8(+) T cell exhaustion[J]. Nature, 2019, 571(7764): 211-218. DOI: 10.1038/s41586-019-1325-x.

[6] IM S J, HA S J. Re-defining T-cell exhaustion: Subset, function, and regulation[J]. Immune Netw, 2020, 20(1): e2. DOI: 10.4110/in.2020.20.e2.

[7] WINKLER F, BENGSCH B. Use of mass cytometry to profile human T cell exhaustion[J]. Front Immunol, 2019, 10: 3039. DOI: 10.3389/fimmu.2019.03039.

[8] HUDSON W H, GENSHEIMER J, HASHIMOTO M, *et al*. Proliferating transitory T cells with an effector-like transcriptional signature emerge from PD-1(+) stem-like CD8(+) T cells during chronic infection[J]. Immunity, 2019, 51(6): 1043-1058. e1044. DOI: 10.1016/j.immuni.2019.11.002.

[9] BLANK C U, HAINING W N, HELD W, *et al*. Defining 'T cell exhaustion'[J]. Nat Rev Immunol, 2019, 19(11): 665-674. DOI: 10.1038/s41577-019-0221-9.

[10] GHONEIM H E, FAN Y, MOUSTAKI A, *et al*. De novo epigenetic programs inhibit PD-1 blockade-mediated T cell rejuvenation[J]. Cell, 2017, 170(1): 142-157. e119. DOI: 10.1016/j.cell.2017.06.007.

[11] 张红梅. 沉默组蛋白去乙酰化酶11对NKG2D-CAR-T免疫功能影响的研究[D]. 上海:华东师范大学, 2019.

[12] ALFEI F, KANEV K, HOFMANN M, *et al*. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection[J]. Nature, 2019, 571(7764): 265-269. DOI: 10.1038/s41586-019-1326-9.

[13] KIM K, PARK S, PARK S Y, *et al*. Single-cell transcriptome analysis reveals TOX as a promoting factor for T cell exhaustion and a predictor for anti-PD-1 responses in human cancer[J]. Genome Med, 2020, 12(1): 22. DOI: 10.1186/s13073-020-00722-9.

[14] SHIN H, BLACKBURN S D, INTLEKOFER A M, *et al*. A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection[J]. Immunity, 2009, 31(2): 309-320. DOI: 10.1016/j.immuni.2009.06.019.

[15] LYNN R C, WEBER E W, SOTILLO E, *et al*. c-Jun overexpression in CAR T cells induces exhaustion resistance[J]. Nature, 2019, 576(7786): 293-300. DOI: 10.1038/s41586-019-1805-z.

[16] CHEN J, LOPEZ-MOYADO I F, SEO H, *et al*. NR4A transcription factors limit CAR T cell function in solid tumours[J]. Nature, 2019, 567(7749): 530-534. DOI: 10.1038/s41586-019-0985-x.

[17] SEO H, CHEN J, GONZALEZ-AVALOS E, *et al*. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8(+) T cell exhaustion[J]. Proc Natl Acad Sci U S A, 2019, 116(25): 12410-12415. DOI: 10.1073/pnas.1905675116.

[18] LAKINS M A, GHORANI E, MUNIR H, *et al*. Cancer-associated fibroblasts induce antigen-specific deletion of CD8 T Cells to protect tumour cells[J]. Nat Commun, 2018, 9(1): 948. DOI: 10.1038/s41467-018-03347-0.

[19] LI L, WANG L, LI J, *et al*. Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian cancer[J]. Cancer Res, 2018, 78(7): 1779-1791. DOI: 10.1158/0008-5472.CAN-17-2460.

[20] THOMMEN D S, SCHUMACHER T N. T cell dysfunction in cancer[J]. Cancer Cell, 2018, 33(4): 547-562. DOI: 10.1016/j.ccell.2018.03.012.

[21] YU T, JU Z, LUO M, *et al*. Elevated expression of miR-146a correlates with high levels of immune cell exhaustion markers and suppresses cellular immune function in chronic HIV-1-infected patients[J]. Sci Rep, 2019, 9(1): 18829. DOI: 10.1038/s41598-019-55100-2.

[22] STELEKATI E, CHEN Z, MANNE S, *et al*. Long-term persistence of exhausted CD8 T cells in chronic infection is regulated by microRNA-155[J]. Cell Rep, 2018, 23(7): 2142-2156. DOI: 10.1016/j.celrep.2018.04.038.

[23] ZHANG T, ZHANG Z, LI F, *et al*. miR-143 regulates memory T cell differentiation by reprogramming T cell metabolism[J]. J Immunol, 2018, 201(7): 2165-2175. DOI: 10.4049/jimmunol.

1800230.

[24] WALSH Z, ROSS S, FRY T J. Multi-specific CAR targeting to prevent antigen escape[J]. Curr Hematol Malig Rep, 2019, 14(5): 451-459. DOI:10.1007/s11899-019-00537-5.

[25] WEI J, ZHU X, MAO X, *et al*. Severe early hepatitis B reactivation in a patient receiving anti-CD19 and anti-CD22 CAR T cells for the treatment of diffuse large B-cell lymphoma[J]. J Immunother Cancer, 2019, 7(1): 315. DOI:10.1186/s40425-019-0790-y.

[26] ZAH E, LIN M Y, SILVA-BENEDICT A, *et al*. T cells expressing CD19/CD20 bispecific chimeric antigen receptors prevent antigen escape by malignant B cells [J]. Cancer Immunol Res, 2016,4(6):498-508. DOI:10.1158/2326-6066.CIR-15-0231.

[27] HU W, ZI Z, JIN Y, *et al*. CRISPR/Cas9-mediated PD-1 disruption enhances human mesothelin-targeted CAR T cell effector functions [J]. Cancer Immunol Immunother, 2019, 68(3): 365-377. DOI:10.1007/s00262-018-2281-2.

[28] GUO X, JIANG H, SHI B, *et al*. Disruption of PD-1 enhanced the anti-tumor activity of chimeric antigen receptor T cells against hepatocellular carcinoma[J]. Front Pharmacol, 2018, 9: 1118. DOI:10.3389/fphar.2018.01118.

[29] EYQUEM J, MANSILLA-SOTO J, GIAVRIDIS T, *et al*. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection [J]. Nature, 2017, 543(7643): 113-117. DOI:10.1038/nature21405.

[30] BIELAMOWICZ K, FOUSEK K, BYRD T T, *et al*. Trivalent CAR T cells overcome interpatient antigenic variability in glioblastoma [J]. Neuro Oncol, 2018, 20(4): 506-518. DOI: 10.1093/neuonc/nox182.

[31] MA L, DICHWALKAR T, CHANG J Y H, *et al*. Enhanced CAR-T cell activity against solid tumors by vaccine boosting through the chimeric receptor [J]. Science, 2019, 365(6449): 162-168. DOI:10.1126/science.aav8692.

[32] MA X, SHOU P, SMITH C, *et al*. Interleukin-23 engineering improves CAR T cell function in solid tumors [J]. Nat Biotechnol, 2020, 38(4): 448-459. DOI:10.1038/s41587-019-0398-2.

[33] ADACHI K, KANO Y, NAGAI T, *et al*. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor [J]. Nat Biotechnol, 2018, 36(4): 346-351. DOI:10.1038/nbt.4086.

[34] LONG A H, HASO W M, SHERN J F, *et al*. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors[J]. Nat Med, 2015, 21(6): 581-590. DOI:10.1038/nm.3838.

[35] GUEDAN S, MADAR A, CASADO-MEDRANO V, *et al*. Single residue in CD28-costimulated CAR T cells limits long-term persistence and antitumor durability[J]. J Clin Invest, 2020, 130(6):3087-3097. DOI:10.1172/JCI133215.

[36] HU W, HUANG X, HUANG X, *et al*. Chimeric antigen receptor modified T cell (CAR-T) co-expressed with ICOSL-41BB promote CAR-T proliferation and tumor rejection[J]. Biomed Pharmacother, 2019, 118: 109333. DOI: 10.1016/j.biopha.2019.109333.

[37] CHOI B D, YU X, CASTANO A P, *et al*. CRISPR-Cas9 disruption of PD-1 enhances activity of universal EGFRv Ⅲ CAR T cells in a preclinical model of human glioblastoma[J]. J Immunother Cancer, 2019, 7(1): 304. DOI:10.1186/s40425-019-0806-7.

[38] ZHAO J, LIN Q, SONG Y, *et al*. Universal CARs, universal T cells, and universal CAR T cells[J]. J Hematol Oncol, 2018, 11(1): 132. DOI:10.1186/s13045-018-0677-2.

[39] GARGETT T, TRUONG N, EBERT L M, *et al*. Optimization of manufacturing conditions for chimeric antigen receptor T cells to favor cells with a central memory phenotype[J]. Cytotherapy, 2019,21(6): 593-602. DOI: 10.1016/j.jcyt.2019.03.003.

[40] XU Y, ZHANG M, RAMOS C A, *et al*. Closely related T-memory stem cells correlate with in vivo expansion of CAR. CD19-T cells and are preserved by IL-7 and IL-15[J]. Blood, 2014, 123(24): 3750-3759. DOI:10.1182/blood-2014-01-552174.

[41] ALIZADEH D, WONG R A, YANG X, *et al*. IL15 enhances CAR-T cell antitumor activity by reducing mTORC1 activity and preserving their stem cell memory phenotype[J]. Cancer Immunol Res, 2019, 7(5): 759-772. DOI: 10.1158/2326-6066.CIR-18-0466.

[42] WEI J, LONG L, ZHENG W, *et al*. Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy[J]. Nature, 2019, 576(7787): 471-476. DOI:10.1038/s41586-019-1821-z.

[43] ZOU F, LU L, LIU J, *et al*. Engineered triple inhibitory receptor resistance improves anti-tumor CAR-T cell performance via CD56[J]. Nat Commun, 2019, 10(1): 4109. DOI:10.1038/s41467-019-11893-4.

[44] KLOSS C C, LEE J, ZHANG A, *et al*. Dominant-negative TGF-β receptor enhances psma-targeted human CAR T cell proliferation and augments prostate cancer eradication[J]. Mol Ther, 2018,26(7):1855-1866. DOI:10.1016/j.ymthe.2018.05.003.

[45] TANG N, CHENG C, ZHANG X, *et al*. TGF-β inhibition via CRISPR promotes the long-term efficacy of CAR T cells against solid tumors[J]. JCI Insight, 2020, 5(4): e133977. DOI: 10.1172/jci.insight.133977.

[46] NAKAJIMA M, SAKODA Y, ADACHI K, *et al*. Improved survival of chimeric antigen receptor-engineered T (CAR-T) and tumor-specific T cells caused by anti-programmed cell death protein 1 single-chain variable fragment-producing CAR-T cells[J]. Cancer Sci, 2019, 110(10): 3079-3088. DOI: 10.1111/cas.14169.

[47] GARGETT T, YU W, DOTTI G, *et al*. GD2-specific CAR T cells undergo potent activation and deletion following antigen encounter but can be protected from activation-induced cell death by PD-1 blockade[J]. Mol Ther, 2016, 24(6): 1135-1149. DOI:10.1038/mt.2016.63.

[48] JADHAV R R, IM S J, HU B, *et al*. Epigenetic signature of PD-1$^+$ TCF1$^+$ CD8 T cells that act as resource cells during chronic viral infection and respond to PD-1 blockade[J]. Proc Natl Acad Sci U S A, 2019, 116(28): 14113-14118. DOI: 10.1073/pnas.1903520116.

[49] SINGH S, HASSAN D, ALDAWSARI H M, *et al*. Immune checkpoint inhibitors: A promising anticancer therapy[J]. Drug Discov Today, 2020, 25(1): 223-229. DOI: 10.1016/j.drudis.2019.11.003.

[50] EGGERMONT A M, CHIARION-SILENI V, GROB J J, *et al*. Prolonged survival in stage Ⅲ melanoma with ipilimumab adjuvant therapy[J]. N Engl J Med, 2016, 375(19): 1845-1855. DOI:10.1056/NEJMoa1611299.

[51] HAMID O, ROBERT C, DAUD A, *et al*. Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001[J]. Ann Oncol, 2019, 30(4): 582-588. DOI:10.1093/annonc/mdz011.

[52] HUANG A C, POSTOW M A, ORLOWSKI R J, *et al*. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response[J]. Nature, 2017, 545(7652): 60-65. DOI:10.1038/nature22079.

[53] TANG B, YAN X, SHENG X, *et al*. Safety and clinical activity with an anti-PD-1 antibody JS001 in advanced melanoma or urologic cancer patients[J]. J Hematol Oncol, 2019, 12(1): 7. DOI:10.1186/s13045-018-0693-2.

[54] YANG J, DONG L, YANG S, *et al*. Safety and clinical efficacy of toripalimab, a PD-1 mAb, in patients with advanced or recurrent malignancies in a phase Ⅰ study[J]. Eur J Cancer, 2020, 130:182-192. DOI: 10.1016/j.ejca.2020.01.028.

[55] LARKIN J, CHIARION-SILENI V, GONZALEZ R, *et al*. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma[J]. N Engl J Med, 2019, 381(16): 1535-1546. DOI:10.1056/NEJMoa1910836.

[56] KON E, BENHAR I. Immune checkpoint inhibitor combinations: Current efforts and important aspects for success [J]. Drug Resist Updat, 2019, 45:13-29. DOI: 10.1016/j.drup.2019.07.004.

[57] D'ANGELO S P, MAHONEY M R, TINE B AVAN, *et al*. Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): Two open-label, non-comparative, randomised, phase 2 trials[J]. Lancet Oncol, 2018, 19(3): 416-426. DOI: 10.1016/S1470-2045(18)30006-8.

[58] FOURCADE J, SUN Z, PAGLIANO O, *et al*. PD-1 and Tim-3 regulate the expansion of tumor antigen-specific $CD8^+$ T cells induced by melanoma vaccines[J]. Cancer Res, 2014, 74(4): 1045-1055. DOI:10.1158/0008-5472.CAN-13-2908.

[59] MAUDE S L, LAETSCH T W, BUECHNER J, *et al*. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia[J]. N Engl J Med, 2018, 378(5): 439-448. DOI: 10.1056/NEJMoa1709866.

[60] BOUCHKOUJ N, KASAMON Y L, DE CLARO R A, *et al*. FDA approval summary: Axicabtagene ciloleucel for relapsed or refractory large B-cell lymphoma[J]. Clin Cancer Res, 2019, 25(6): 1702-1708. DOI:10.1158/1078-0432.CCR-18-2743.

[61] DEPIL S, DUCHATEAU P, GRUPP S A, *et al*. 'Off-the-shelf' allogeneic CAR T cells: Development and challenges[J]. Nat Rev Drug Discov, 2020, 19(3): 185-199. DOI: 10.1038/s41573-019-0051-2.

[62] MARDIANA S, SOLOMON B J, DARCY P K, *et al*. Supercharging adoptive T cell therapy to overcome solid tumor-induced immunosuppression[J]. Sci Transl Med, 2019, 11(495): eaaw2293. DOI:10.1126/scitranslmed.aaw2293.

[63] XU J, CHEN L J, YANG S S, *et al*. Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen in relapsed/refractory multiple myeloma[J]. Proc Natl Acad Sci U S A, 2019, 116(19): 9543-9551. DOI:10.1073/pnas.1819745116.

[64] SAMSON A, SCOTT K J, TAGGART D, *et al*. Intravenous delivery of oncolytic reovirus to brain tumor patients immunologically primes for subsequent checkpoint blockade[J]. Sci Transl Med, 2018, 10(422): eaam7577. DOI: 10.1126/scitranslmed.aam7577.

[65] WING A, FAJARDO C A, POSEY A D, *et al*. Improving CART-cell therapy of solid tumors with oncolytic virus-driven production of a bispecific T-cell engager[J]. Cancer Immunol Res, 2018, 6(5): 605-616. DOI: 10.1158/2326-6066.CIR-17-0314.

[66] GARGETT T, YU W, DOTTI G, *et al*. GD2-specific CAR T cells undergo potent activation and deletion following antigen encounter but can be protected from activation-induced cell death by PD-1 blockade[J]. Mol Ther, 2016, 24(6): 1135-1149. DOI: 10.1038/mt.2016.63.

[67] NAKAZAWA T, NATSUME A, NISHIMURA F, *et al*. Effect of CRISPR/Cas9-mediated PD-1-disrupted primary human third-generation CAR-T cells targeting EGFRvⅢ on in vitro human glioblastoma cell growth[J]. Cells, 2020, 9(4): 998. DOI: 10.3390/cells9040998.

[68] KHALIL D N, SMITH E L, BRENTJENS R J, *et al*. The future of cancer treatment: Immunomodulation, CARs and combination immunotherapy[J]. Nat Rev Clin Oncol, 2016, 13(5): 273-290. DOI:10.1038/nrclinonc.2016.25.

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