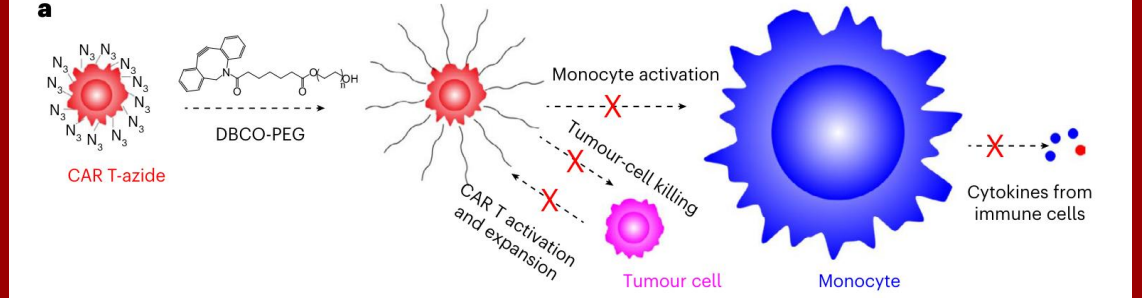

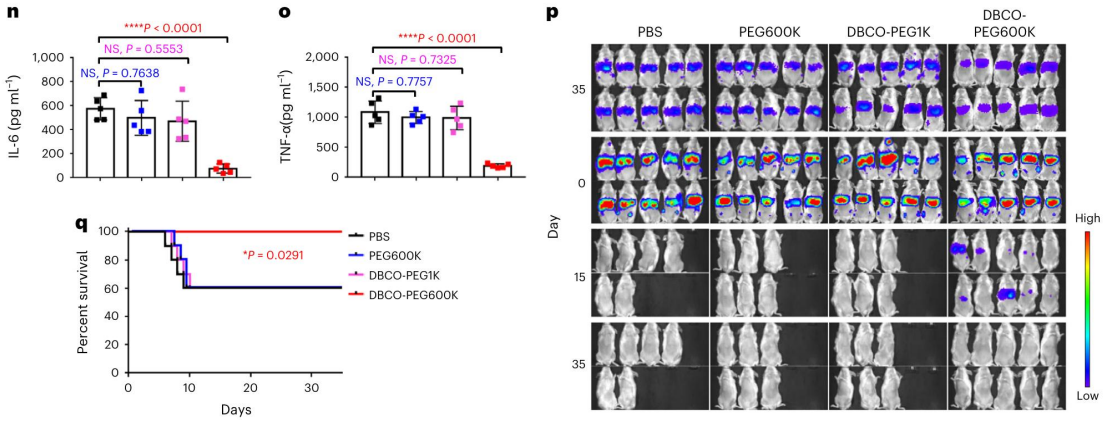

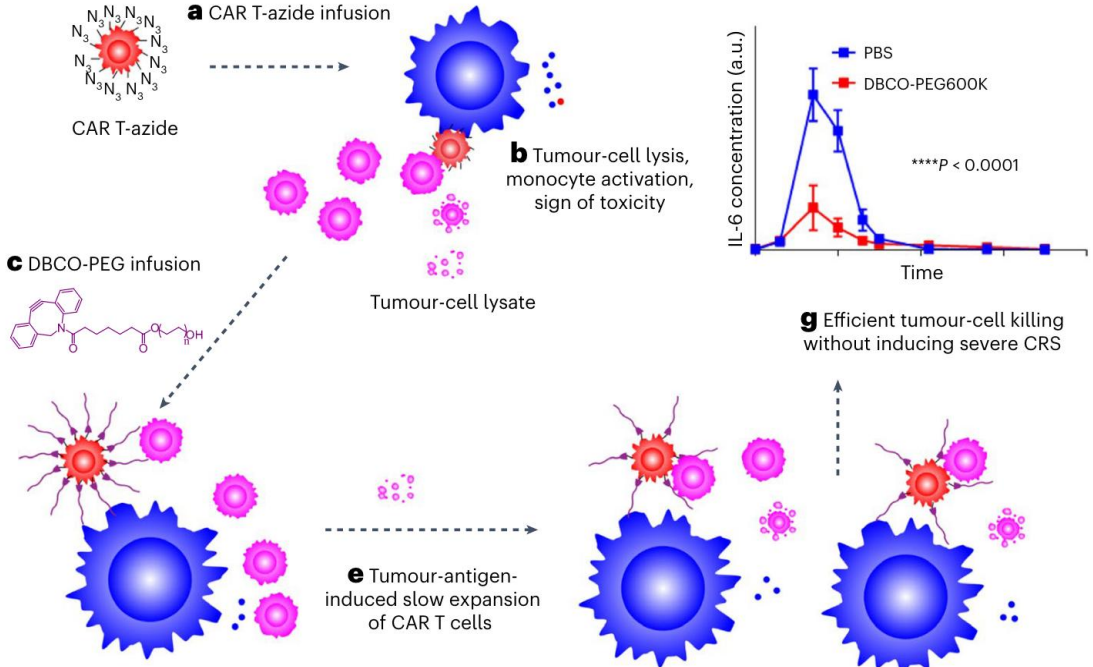

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Reduce the risk of CAR-T cell therapy with click chemistry

汇报人 刘家希

2024年5月30日

CONTENTS

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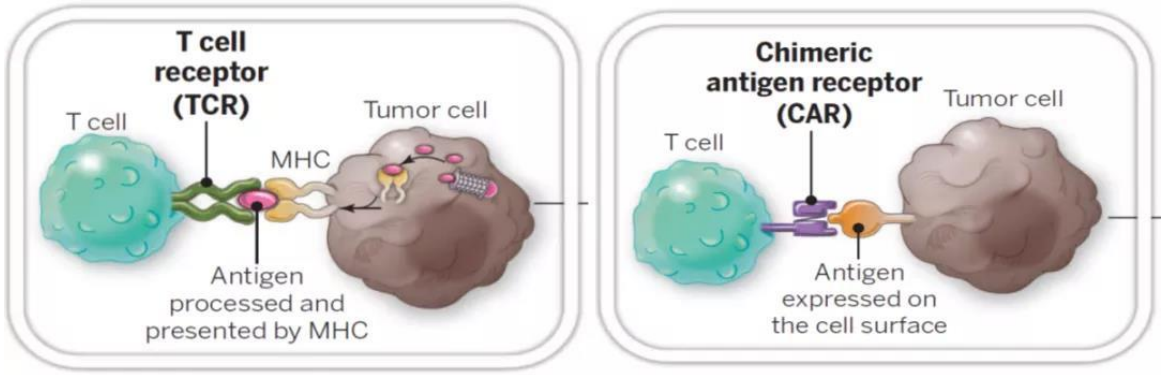
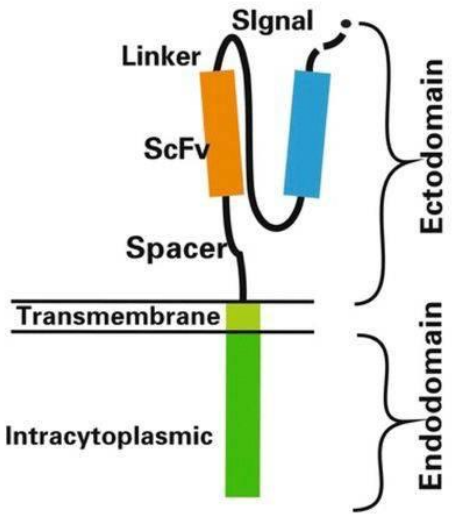
01

Background

1.1 CAR-T cell therapy



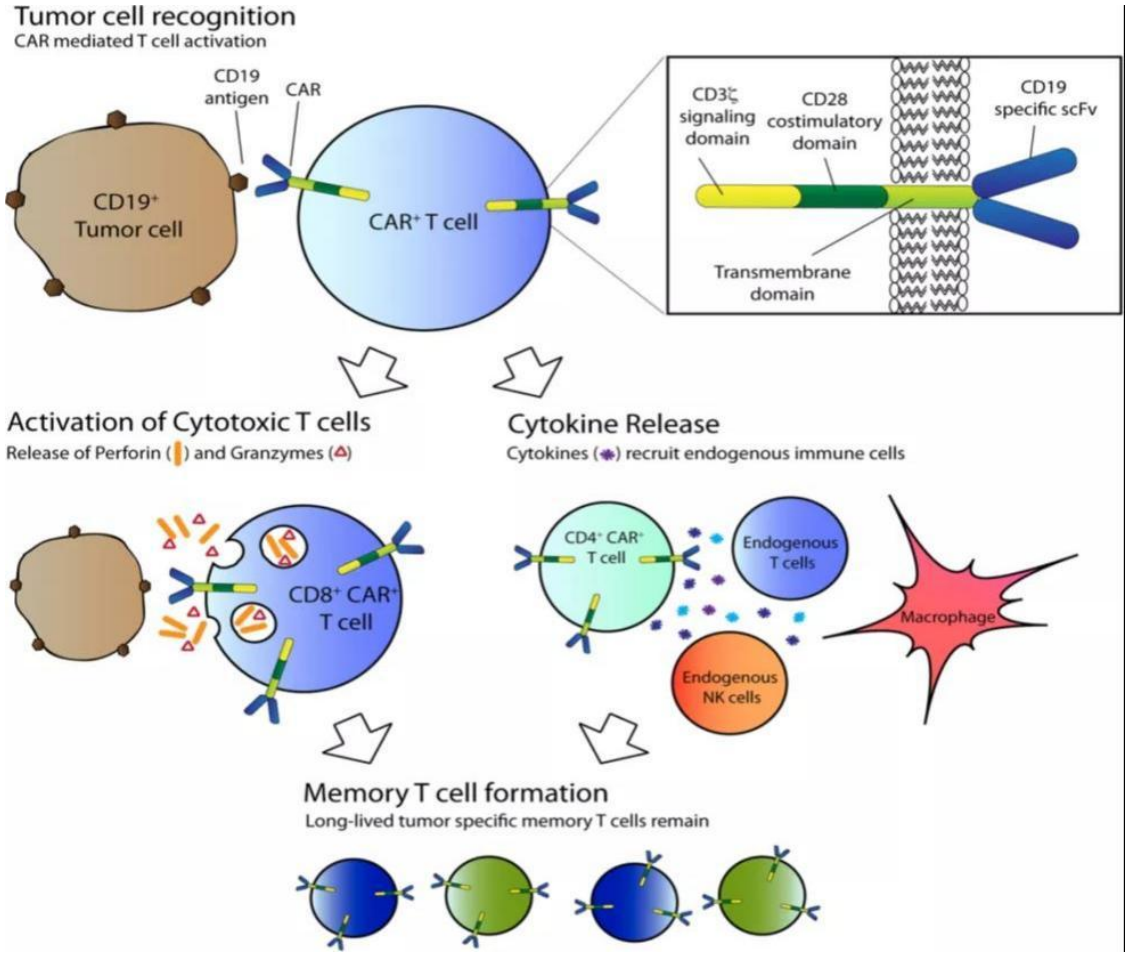


CARs由胞外区、胞内区和跨膜区构成，对每个区域的不同设计影响CAR-T细胞功能的发挥

胞外区源于单链抗体ScFv，由轻链和重链共同组成。胞外区负责抗原的识别，这种识别本质上是抗体与抗原的特异性结合。目前已设计出的ScFv可识别抗原包括CD19,CD20,EGFR,GD2等

跨膜区连接胞内区和胞外区，影响导入的CAR基因的表达能力。有文献证实包含CD28的TM区的CAR表达能力最强[1]

胞内区负责信号的传导，比如TCRζ（CD-3ζ）或FcRγ。当ScFv与其识别的抗原结合时，会通过TCRζ向胞内传导TCR样信号根据胞内区结构不同，可将CAR-T分为三代。第三代CAR-T增加了更多共刺激分子结构域（如CD28+4-1BB)来增强T细胞能力



图源：Biomarker Research；资料来源：[1] Molecular Therapy

1.2 Treatment risk

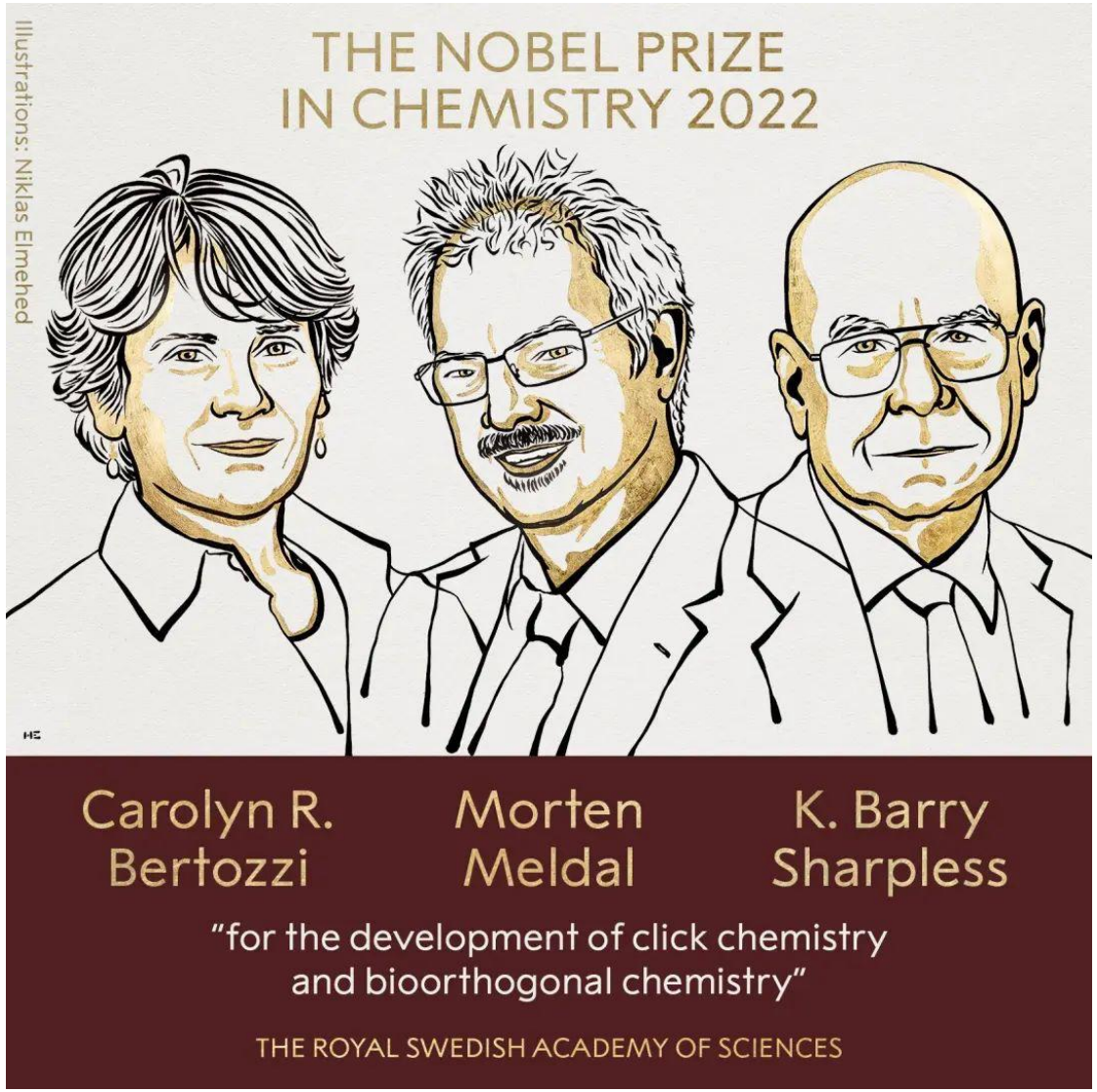
- **脱靶效应（off-target toxicity）**
  正常组织毒性指基因修饰T细胞对同样表达其靶点的正常组织的毒性。脱靶效应是指基因修饰T细胞对不表达这些靶分子的正常组织或器官的毒性。

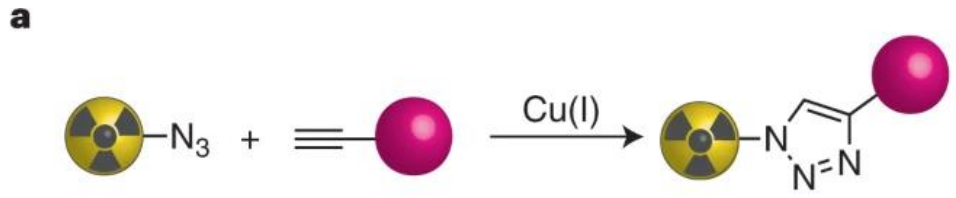
- **细胞因子风暴(cytokine release syndrome，CRS)**
  当CAR-T细胞被回输至体内，细胞治疗过程中会释放的大量细胞因子，包括 IL-6、TNFα 和 IFNγ，这些炎性介质促发急性炎症反应诱导上皮及组织损伤，导致微血管渗漏等机体多种免疫炎症反应。

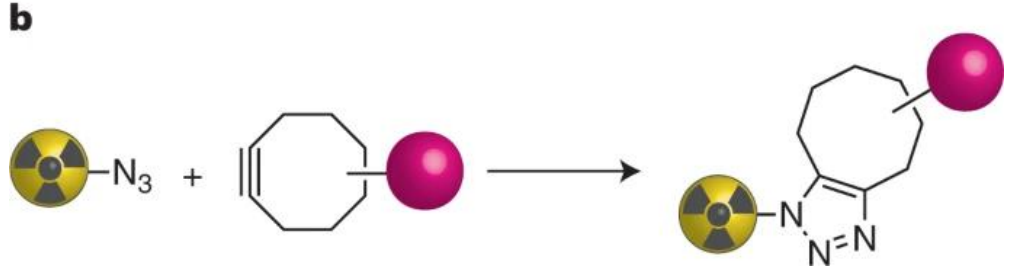
- **神经毒性（neurotoxicity）**
  在成功利用托珠单抗治疗CRS之后，神经毒性已成为CAR-T治疗过程中的主要生命威胁事件，其发生机制仍有待探索。多项临床试验曾报道包括脑梗死、谵妄、意识不清、抽搐、神经麻痹、视野缺损、共济失调、言语障碍在内的多种神经系统异常症状。

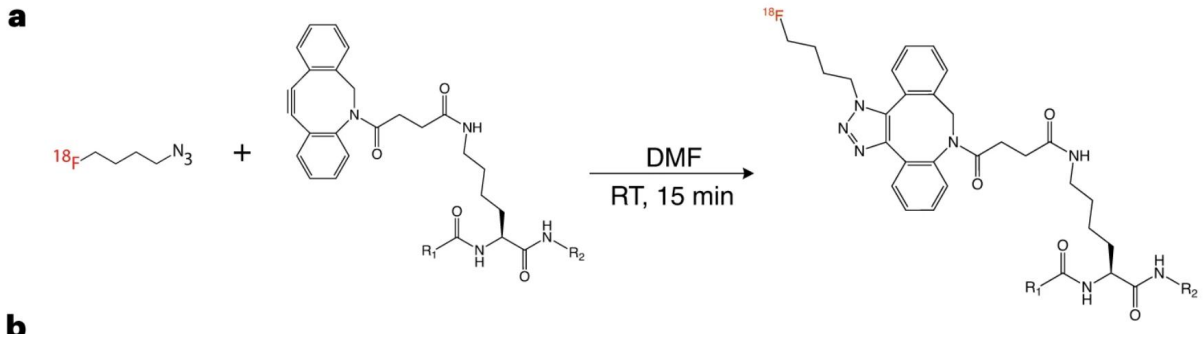
- **其他毒副作用**
  CAR-T尚有某些其他的毒副作用，如出凝血功能障碍、B细胞发育不良等。当CAR-T应用于实体瘤治疗时，还可能造成CAR-T细胞归巢和免疫抑制肿瘤微环境等问题。

1.3 Click chemistry









David et.al, *Nature*, 2023

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02

Reseach

2.0 Brief

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nature materials

Article

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In situ PEGylation of CAR T cells alleviates cytokine release syndrome and neurotoxicity

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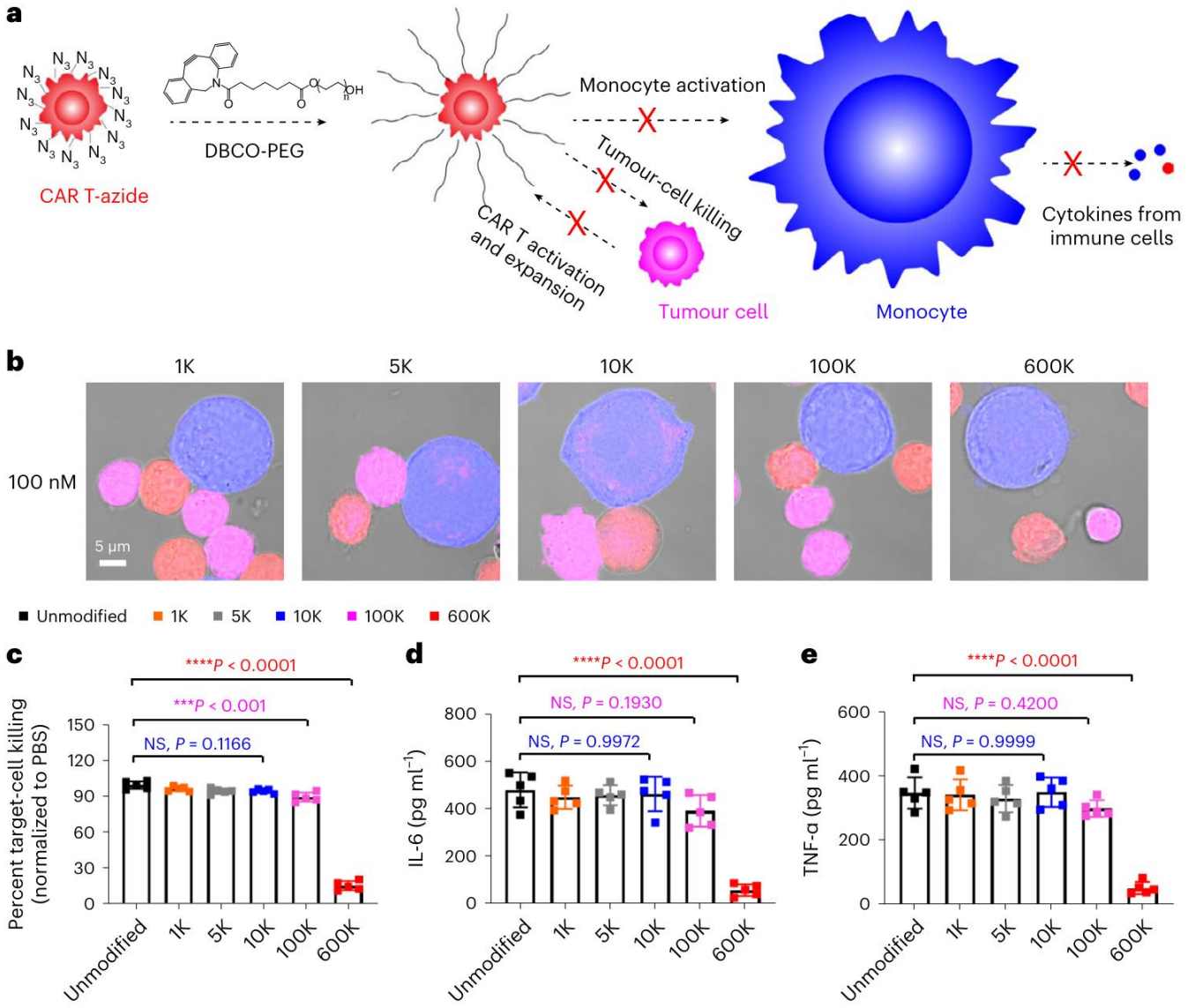
Ningqiang Gong[1,7], Xuexiang Han[1,7], Lulu Xue[1,7], Rakan El-Mayta[1], Ann E. Metzloff[1], Margaret M. Billingsley[1], Alex G. Hamilton[1] & Michael J. Mitchell[1,2,3,4,5,6]

2.1 In vitro interaction



- Activated mononuclear macrophages are known to produce cytokine IL-6
- Activated CAR T cells produce cytokine TNF-α

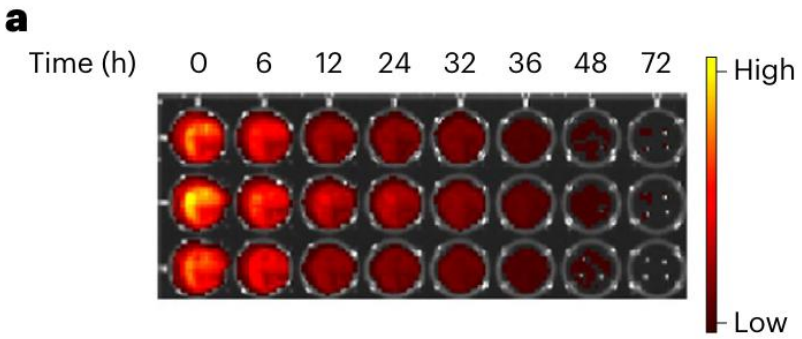
PEG MW600K CAR T cells significantly blocked all cell-cell interactions

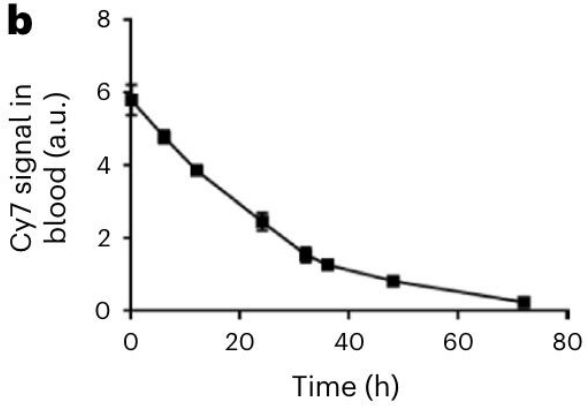
2.2 Pharmacokinetics

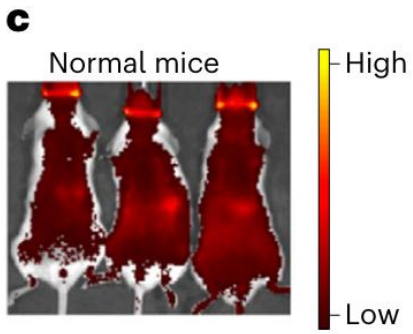
DBCO-PEG600K-Cy7
was injected in vivo（a-c）

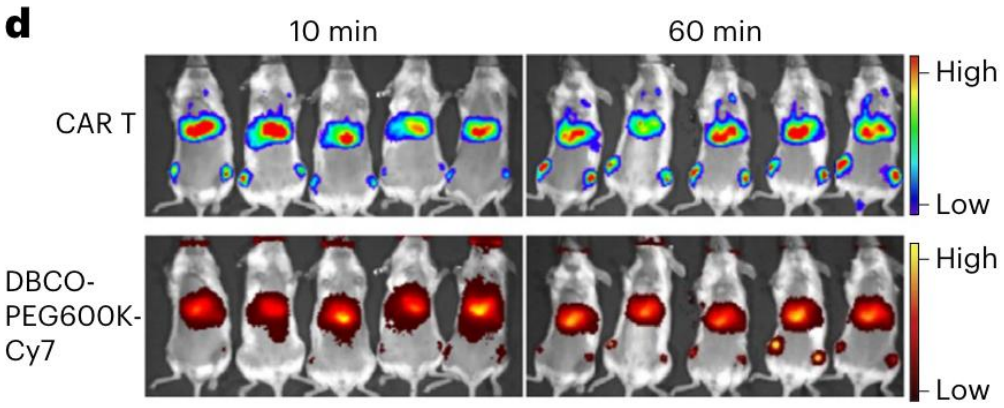
The injection can spread throughout the body

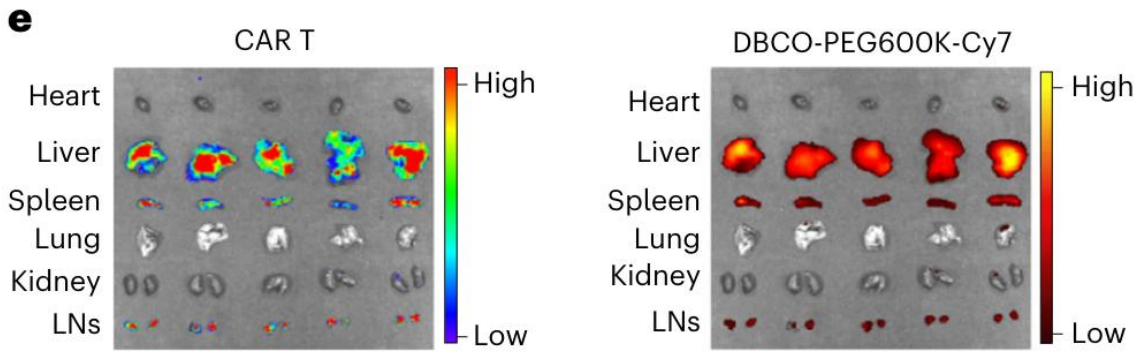
DBCO-PEG600K-Cy7
and **CAR T-CSFE cells**
were injected in vivo（d-e）







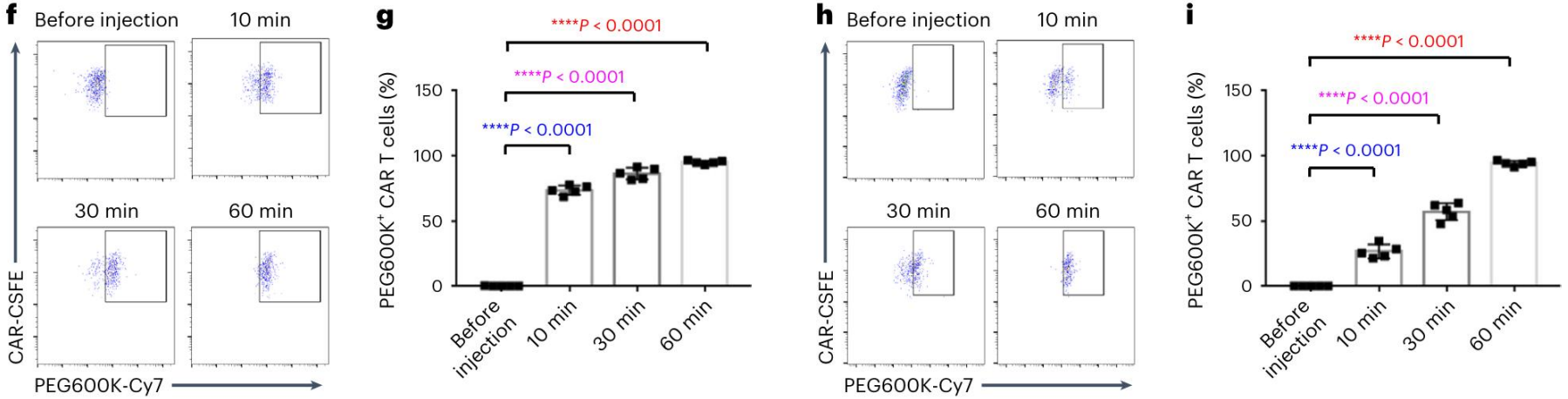




2.3 In vitro interaction

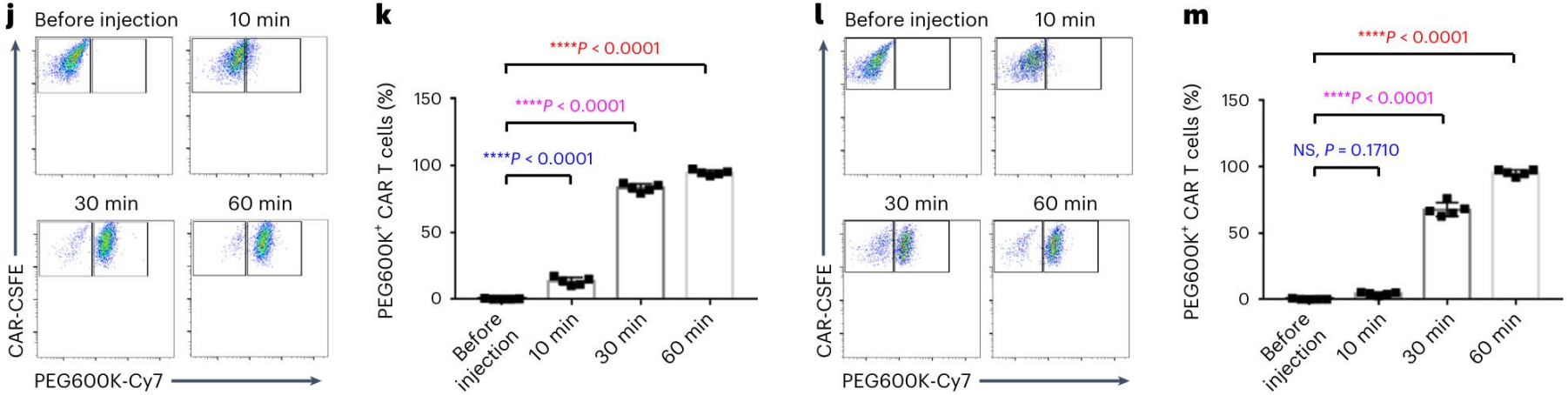
PEG-600K+ CAR T cells are enriched at tumor sites in vivo

blood



liver

spleen



LNs

2.4 Remission of CRS response

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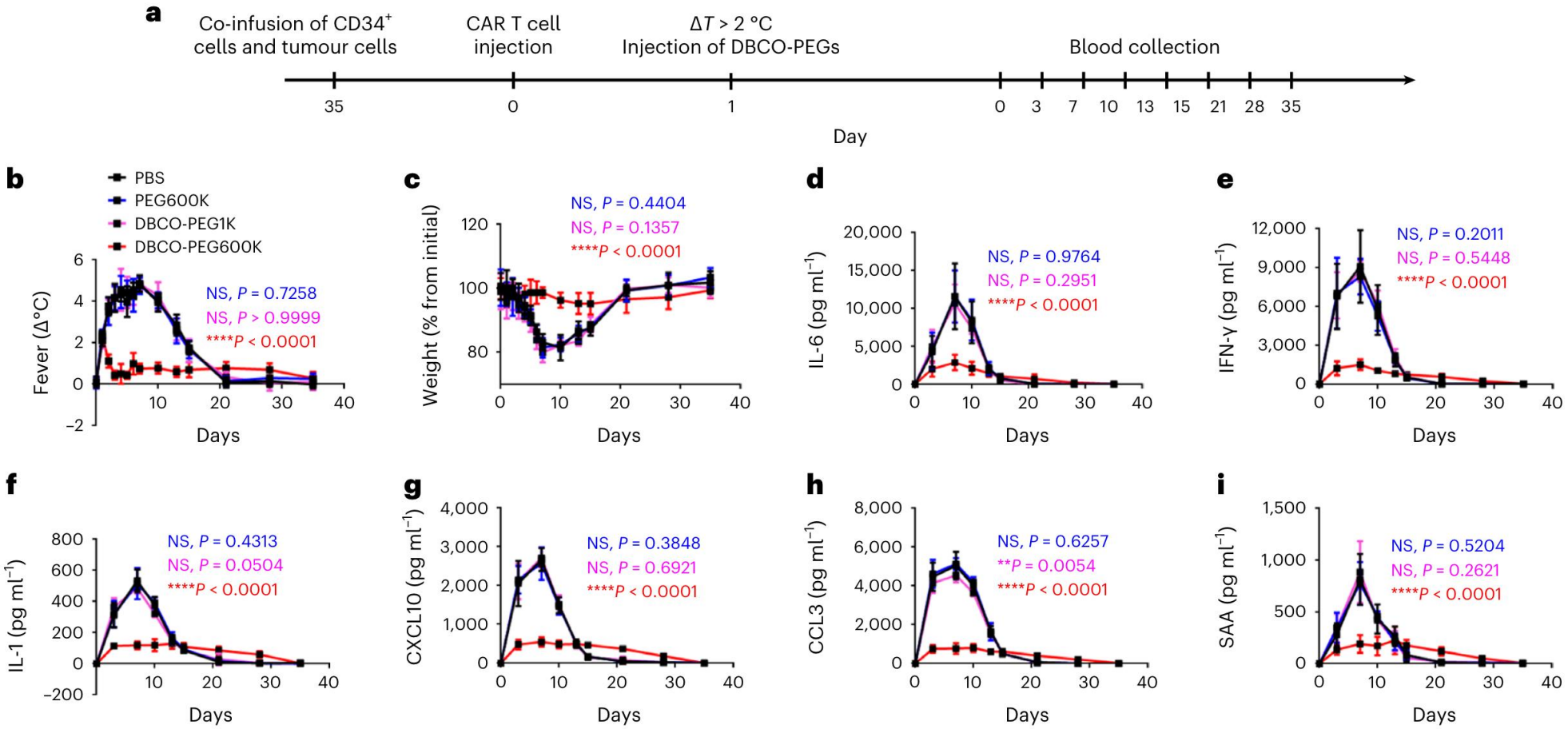
Treatment with DBCO-PEG600K dramatically reversed high fever (b), weight loss (c), and cytokine release (d-i)



2.4 Remission of CRS response

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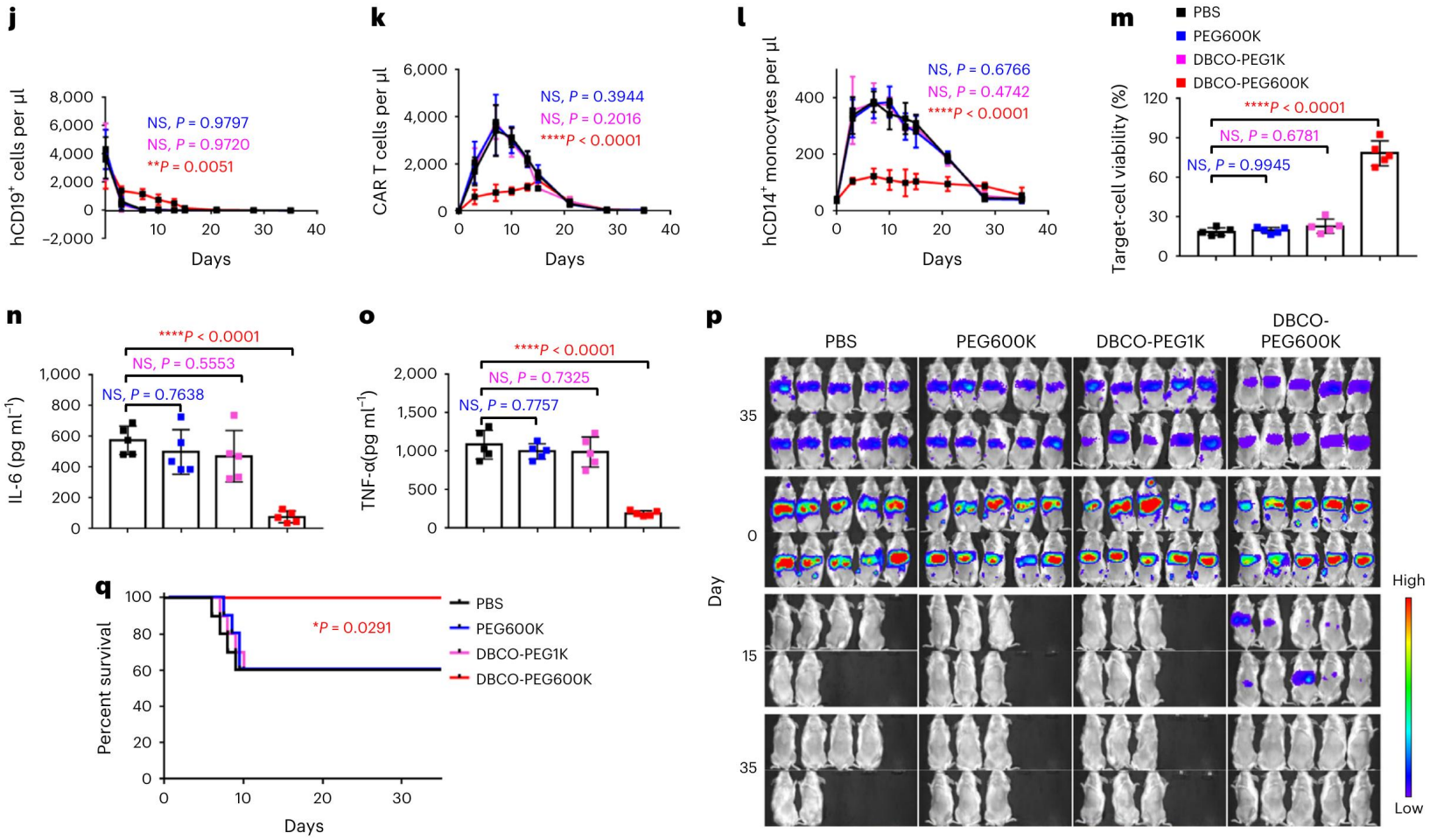
- hCD19+ : tumor cells
- hCD14+ : monocyte

- Tumor cell clearance was significantly delayed **(j, p-q)**
- CAR-T cell expansion was greatly reduced **(k)**
- Monocyte expansion was greatly reduced **(l)**
- Target cells were not highly killed**(m)**

DBCO-PEG600K CAR T cells greatly relieved the symptoms of CRS



2.5 Remission of Neurotoxic

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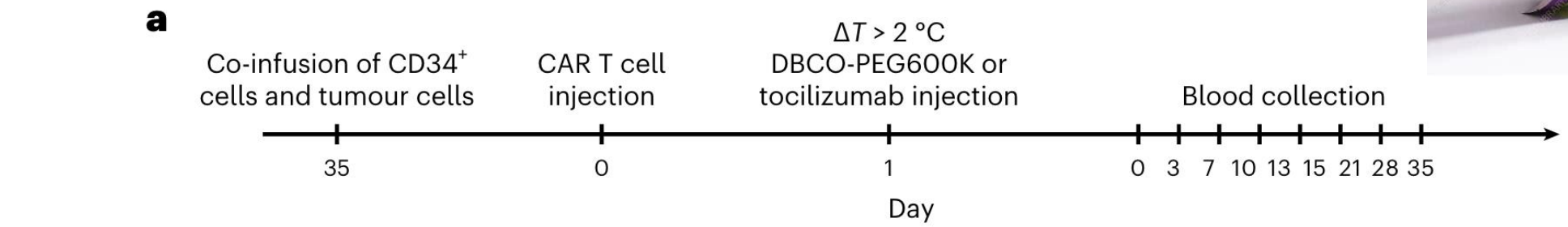
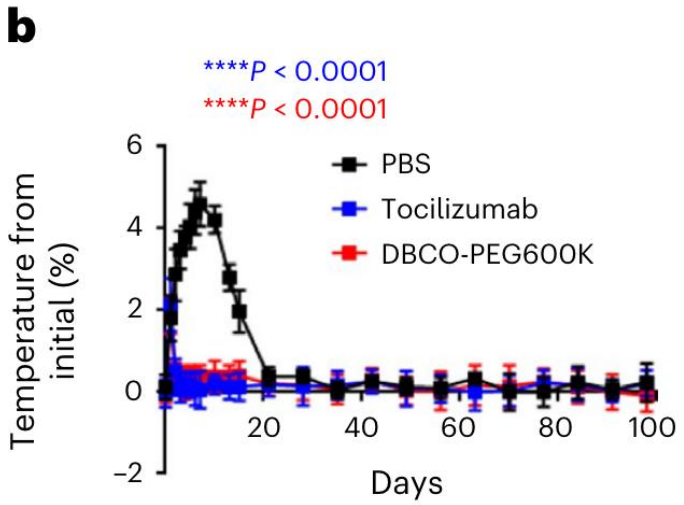
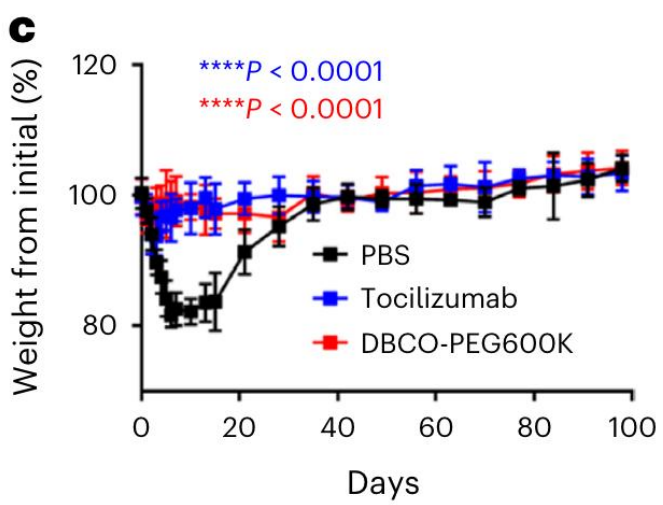
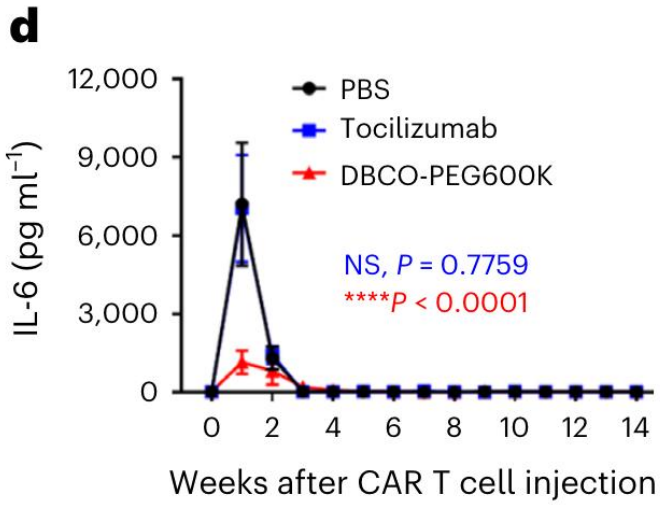
Both tocilizumab and DBCO-PEG600K can relieve the symptoms of CRS









2.5 Remission of Neurotoxic

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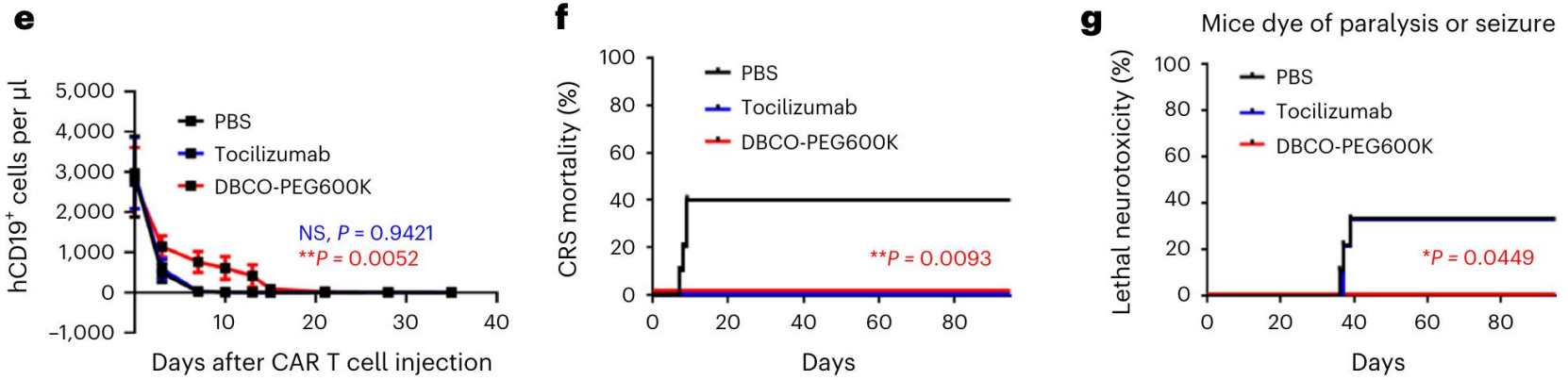
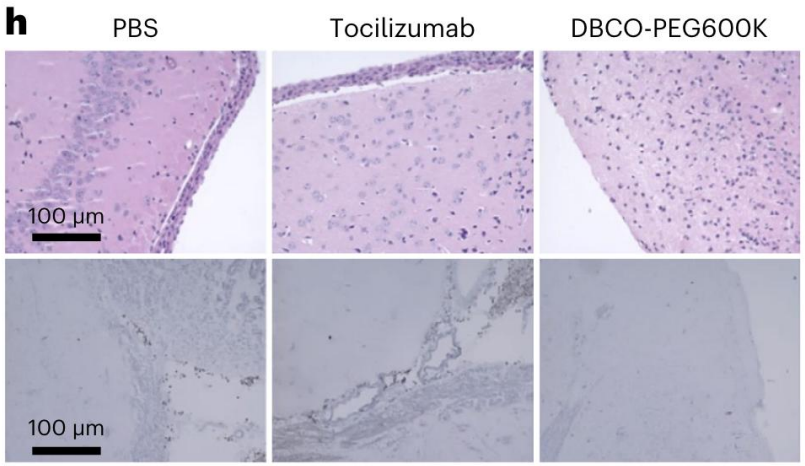
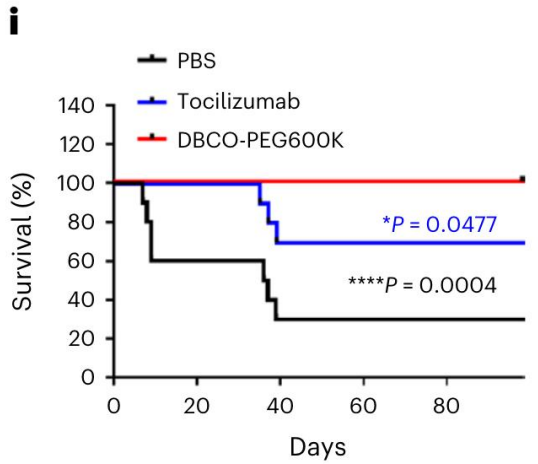
DBCO-PEG600K can protect mice from severe neurotoxicity that is not protected by tocilizumab

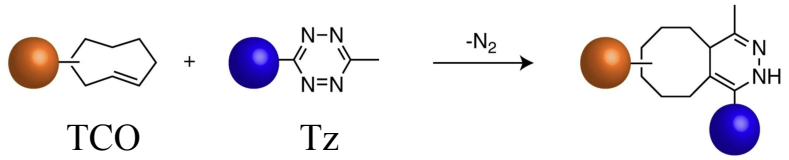


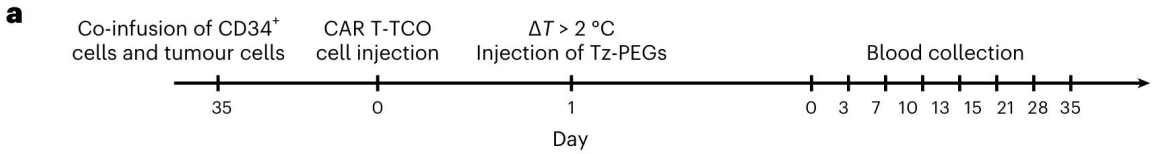
- Meningeal thickening（脑膜增厚）
- infiltration of monocytes in the subarachnoid space（蛛网膜下腔的单核细胞浸润）

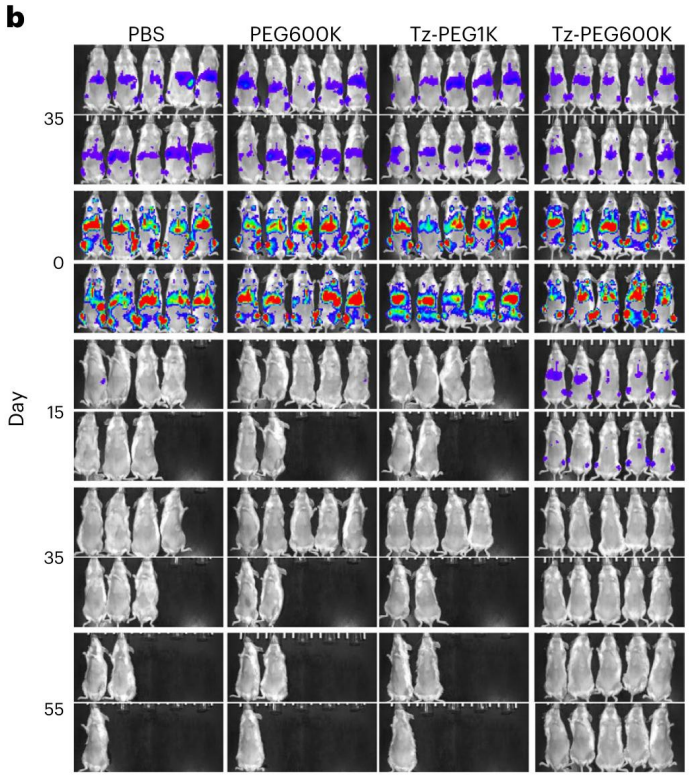


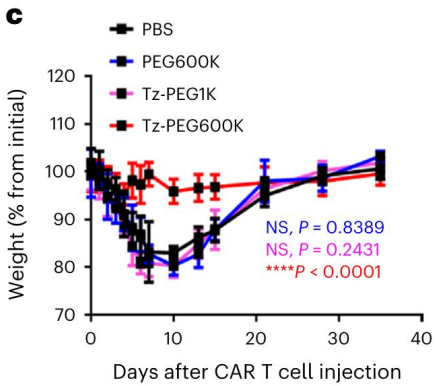


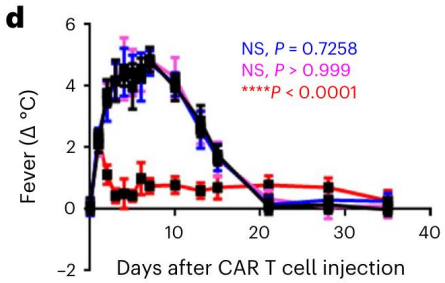
2.6 Model extension

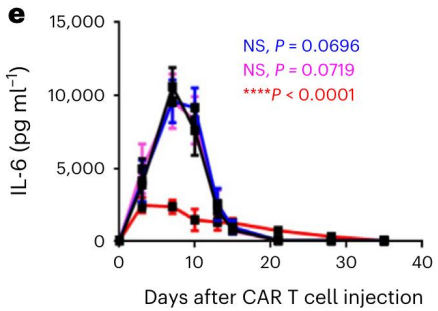


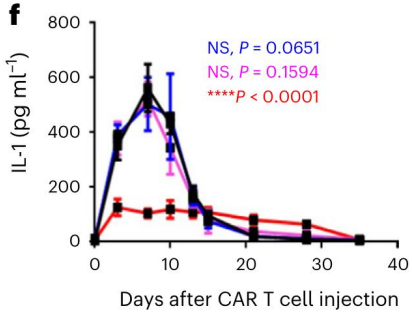


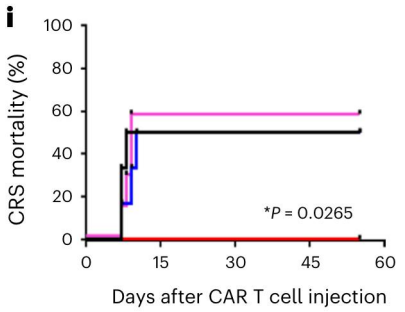


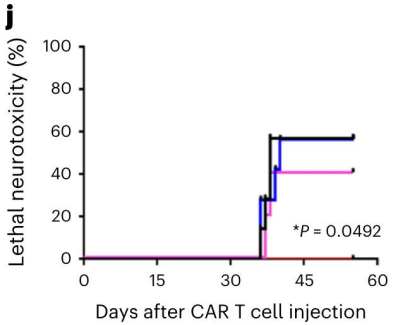


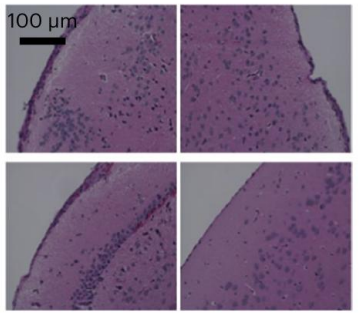


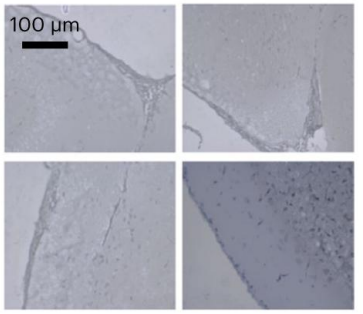












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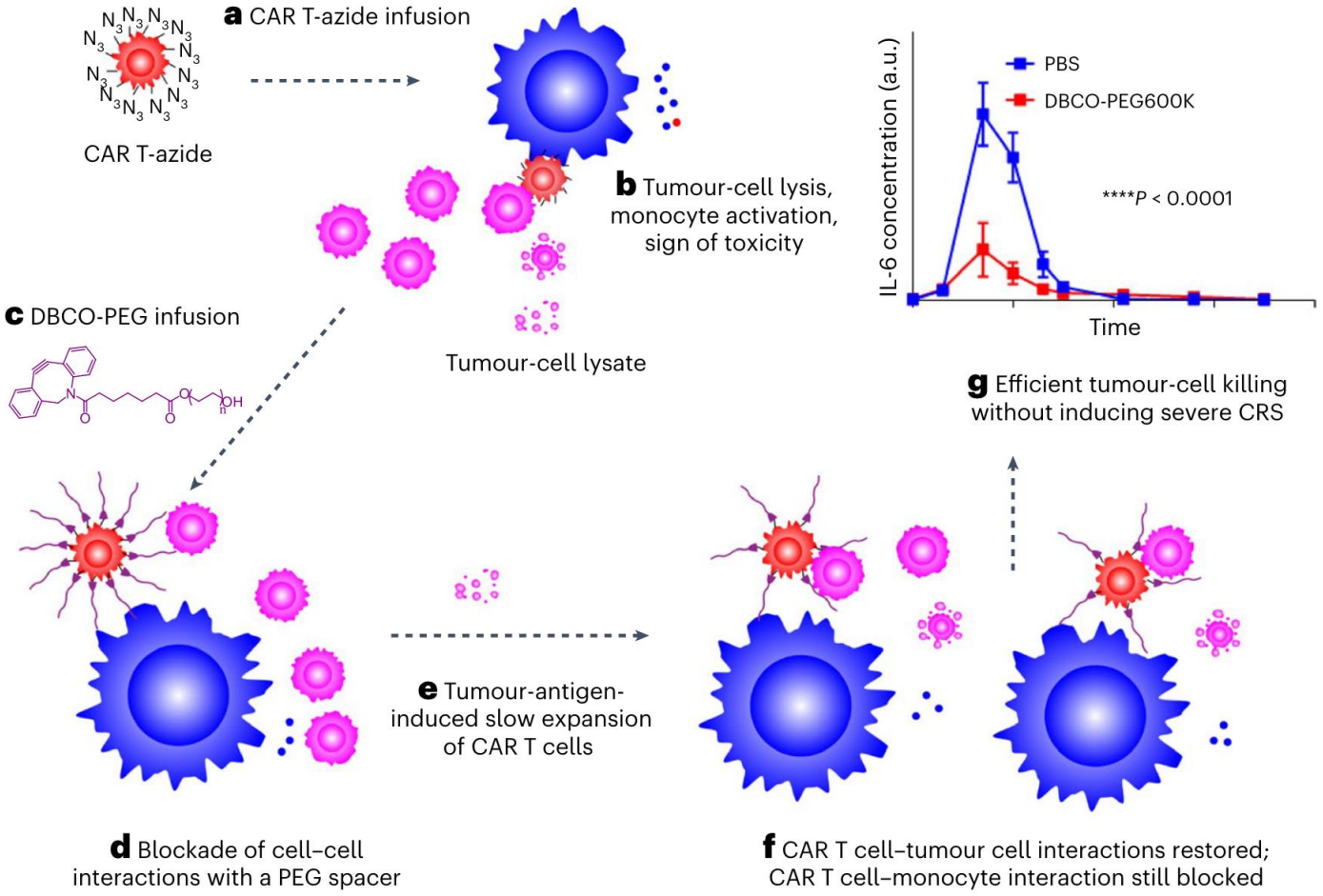
03

Discussion

Discussion

- **Summary**

本研究发现CAR-T细胞的原位PEG化（a-c）创造了一个聚合间隔物，可以大大减少CAR T细胞、肿瘤细胞和单核细胞之间的相互作用，从而整体降低CAR-T细胞的密集肿瘤细胞裂解和单核细胞过度活化（d），从而减少毒性细胞因子的分泌。随着时间的推移，CAR-T细胞缓慢扩张，PEG600K间隔层被稀释，逐渐恢复细胞间的相互作用（e、f）。由于单核细胞较大，肿瘤细胞较小，CAR-T细胞-肿瘤细胞相互作用比CAR-T细胞-单核细胞相互作用更早恢复，为单核细胞过度激活之前的CAR-T细胞杀伤肿瘤打开一个治疗窗口。通过这种方式，CAR-T细胞完全清除肿瘤细胞，但不会诱导过度的IL-6产生（g）。与治疗性抗体托珠单抗治疗相比，致命的神经毒性也较低，这表明**CAR-T细胞的原位聚乙二醇化为更安全的细胞免疫治疗提供一种新策略。**



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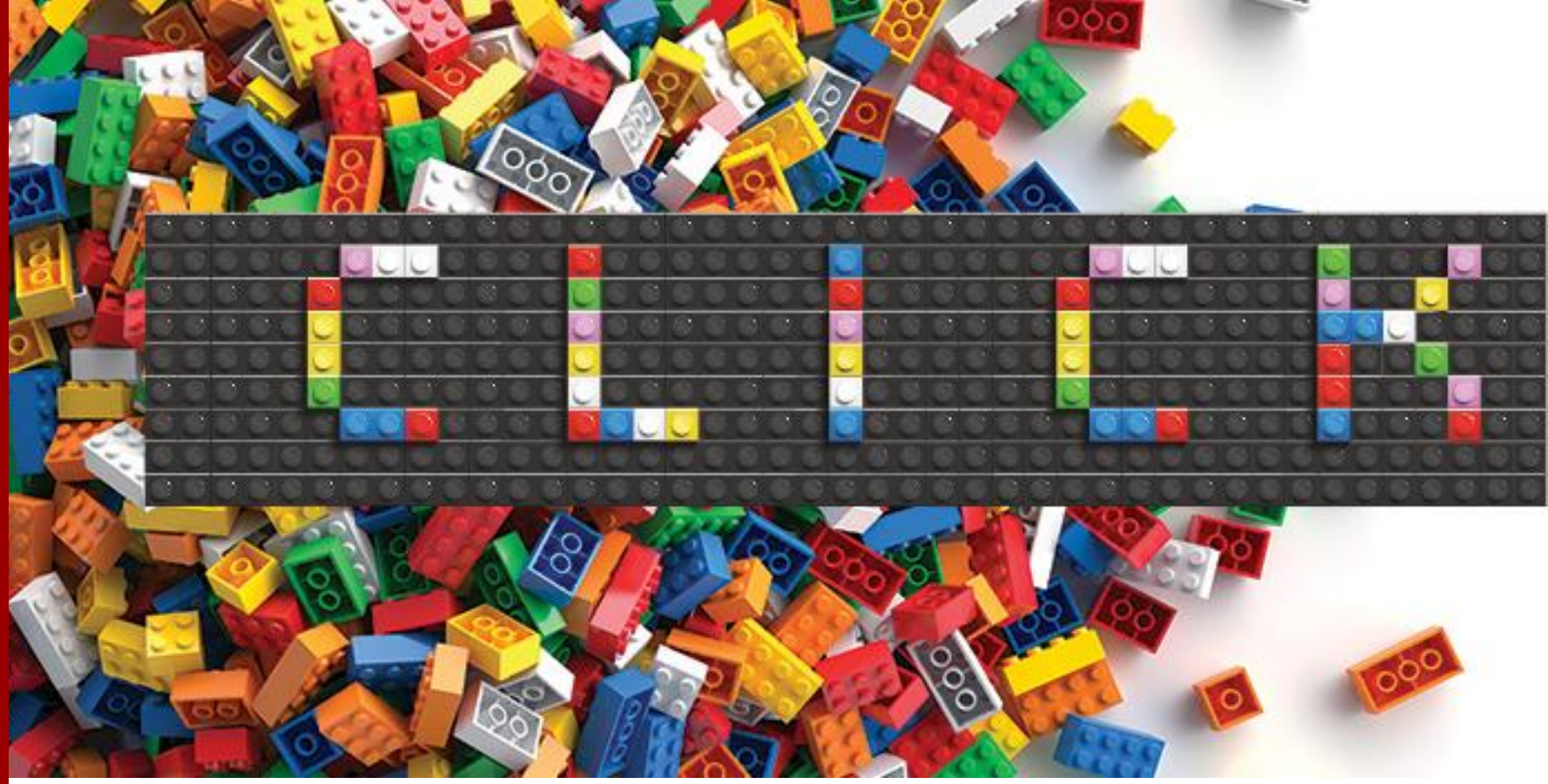
Q&A

谢谢大家



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Reduce the risk of CAR-T cell therapy with click chemistry

主讲人 刘家希

2024年5月30日