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免疫抑制和肝移植

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摘要

完美的手术技术和充分的免疫抑制是确保最佳移植物和患者存活的关键。不同药物的可用性导致了一些通常由行业驱动的不同类型的临床试验，以寻找理想的免疫抑制方案。然而，大量概念不同的研究设计未能明确定义最佳免疫抑制方案。基于钙调神经磷酸酶抑制剂他克莫司、抗代谢药物霉酚酸酯或硫唑嘌呤和短期类固醇（除了可能的诱导外）的三联免疫抑制方案仍然是目前公认的肝移植标准免疫抑制方案。然而，鉴于排斥定义的变化、免疫抑制负荷的定制以及由于慢性免疫抑制引起的长期副作用，未来的试验最好包括一个以上的终点，而不是急性T细胞介导的急性排斥（a-TCMR）或肾衰竭。相反，需要一个涵盖患者和移植物存活率以及急性和慢性排斥反应发生率的综合终点。这些免疫现象应根据一系列长期的生物学和组织学随访进行检查。临床相关α-TCMR的诊断和治疗应基于综合生物学、免疫学和组织病理学的发现。这两个要素对于朝着更谨慎的免疫抑制处理和有利于临床操作耐受性的方向发展至关重要。

1. 引言

外科技术和围手术期护理的改进逐渐提高了实体器官移植的效果。免疫抑制处理对同种异体移植和患者存活至关重要。在移植的早期，类固醇和硫唑嘌呤是唯一能控制宿主对移植物免疫反应的药物；目前，几种化合物可以指导供体-受体的相互作用[1–2]。

许多研究已经确定了最有效和毒性较低的免疫抑制方案来保护移植物和受体[3–5]。但是，很少有研究遵循Jadad定义的5个标准：随机化、盲化、充分描述随机化和盲化过程，以及意向治疗随访，并提及研究中退出或撤回的情况。这在一定程度上解释了目前对理想治疗方案的探索[6]。对2001—2021年期间的详细文献进行回顾，发现只有7项双盲、前瞻性和随机对照试验（RCT）的参与者为50人或更多；4项未能为临床实践提供任何相关结论（表1 [7–14]）。尽管Starzl [1]最初对大型动物和人类对移植物的接受性进行了观察，但多药免疫抑制是防止同种异体移植物“排斥”的最佳方法。这项政策经常产生过度的免疫抑制，这是导致高比例受者发生潜在致命代谢（40%）、心血管（20%）、肾脏（20%）以及肿瘤和感染并发症（10%~20%）的原因[15–16]。这些副作用解释了为什么移植后的长期结果在过去20年中没有显著改善，以及为什么受者在移植物功能正常的情况下死亡是晚期移植物丢失的最常见原因[3–4]。

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表1 2000—2021年间肝移植(LT)的双盲和(或)安慰剂对照随机对照试验

Reference	IS	Design	No. of pts	Study completion/exclusion criteria	Endpoints	BPAR	CR	HCV evolution/metabolic impact	GS	PS	Composite endpoint
Wiesner et al., 2001 [7]	MMF vs. AZA in triple CyA-based IS	MUC and TC	278 vs. 287	+/–62% at 6 months and +/–55% at 12 months/yes	BPAR + TBPAR within 6 months; GS + PS at 12 months;HCV evolution	38.5% vs. 47.7%, MMF better, $P < 0.025$; 31.0% vs. 40.0%, graft loss censored, $P < 0.060$, at 6 months; 30.6% vs. 41.1% in HCV pts, $P < 0.040$	3.8% vs. 8.2%, MMF better, $P < 0.020$	MMF better in HCV-negative pts at 6 months but NS	Similar at 12 months, NS	Similar at 12 months, NS	NA
Neuhaus et al., 2002 [8]	Basiliximab vs. placebo in triple CyA-based IS	MUC and TC	188 vs. 196	+/–83%/yes	BPAR + GS + PS at 6 and 12 months; HCV evolution; composite: BPAR + GS + PS	Similar; NS	Similar, NS	Basiliximab better in HCV-negative pts but NS	Similar, NS	Similar, NS	Similar at 6 and 12 months, NS
Pageaux et al., 2004 [9]	Steroids vs. placebo in triple CyA-based IS	MUC and France	90 vs. 84	+/–75%/yes	BPAR + TBPAR; GS + PS; HCV evolution; metabolic impact; all at 6 months	24.1% vs. 38.1%, steroids better, $P = 0.030$	Similar, NS	Similar, NS; similar, NS; similar, NS	Similar at 6 months, NS	Similar, NS	NA
Filipponi et al., 2004 [11]	Steroids vs. placebo in triple CyA- and Basiliximab-based IS	MUC and Italy	74 vs. 66	+/–75%/yes	BPAR; TBPAR; GS + PS; HCV evolution; composite (pts + graft loss + withdrawal); all at 12 months	Similar; NS	NA	Similar, NS	Similar, NS	Similar, NS	8.0% vs.15.6%, $P = 0.030$
Moench et al., 2007 [10]	Steroids vs. placebo at 14 d after LT in TAC-based IS	MOC and Mainz	54 vs. 56	+/–64%/no	TBPAR; CR; GS + PS; metabolic impact; all at 12 months	Placebo better, $P = 0.016$	Similar, NS	NA, LDL cholesterol placebo better at 6 months, $P = 0.033$; similar at 12 months, NS	Similar, NS	Similar, NS	Similar, NS
Lerut et al., 2008 [12] and Lerut et al., 2014 [13][a]	Steroids vs. placebo in TAC-based double IS	MOC and Brussels	78 vs. 78	100%/no	BPAR; TBPAR; GS + PS; TAC monotherapy; metabolic/renal impact; all at 3 and 12 months	Similar; NS; similar at 3 and 12 months; NS	Similar, NS, TBPAR better in steroids group at 3 months, $P = 0.040$; similar at 12 months, NS	NA; placebo better at 12 months but NS; similar, NS	Placebo better at 12 months, $P = 0.03$	Similar, NS	NA

续表

Reference	IS	Design	No. of pts	Study completion/exclusion criteria	Endpoints	BPAR	CR	HCV evolution/metabolic impact	GS	PS	Composite endpoint
Iesari et al., 2018 [14] [b]	rATG single shot vs. no induction in TAC-based monotherapy IS	MOC and Brussels	97 vs. 109	100%/no	BPAR; TBPAR; GS + PS; TAC monotherapy; all at 3 and 12 months	Similar, NS; similar, NS at 3 and 12 months; similar, NS; similar, NS	Similar, NS	NA	Placebo better at 3 and 12 months but NS	Placebo better at 3 and 12 months but NS	NA

underlined items represent the primary endpoint of the respective study.
IS: immunosuppression; MMF: mycophenolate mofetil; AZA: azathioprine; CyA: ciclosporin; TAC: tacrolimus; rATG: rabbit-antilymphocyte globulin; MUC: multicentric study; TC: transcontinental study; MOC: monocentric study; pts: patients; BPAR: biopsy-proven acute rejection; TBPAR: treated BPAR; CR: chronic rejection; GS: graft survival; PS: patient survival; HCV: hepatitis C virus; NA: not applicable; NS: not significant; LDL: low-density lipoprotein.
[a] Ref. [13] reports the long-term results of TAC monotherapy concept.
[b] Not placebo-controlled.

在此，我们对排斥反应和最佳免疫抑制的定义进行综述，旨在提出在肝移植领域中更合理地使用免疫抑制的建议，并为肝移植领域未来的临床研究提供指导。

2. 关于免疫抑制的历史记录

在丹佛最初的肝脏和肾脏移植经验中，免疫抑制主要包括“秘密鸡尾酒BW322”，即泼尼松和硫唑嘌呤。随后，局部产生的抗淋巴细胞球蛋白被用作类固醇节约剂[1]。这些开创性的系列研究显示了不令人满意的存活率（长期存活率约为20%），从而引发了对更强免疫抑制剂的研究。对这些报道的回顾性分析表明，需要对这些结果进行更复杂的解释。80%的移植物会由于技术原因、器官保存不良和心肺并发症而丢失，其中20%的丢失归因于免疫因素。更重要的是，由于这种“轻度”免疫抑制方案，一些患者在20多年后达到耐受状态[17–18]。

20世纪60年代的非特异性“类固醇-硫唑嘌呤”组合在20世纪80年代被基于钙调磷酸酶抑制剂（CNI）的免疫抑制取代，随后在2000年代出现了基于雷帕霉素（mTOR）抑制机制靶点、共刺激抑制剂和单克隆抗体的方案[19–21]。CNI环孢菌素和他克莫司因其选择性作用机制而改变了该领域，从而实现了免疫抑制的最小化和移植耐受性。一年和五年的患者和移植物存活率分别迅速跃升至75%~90%和60%~70% [2]。来自制药行业个人通讯的信息显示，全球范围内已进行了500多项基于CNI和mTOR抑制剂（mTORi）的多中心免疫抑制研究，目的是减少同种异体移植排斥反应的发生率或改善肾功能。尽管几种最小化方案的前景很大，但四联和三联药物方案通常用于临床实践[22]。在Starzl于2017年去世后，研究者对免疫抑制最小化方案的推广以及更大规模的耐受性免疫抑制策略的兴趣已经消退。通过对电子数据库Medline-PubMed进行系统搜索，从2012年至2022年6月期间，一组选定的研究人员进行了关于耐受性的研究，使用的医学主题标题为：临床研究/试验、耐受性、细胞疗法、免疫抑制和LT。搜索只找到了19篇论文。不出所料，免疫耐受网络决定在2022年优先进行耐受试验（个人信息）。

当重新评估排斥反应和标准免疫抑制预防和治疗的定义时，需要考虑过去的临床观察和经验。

3. 重新考虑肝脏接受度

在LT免疫学中，第一个发现是排斥和耐受是同一连续体的步骤。因此，消除早期急性T细胞介导的排斥反应（a-TCMR）可能会对移植物的长期存活产生反作用[1,23–24]。第二，免疫抑制期间器官移植时间延长是部分耐受的标志。供体和受体免疫系统之间的相互作用已被证明对结果有利[25]。1993年，Starzl等[26]对实体器官移植后的细胞迁移和嵌合体的研究表明，在没有长期免疫抑制的情况下，移植物适应和接受不止是一个空想。1969年，Starzl指出：“几乎可以肯定的是，接受免疫抑制治疗的宿主体内移植器官的持续存在，通常会导致对同种移植物抗原的选择性反应性丧失”和“移植后早期大剂量的免疫抑制剂可能会侵蚀耐受机制，因此排除了对长期免疫抑制治疗的最小依赖（或独立）的目标”[1,26–29]。移植接受是基于特异性克隆激活、缺失和衰竭之间的动态相互作用（图1 [29]）。消除这种相互作用会中止移植物的接受途

径。对临时低剂量他克莫司给药后移植物功能正常的小动物的观察和免疫抑制最小化的临床经验，鼓励移植医生追求这种耐受性策略[29–32]。第三，肝脏是免疫特权器官。然而，这一优势在很大程度上被忽视了，因为缺乏对药物最小化和耐受性诱导的研究以及缺乏完整的有记录的生物学和组织病理学长期随访。Demetris 的名言："活检是移植的科学"，必须在这种背景下考虑[13,32–38]。这三项历史观察对于选择停用免疫抑制药物的患者至关重要[39]。

4. 重新考虑肝脏排斥反应

准确的排斥定义对于从检测免疫抑制的临床研究中得出相关结论是至关重要的。A-TCMR 的发生率在术后第一周达到高峰，第二年下降（约 5%），随后几年进一步下降（约 2%）[2,12,23–24,36]。不同报道之间的异质性、不同的排斥定义以及不同的纳入和排除标准阐明了 TCMR 发病率的广泛范围（中位数约为 40%，范围从 10% 到 80% 不等）[31,39–41]。例如，年龄较大、身体虚弱以及同乙肝病毒和酒精相关的肝病与免疫能力降低有关。更好的移植物质量，特别是来自活体捐献的移植物质量，以及作为诱导剂的枯竭抗体的管理，降低了免疫反应的风险，而自身免疫性肝病则增加了不良免疫事件的风险[23–24,42–47]。

早期 a-TCMR 的临床过程通常是良性的，因为大多数患者对增加剂量的 CNI 和（或）高剂量的类固醇有反应。晚期 TCMR，通常被定义为肝移植后 3~6 个月发生的事件，使受体面临更高的发生慢性排斥（CR）和移植物丢失的风险[23–24]。此外，皮质类固醇的预防性和治疗性应用在研究类型、剂量、次数、给药途径和持续时间方面存在显著差异[48–49]（表 2 [14,21]）。免疫抑制剂剂量或方案的差异会影响 TCMR 的发生率，更重要的是，会影响皮质类固醇抵抗排斥反应的发生率，皮质类固醇抵抗排斥反应定义为对给定的中枢依赖性甲基强的松龙剂量无反应的排斥反应，最终会导致或不会导致皮质类固醇减量[8–10,12,14,23,48–49]。总的来说，这些因素阐明了对特定免疫抑制方案疗效的不同解释（表 2）。

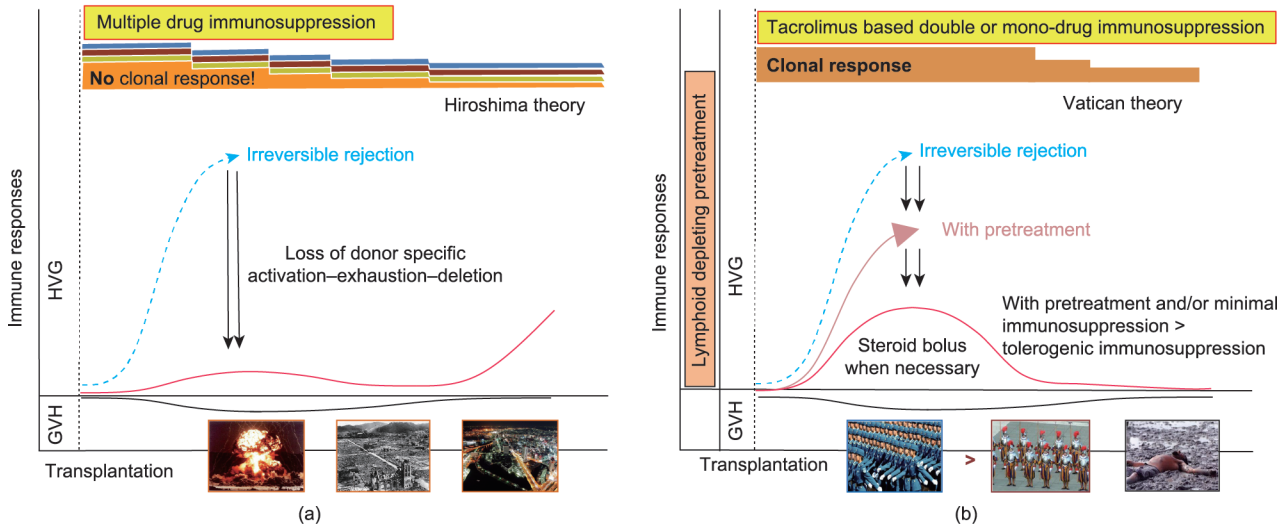

图 1.（a）强多药免疫抑制消除了供体特异性激活-耗竭-缺失过程。因此，取消免疫抑制时，免疫抑制撤销可能导致延迟（慢性）排斥反应。这种情况相当于原子弹爆炸的后果。罕见的幸存者是核战争（广岛理论）的最强反对者。（b）基于低 CNI 效应的免疫抑制有利于供体-受体相互作用，最终需要类固醇推注。预处理可能通过减少 T 和 B 细胞"大军"（梵蒂冈理论）来减弱这种相互作用。随后发生的供体-受体受控相互作用可能导致之后的耐受性状态。粉红色和黑色曲线分别表示通常观察到的移植物抗宿主（GVH）和宿主抗移植物（HVG）反应的时间分布[29]。Tr：移植。

表 2 肝移植免疫抑制临床研究中的混杂因素

Confounding factor	Weak study design	Strong study design
Study design	Multicentre	Uni-/pluri-centre
	Transcontinental	National or regional
	Industry-driven study	Investigator-driven study
	Non-randomised [a]	Randomised
	Inappropriate method of randomisation [a]	Appropriate method of randomisation
	Absence of double-blinding [a]	Double-blinding
	Inappropriate method of blinding [a]	Appropriate method of blinding

续表

Confounding factor	Weak study design	Strong study design
	Absence of placebo controls	Presence of placebo controls
	No information about withdrawals and dropouts [a]	Description of withdrawals and dropouts
Prophylactic immunosuppression [b]	Induction therapy (delaying rejection) [c]	No induction therapy
	Corticosteroid administration	No or short-term (2-to-3-month) corticosteroid administration
	Triple- or quadruple-drug therapy	Mono- or bi-drug therapy
	No harmonisation of immunosuppressive regimen between study arms	Harmonisation of concomitant immunosuppressive drugs
Donor and recipient selection	(Highly) selected patients	Unselected consecutive patients
	Exclusion of auto-immune, HCV-infected, and acute-liver-failure patients	Inclusion of auto-immune, HCV-infected, and acute-liver-failure patients
	Exclusion of high-MELD-score patients	Inclusion of all patients, regardless of MELD
	Exclusion of ICU patients	Inclusion of all UNOS categories
	Exclusion of patients dependent on organ support (renal replacement therapy, ventilation, etc.)	Inclusion of all patients, regardless of organ support needs
	Exclusion of patients with renal failure (exclusion of patients with creatinine > 1.5 $mg \cdot dL^{-1}$ or creatinine clearance < 40 $mL \cdot min^{-1}$ per 1.73 m^2)	Inclusion of all renal conditions
	Exclusion of fragile patients	Inclusion of all patients, regardless of nutritional status
	Exclusion of younger and older adult donors and recipients	Inclusion of all categories of age
	Exclusion of long ischaemia times	Inclusion independent from ischaemia time
	Exclusion of EBV negative CMV, HBV, and HCV positive recipients and donors	Inclusion of all patients regardless of viral status
	Exclusion of grafts from DCD	Inclusion of all donor types
Definition of rejection	Clinically suspected rejection	BPAR
	Only per-cause biopsies	Per-protocol and per-cause biopsies
	Absence of Banff score and RAI	Banff score or RAI
	Absence of immunostaining (C4d, CK19, etc.)	Specialised transplant pathology reading
	Local pathology reading	Centralized pathology reading
	Counting of all rejection episodes	Rejection episodes within delay of 14 d considered as one single rejection episode
	Local biopsy reading	Central biopsy reading
Definition of steroid-resistant rejection	No response to steroid pulses	No response to 250–1000 mg methylprednisolone pulses
	5.00 g methylprednisolone	—
	3.00 g methylprednisolone	—
	1.00 g methylprednisolone	—
	0.50 g methylprednisolone	—
	0.25 g methylprednisolone	—
	Intravenous vs. oral pulse	—
	Single or two courses	Single course
	Steroid pulse followed by tapering (200–160–120–80–40–20 mg)	No tapering

MELD: the Model for End-Stage Liver Disease; ICU: intensive care unit; UNOS: United Network for Organ Sharing; EBV: Epstein–Barr virus; CMV: cytomegalovirus; DCD: donation after cardiac death; CK19: cytokeratin 19.

[a] Five items that correspond to the Jadad scale.

[b] Prophylaxis, as opposed to therapy, implies the use of immunosuppressants to prevent, rather than to treat, an immunological event.

[c] Induction therapy is considered as a potential confounder in case the primary endpoint of a trial is only BPAR (scored following Banff or rejection activity index (RAI)). Induction is known to reduce tissue inflammatory changes compared to no-induction regimen. When considering both histopathology and clinical evolution (e.g., the necessity to treat), the advantage of induction therapy may disappear [14,21].

1%~5%的肝移植受者可以发展成CR。CR的定义与LT [12–13,24,39,50]之后的时间无关。标志性病变是胆管缺失综合征（VBDS），在至少包含10个汇管区的代表性组织样本中，50%的汇管区胆管消失。由于其在治疗中的影响，在临床中应始终考虑这一点。在大约30%的肝移植受者中观察到胆管并发症，导致一定程度肝毒性的药物，如常用的阿莫西林-克拉维酸盐、环丙沙星、甲氧苄啶-磺胺甲噁唑和卡马西平，可以模拟CR的组织病理学[51–52]（表3）。VBDS也可导致免疫抑制减少或停药，无论是出于医学原因还是由受者决定。在接受肝移植的患者中，不遵从或不依从的发生率最高（高达14%）[53]。幸运的是，在大多数情况下，重新引入先前的免疫抑制水平可以解决这种“诱发的”免疫抑制不足[29,32,54–55]。

表3 肝移植术后药物性小胆管损伤

Drug	Induced bile duct lesions	
	Acute	Chronic[b]
Allopurinol	+	(–)
Amitriptyline	+	+
Amoxicillin-clavulanate[a]	+	(–)
Ampicillin	+	(–)
Azathioprine	+	(–)
Barbiturates	+	+
Carbamazepine	+	+
Chlorothiazide	+	+
Chlorpromazine	+	+
Cimetidine	(–)	+
Ciprofloxacin	+	(–)
Erythromycin	+	+
Fenofibrate	+	(–)
Flucloxacilline	(–)	+
Glibenclamide	+	(–)
Glycyrrhizin	+	+
Chlorpromazine	+	+
Haloperidol	(–)	+
Ibuprofen	(–)	+
Imipramine	(–)	+
Itraconazole	+	(–)
Propafenone	+	(–)
Saint John’s Wort	+	+
Terbinafine	+	+
Ticlopidine	+	(–)
Trimethoprim-sulfamethoxazole	(–)	+

[a] Amoxicillin-clavulanate (Augmentin®; GlaxoSmithKline, Belgium) is one of the most prescribed drugs worldwide; ciprofloxacin, erythromycin, itraconazole, carbamazepine, and trimethoprim-sulfamethoxazole are used very frequently after LT.

[b] Chronic bile duct injury can mimic chronic allograft rejection.

严重的移植物功能障碍、实质坏死、组织学上的胆管破坏、C4d阳性、免疫荧光和血浆测定中的供体特异性抗体有助于识别抗体介导的排斥反应（AMR）。早期AMR，也称为超急性或暴发性排斥、出血性坏死或第七天综合征，在轻度和强烈免疫抑制下均可观察到[1,24,56–58]。与包括急性排斥在内的严重同种异体移植物功能障碍的其他原因相比，组织学检查显示有明显大量的凋亡肝细胞。在排除导致类似损伤模式的其他损伤（如肝动脉血栓形成）后，该诊断可通过供体特异性抗体（DSA）和组织补体激活（即微血管系统C4d免疫组织化学阳性）的证据来确认。在ABO血型相合的肝移植中，AMR非常罕见（小于1%），而在ABO血型不相合的肝移植中，AMR的发生率在7%~10%之间。在韩国和日本的几个移植中心，ABOi肝移植目前占所有肝移植的30%，这些经验极大地增加了这种情况下免疫处理的知识[59]。免脾切除术结合术前利妥昔单抗（一种单克隆抗CD20抗体）和多次血浆置换治疗来减少自然循环中的ABO抗体，将ABO活体肝移植（LDLT）转化为一个有效的机会，分别提供90%和80%的一年和三年生存率[25]。

TCMR发生率的范围很广，需要建立更精确的定义[23–24,40]。因此，必须贯彻四项原则。首先，活检是区分排斥反应和其他同种异体移植物功能障碍原因以及验证排斥反应治疗的“金”标准[25,35,37,39,50]。尽管活检在免疫抑制管理决策中起着关键作用，但其侵入性和严重并发症的风险阻碍了按方案活检的广泛使用。基于符合方案的活组织检查的研究表明，只有三分之一的肝脏检查正常的受者表现出正常的组织学特征，这为调整免疫抑制提供了指导[35–37]。鉴于移植医生之间对临床疑似排斥反应的意见不一致，仍盲目追求几种治疗方法，这造成了严重并发症的风险，其通常由随后不必要的免疫抑制负担加重引起[60]。这些观察大大超过了对空白活检的恐惧。几项研究表明，监测活检可以安全地进行。在0.35%~5.50%的手术中报道的并发症通常可以在一周内解决。出血并发症通常可以使用介入放射学进行控制，而活检相关的胆管炎通常是由潜在的未知胆管问题引起的[40,61–63]。其次，Banff分类不仅有助于排异反应的分级，也有助于比较不同经验的结果，其方式类似于肿瘤学中肿瘤-淋巴结-转移（TNM）分类的作用[35,60]。第三，应提倡集中式活检读数，特别是在大型跨洲多中心研究中，这些研究经常汇集几十个具有不同移植专业知识的中心[7–8,64]。在大多数免疫抑制试验中，多名病理学家的活检读数会导致终点出现重大偏差。第四，在做出任何治疗决定之前，应联合生物学（作为替代血浆分析物的演变）和组织病理学进行分

析。一般来说，强有力的免疫抑制可以减少炎症浸润，从而降低Banff评分。在这种情况下，被评估的药物或方案可能被错误地解释为更有效，尽管其对临床实践没有影响。在肝移植中，如果生物学参数未证实临床损害，则按方案活检的TCMR不一定需要额外的免疫抑制[24,41]。并非每一个经活检证实的严重急性排斥（BPAR）病例都值得治疗[12,14,23–24,65–66]。值得注意的是，急性排斥反应的发作并不一定导致患者或移植物存活率下降。这一证据强调了受控同种异体反应的致耐受性潜力，这使得移植物对进一步的免疫攻击不太敏感[23–24,67]。

CNI已经改变了传统的排斥症状，包括发热、腹痛、压痛和移植物肿胀，这些都是罕见和不可靠的。因此，需要开发非侵入性、经济有效、快速、可重复性、敏感性和特异性的排斥反应生物标志物。肝脏测试、细胞因子谱、炎症标志物、三磷酸腺苷（ATP）活性、外周T细胞聚集和复杂的‘组学’特征已经被研究过了[34,68–69]。最近，循环microRNAs（MiRNAs）和供体来源的无细胞DNA等无细胞生物标志物也被纳入诊断库。这些生物标记物有望成为监测和预测同种异体移植排斥反应的无创活检替代物[70–71]。在一项检查耐受性的试验中，Shaked等[72]报道，在临床症状出现前40天，通过miRNA检测可检测到排斥反应。尽管如此，理想的候选生物标志物仍然很难识别，因为排斥反应与几个混杂因素之间存在明显的重叠，如移植物脂肪变性、缺血-再灌注损伤（IRI）、局灶性或系统性感染、胆管和血管并发症、新发或复发性病毒感染以及药物诱导的肝脏异常。细胞溶解酶（天冬氨酸和丙氨酸氨基转移酶）和胆汁淤积酶（γ-谷氨酰转移酶和碱性磷酸酶）的准确性较低，与排斥反应的严重程度相关性较差。它们的动态是IRI修复的替代品[24]。进行性增加的血清胆红素和外周嗜酸性粒细胞计数是早期TCMR标记物。嗜酸性粒细胞增多与中度至重度TCMR密切相关[73–76]。尽管血小板在肝再生和IRI反应中起着关键作用，但在a-TCMR中对血小板的研究很少[77–79]。无论选择何种免疫抑制方案，血小板计数通常在移植后5天开始增加。当避免使用诱导血小板减少的药物（如硫唑嘌呤、mTORi和抗病毒药物）时，肝脾隔离和免疫介导的内皮移植物损伤解释了已知的术后初期血小板计数下降。通过从血小板表面释放血管性假血友病因子，胞吐触发循环血小板消耗[80]。血小板计数增加表明内皮修复；相反，内皮损伤会进一步降低血小板计数。这种类型的血小板动力学已在AMR和异种移植中得到充分证明[25,59,81]。几种细胞因子在急性排斥反应中表达，包括募集嗜酸性粒细胞的白细胞介素6（IL6）。这些细胞因子的动力学不能清楚地区分排斥和感染，使得它们的临床应用不可行。这同样适用于识别细胞色素多态性和分化蛋白的T细胞簇[68]。

自1995年以来，来自布鲁塞尔天主教鲁汶大学的团队前瞻性地研究了按方案或按原因活检的Banff评分与上述生物标志物之间的相关性，以客观地定义LT中的早期a-TCMR。选定的生物学参数包括血清胆红素升高、进行性嗜酸性粒细胞增多、血小板计数减少以及LT后第5~7天嗜酸性粒细胞绝对计数大于600个细胞·L^{-1} [11–14,73]。在这个模型中，两个以上的生物标记加上Banff评分6（表示中度至重度排斥）将TCMR的组织学图片转变为临床相关描述。临床排斥意味着通过作用于CNI谷水平或分配高剂量类固醇推注来增强免疫抑制负荷。在无反应的情况下，抗淋巴细胞血清是最常用的处方[12,23–24,65,82–83]。这种方法大大减少了抗淋巴细胞抗体在皮质类固醇耐药排斥反应中的应用。这个“7-up分数”是根据时间命名的，即术后第7天，此时a-TCMR最常发生并执行方案活检。在布鲁塞尔圣卢克大学医院开展的两项前瞻性、全面性、研究者驱动的随机对照试验中，对该评分的有用性进行了调查。这些研究比较了他克莫司单一疗法加安慰剂与他克莫司加为期两个月的短期类固醇疗法；以及他克莫司单一疗法与他克莫司加上一种单一的术中高剂量多克隆兔抗淋巴细胞球蛋白（rATG）[12,14]。第二项研究没有安慰剂对照，因为rATG需要强制性皮肤测试；然而，移植团队不知道麻醉师在术中给予rATG（表1 [7–14]）。严格遵守这两项研究方案得出了几个重要结论：①轻度他克莫司单药治疗方案与重度方案相比产生了可比的早期和长期生存率；②只有10%的中重度组织学排斥反应需要治疗；③接受以他克莫司为基础的免疫抑制治疗的患者很少发生皮质类固醇激素抵抗性排斥反应；④免疫抑制的诱导显著降低了第7天的Banff评分，但不影响临床排斥反应的发生率，因此不影响治疗的需要；⑤最小的免疫抑制提供肾脏保护而不危及移植物存活[12–13,55,65,82]。

迄今为止，他克莫司单药疗法已成功用于800多例患者的免疫抑制治疗。这些发现为许多21世纪评估免疫抑制的随机对照试验得出的几个假设提供了明确的解释。

5. 重新考虑肝脏标准免疫抑制

标准免疫抑制被认为是在药物副作用与器官和患者存活率之间取得平衡。一年一度的移植受者科学登记显示，三联免疫抑制方案是肝移植最常用的策略（约三分之二的受者），该方案包含CNI（主要是他克莫司）、抗代谢药（主要是霉酚酸酯或mTORi）和皮质类固醇。这种方案在

肾移植中也很常见[84–85]。在过去的十年中，诱导疗法的使用一直在持续进行；大约三分之一的受者已经选择了这种方法[86]。诱导包括单克隆抗IL2受体抗体、多克隆抗T淋巴细胞抗体或抗胸腺细胞抗体。在少数（10%）受者中，他克莫司单一疗法仍然是一种免疫抑制方案[84]。在多药免疫抑制方案中，他克莫司的最佳血药浓度通常在6~10 ng·mL^{-1}之间，而许多建议、监管机构和制药行业建议在最初几周内达到更高的水平[82,87]。“管理移植中可改变风险的共识”（COMMIT）报告建议，在没有诱导或伴随免疫抑制剂的情况下，避免免疫抑制不足，即他克莫司水平小于6 ng·mL^{-1}。然而，同一报告阻止了免疫抑制最小化[87]。

然而，三联免疫抑制作为肝移植的标准免疫抑制方案，由于多种原因而受到争议。首先，皮质类固醇真的有必要吗？如果是，持续多长时间？霉酚酸酯优于硫唑嘌呤吗？mTORIs能保护肾功能并降低癌症患者的复发率吗？最后，诱导疗法有什么益处吗？一些积累的证据可能会对这些问题有所帮助。首先，多药免疫抑制方案不能从根本上减少临床相关的TCMR事件。即使在对低风险移植候选人进行治疗时，这种方案也可能产生反作用[65–66,83,87–88]。

已经设计了几种药物“鸡尾酒”来抵消在移植后早期和晚期使用的给定药物的副作用。该策略主要集中于避免CNI介导的肾和神经毒性。几种组合已被证明对受检终点有益。不幸的是，长期免疫抑制仍然会损害移植受者的长期预后[15–16,89]。这些令人不安的副作用应该是减少或消除长期免疫抑制负担的主要驱动力。该策略的第一步是尽早停用或完全避免最有害的免疫抑制剂，即皮质类固醇[65,90]。Padbury等（伯明翰小组）[90]首先证明了这种方法是安全的，这一经验已被反复和独立地验证[9–10,12,15,48,55]。三项检查抗IL2受体和抗T淋巴细胞抗体诱导的系统性综述证实，考虑到减少TCMR发作，诱导治疗并无实质性益处[21,91–92]。类似的证据表明霉酚酸酯对抗“老牌”硫唑嘌呤，表明明显较低的成本有利于硫唑嘌呤治疗[93]。迄今为止，由Wiesner等[7]进行的随机对照试验仍然是比较霉酚酸酯和硫唑嘌呤的唯一可用报告。作者表明，在术后的前6个月，麦考酚酸组的BPAR减少（p <0.06）。患者和移植物的一年存活率相似[7]。基于这些发现，在临床实践中，霉酚酸酯几乎普遍取代了硫唑嘌呤。尽管如此，这些发现应根据统计缺陷进行严格的重新评估，如审查排斥反应以外的其他原因的移植物，因不同原因退出研究的比例较高（分别为6个月和12个月时的36%和46%），以及缺乏竞争风险分析。Germani等[93]揭示了霉酚酸酯队列中血小板减少症的发病率显著升高。硫唑嘌呤诱发的肝炎和结节性再生性增生未被记录[94–95]。最近在肾移植中研究了霉酚酸酯与硫唑嘌呤之间的拮抗作用，在排斥方面没有检测到两种药物之间的差异[96]。

mTORi在肾脏保留方法和移植肿瘤学方面也得到重新评估[97–98]。大多数旨在降低CNI肾毒性的研究将标准治疗与延迟CNI和（或）基于抗IL2受体拮抗剂的诱导或mTORi进行了比较[83,91,97]。一项大型多中心随机对照试验，纳入719名随机分组（术后第30天）的基线估计肾小球滤过率（eGFR）相似的受试者，显示接受依维莫司、低剂量他克莫司和皮质类固醇的实验组表现出明显优于标准水平他克莫司组的eGFR [eGFR，12个月时分别为80.9和70.3 mL·min^{-1}·(1.73 m^2)$^{-1}$，36个月时分别为78.7和63.5 mL·min^{-1}·(1.73 m^2)$^{-1}$，p<0.001] [97]。实验组的他克莫司谷浓度维持在约6 ng·mL^{-1}，而对照组的血药浓度显著高于目前临床实践中所追求的水平：前四个月为8~12 ng·mL^{-1}，随后为6~10 ng·mL^{-1} [82,97,99–101]。值得注意的是，De Simone等[97]表明，肾功能在第三个研究组中明显更好，即接受依维莫司和他克莫司清除剂的组。不幸的是，由于BPAR的高比率，这一组不得不提前终止。这些发现证实，应避免早期无CNI免疫抑制[97]。霉酚酸酯也有类似的观察结果。这种药物可能提供一些肾脏保护，以及一些副作用，其中胃肠紊乱和骨髓抑制仍然是特别关注的问题。在单一疗法中，这种药物是次优的，因为排斥反应发生率高[102]。同样，Silver研究[98]是一项基于西罗莫司的大型多中心随机对照试验，包括525名受试者，重点关注肝移植后肝细胞癌的复发。与不含西罗莫司的免疫抑制相比，基于西罗莫司的方案未能显示出长期优势[98]。然而，令人惊讶的是，作者得出结论，基于mTORi的免疫抑制对移植后肾功能和肿瘤复发有益，这是一个全球移植社区内化的策略。相比之下，虽然低剂量CNI方案和避免不必要的TCMR治疗降低了肿瘤复发率，但这两种方法很少在文献中讨论[103–106]。mTORi的附加值已经出现在肝切除标本中持续存在重要肿瘤组织和肿瘤复发的情况下。

6. 重新考虑肝脏最佳免疫抑制

移植物丢失的最重要原因是移植物功能正常的受者死亡。因此，假设免疫抑制引起的合并症是受者死亡事件的主要原因，那么最佳的免疫抑制方法自然意味着将合并症降至最低。目前，他克莫司和环孢霉素的标准谷值范围分别为6~10 ng·mL^{-1}和150~250 ng·mL^{-1} [87,100,107]。最小

化意味着将患者带到尽可能低的耐受性良好的免疫抑制水平[5,12,82,87]。这个过程从基于两种药剂的最佳CNI水平开始，并在3个月、6个月或12个月后逐渐发展。这些适应取决于移植团队的经验和受体的基础疾病，旨在实施单药治疗方案。前期单药治疗是安全有效的[12,29,48,54–55,99]。

免疫抑制的最小化必须包括中枢神经系统，以避免严重的早期a-TCMR发作[54,87,97]。积累的文献表明，当用于单一疗法方案时，标准每日两次制剂和他克莫司缓释制剂之间的疗效没有差异。在稳定的受者中，延长释放制剂的生物利用度增强提供了更一致的暴露和谷值水平。然而，在移植后最初“不稳定”的日子里，使用缓释制剂可能会导致吸收和生物利用度的变化。在最初移植物功能障碍的情况下，这种可变性增加，当供体选择标准逐步扩展以应对移植物短缺时，这是一个值得关注的因素。因此，每天两次他克莫司可以更容易和更快速地适应血浆谷浓度，特别是在肾衰竭的情况下[108]。

延迟单药治疗可包括CNI、抗代谢药或mTORi，最终选择取决于肾毒性或神经毒性、代谢障碍以及新发或复发性肿瘤或同种异体移植疾病的发生情况[10,12–13,29,55,99,109]。

如果肝脏检查长期保持稳定，亚治疗单一疗法是下一级选择[28–29]。这种方法已被证明是安全和有益的，因为它有助于在移植后尽早启动时改善代谢状况、肾功能和生活质量[10,12,82,87,109]。几项研究检测了停用免疫抑制药物对长期免疫抑制药物原有并发症的影响，但未能检测到退化效应。这种效果的缺乏很可能是停药启动过晚的结果。相反，早期停药，最不可避免地会导致药物排斥。选择最佳停药时间需要考虑明显提前和明显延迟的停药[110–111]。

总之，通过克服免疫抑制处理方面的几个固定教条，可以取得进一步的进展，这对于临床操作耐受性（COT）的发展仍然至关重要（表4）。遗憾的是，这条道路已经被低质量的文献和受者多变的长期免疫抑制处理打乱了。关于免疫抑制治疗的决策过程，从未明确分析过护理的连续性。越来越多的长期存活受者、新一代接受过不同培训的移植内科医生和外科医生、许多亚专业对患者护理的管理以及逐渐散居的患者表明护理连续性的缺乏。这一点已被罕见的肝移植后10年、15年和20年随访报告所证实。成功的免疫抑制治疗意味着持续、终生坚持基于可靠和最新文献的统一方案（和理念），最重要的是，基于对受体的熟悉：“了解你的患者”（表5）。这是理想情况下受者应该由同一个移植团队使用一个集中的患者图表进行随访的核心原因。

最佳的免疫抑制应考虑慢性免疫抑制的副作用，并包括具有特定移植指征的单个患者、移植时和移植后的免疫状态以及既往医疗和移植史（如TCMR发作、再次移植的指征、DSA发展和肝内胆管并发症）[24,39,59]。对免疫抑制的类型和数量的独特需求在现有的移植文献中已有所记载。因酒精和HBV相关肝病接受肝移植的患者需要较低的免疫抑制负荷。相比之下，因自身免疫性肝病[包括原发性胆管炎（PBC）、原发性硬化性胆管炎和自身免疫性肝炎]接受肝移植或因免疫学原因接受再次移植的患者需要强有力的免疫抑制。接受肝移植治疗PBC的患者在接受基于环孢菌素的免疫抑制治疗时，无复发生存期延长。HCV感染患者应避免突然改变皮质类固醇剂量[112–114]。

众所周知，免疫抑制剂具有促癌活性。对于既往有肝胆源性或其他癌症病史以及固有的高肿瘤风险（如酒精相关肝病）的患者，药物水平应尽可能保持在较低水平。在肿瘤移植领域，免疫抑制最小化的必要性已经变得迫在眉睫，原发性和继发性肝胆肿瘤的肝移植选择标准正在逐渐

表4 肝移植中需要重新评价的10个免疫抑制教条（或信念）

No.	Immunosuppressive dogma
1	Every episode of moderate-to-severe TCMR (Banff score > 6) requires treatment
2	Per-protocol liver biopsies are not worthwhile in the early or in the long-term post-LT follow-up
3	Immunosuppression including steroids is more effective compared to steroid-free regimens
4	Mycophenolate is more effective than azathioprine
5	Induction therapy offers relevantly increased protection compared to induction-free immunosuppression
6	Multidrug anti-rejection prophylaxis is better than one-drug tacrolimus-based immunosuppression
7	mTORi-based immunosuppression better protects renal function compared to tacrolimus-based minimisation immunosuppression
8	mTORi-based immunosuppression decreases the risk of recurrence of hepatobiliary cancer after LT
9	Tacrolimus-based minimisation immunosuppression is dangerous
10	COT is unrealistic

表5 布鲁塞尔天主教鲁汶大学关于肝移植免疫抑制处理的10条建议

No.	Advice
1	Any degree of immunosuppression, even minimal, is still too much
2	Keep uniformity in the post-transplant care. This is a “conditio sine que non” to reach minimal or no immunosuppression
3	Give priority to immunosuppressive medications. The higher the number of additional drugs, the less compliance to immunosuppressive medications is maintained
4	Avoid continuous changes in immunosuppressive treatment as well as continuous changes by different groups of transplant physicians. This variability favours medical mistakes, and insecurity and non-compliance in patients
5	Excessive immunosuppression manifests itself through medical complications: nephrotoxicity, neurotoxicity, arterial hypertension, hyperuricemia, dyslipidaemia, etc. Complications should not be approached by adding more medications but by reducing immunosuppressive load first and by promoting healthy diet and lifestyle
6	In case of multidrug immunosuppressive regimen, when adaptations are needed change only one drug at a time
7	Do not immediately raise the dose of immunosuppressants in case of low blood levels. Interpret them, instead, within the clinical evolution of the recipient. Low levels and good evolution make very good friends
8	Do not treat rejection based on clinical suspicion but only on integrated histological, biological, and clinical recipient findings
9	Clearly explain to the recipient timing and reasons for any immunosuppression modification and communicate any therapeutic change to the clinical transplant coordinator in order to guarantee compliance and transmission of information to all the caregivers
10	Teach recipients to become their best doctor by filling in detailed follow-up sheets with clinical and biochemical parameters and problem listing. This activity smoothens the post-transplant follow-up, especially in the long term

扩大[103–106,115–116]。

基于其与急性和慢性同种异体移植排斥反应的相关性，预成型或重新DSA（dnDSA）已获得越来越多的关注[117]。移植前和移植后都应进行DSA检查，尤其是原因不明的移植物功能障碍。根据平均荧光强度大于5000的评估，组织病理学发现和DSA水平的增加应驱动免疫抑制强化。此外，免疫抑制最小化或停药方案需要定期dnDSA筛查。发现严重急性排斥反应是dnDSA发展的一个风险因素，最小化期间出现的dnDSA与急性排斥反应有关，阻止了免疫抑制的完全停用[37–38,55,58,72,87,117–118]。

非酒精性脂肪性肝炎（NASH）被认为是增长最快的肝移植适应症，在这组患者中，心血管和代谢风险的增加应通过特定的免疫抑制方法来解决。对于血糖控制不佳的受试者，建议从他克莫司改用环孢霉素，而对于出现难治性高脂血症的受试者，建议从环孢霉素改用他克莫司[87,119]。

除了定制的免疫抑制外，在考虑免疫抑制剂的给药时，还需要精细的决策，这应该基于创新的方法。特定药物的谷值水平或副作用的存在并不能描述对整体免疫力或个体免疫抑制需求的实际影响[120–121]。

与术后早期的过度免疫抑制和患者内高变异性相反，他克莫司累积暴露量的增加（通过谷浓度曲线下面积计算）会影响长期结果。这种监测免疫抑制负荷的方法是一种有价值的工具，可以适当地个体化调节免疫抑制，以降低潜在致命副作用的风险[99–101]。

确定移植组织和外周血单核细胞中的药物水平是解决这些未满足需求的另一种创新策略[120–121]。组织和单核细胞中的他克莫司浓度已显示与Banff评分相关，与血液水平中的高变异性相反（图2）。关于“原位”免疫抑制、生物标志物和组织病理学发现的同步信息代表了一种准确确定免疫抑制确切需求的方法。从逻辑的角度来看，在血清和组织取样的1~2天内获得所有这些信息是可能的。

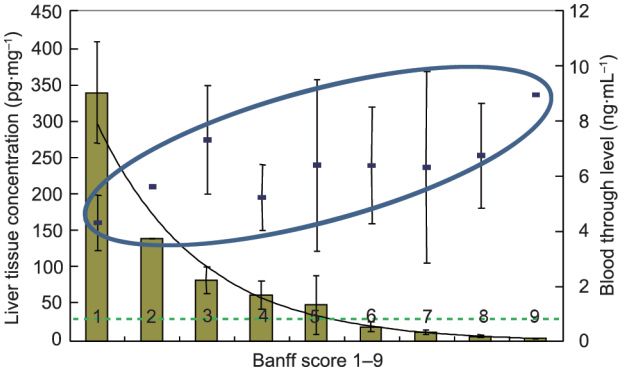

图2. 原位免疫抑制和LT：他克莫司组织水平与第7天活检的Banff评分密切相关，与谷血水平相反。移植物中他克莫司浓度小于30 pg·mg^{-1}与TCMR显著相关（敏感性，89%；特异性，98%）[120]。

移植的“圣杯”是在没有免疫抑制的患者中，获得一个功能正常的移植物，其在组织病理学分析中没有活动性疾病的迹象，这就是COT的定义。只要进行了详细的生

物学、组织病理学和免疫学监测，至少20%的精心挑选的患者可以安全地实现这一目标[28,32]。临床上（LT年龄较大、男性、无自身免疫性疾病、LT与开始断药之间间隔较长）、组织病理学（正常的基线活检和无炎症）以及细胞和分子转录信号已被认为是COT的良好选择因素。在前瞻性研究中：接受肝移植的患者逐步停用免疫系统抑制药物（A-WISH）、免疫抑制停用疗法（I-WITH）、免疫系统重新编程以建立耐受性（RISET）、小儿肝移植受者停用免疫抑制药（WISP-R）和T调节细胞（Treg）研究中，COT的发生率分别为13%、38%、40%、60%和70%[37–38,122–126]。细胞疗法将在耐受性试验中发挥关键作用，并赋予机器灌注技术优势，这是一个移植前免疫调节和修复同种异体移植物的平台[127]。间充质和调节性T细胞输注的安全性和有效性此前已经在临床实践中进行了测试。迄今为止，由于选择性纳入标准[126,128–131]，细胞疗法仅用于极少数患者。Todo等[125]在一项试验中检验了细胞疗法的潜力，在该试验中，考虑到最初的四重免疫抑制，在给予共培养的供体和受体T调节性淋巴细胞后，10名LDLT登记的受体中有7名成功解除了免疫抑制。

另一种方法是共刺激阻断，这主要是由于缺乏共刺激信号，导致无反应的供体-受体识别。这种策略，加上对非抗原特异性信号分子的暂时干扰，使T细胞对抗原呈递细胞无能或无活性。这个概念是1997年由天主教鲁汶大学的团队在人类第一次联合刺激阻断随机对照试验中首创的[132]。从移植后第1~10天开始输注当地开发的抗CD2单克隆抗体（Lo-Cd2, Biotransplant, USA），同时输注基于他克莫司的免疫抑制剂。18名长期存活者中没有一人需要TCMR发作的治疗。移植后5年多，5名患者免于免疫抑制，而9名患者处于低水平、间隔免疫抑制状态（未发表的个人结果）。最近，这一概念已成功应用于骨髓和肾脏移植，以及治疗$CD2^+$ T细胞淋巴瘤和银屑病。计划在不久的将来进行肝移植试验[133]。

总之，需要更大规模的研究者主导的研究来确保从临床操作耐受性试验中得出精确的结论[122–123,134]。

7. 重新评价关于免疫抑制的文献

综上所述，需要一种标准的多药免疫抑制方案。支持这一方法的文献包含许多临床试验，这些试验在设计、终点和对结果的解释方面存在缺陷[135–137]。此外，许多试验未能将实验组与已建立的免疫抑制策略进行比较，即从LT当天开始的两种或一种药物、低水平（6~8 ng, ML1）、基于他克莫司的方案。

试验的终点应该包括不止一个特定的参数，如a-TCMR或肾功能衰竭的发生率（图3）。根据患者和移植物的存活率以及免疫相关的结果实施联合终点将是有益的，其中通过进行连续的长期生物学和组织病理学随访（图3）[41,80,125]来阐明排斥的全谱（不仅考虑急性，而且考虑CR）。事实上，a-TCMR不是移植物或患者存活率不佳的同义词，反之亦然[7–14]。

Single endpoints	
Survival	Renal function
• Graft survival	Metabolic complications
• Patient survival	• Dyslipidaemia
Rejection	• Diabetes
• BPAR	• High blood pressure
• Clinically relevant or treated BPAR	Infectious complications
• Steroid-resistant rejection	• CMV
• CR	• EBV
Cancer	• HCV, HBV (reinfection or progression)
• Recurrence	• Bacterial infections
• *De novo* tumours	• Fungal infections
Minimisation immunosuppression	• Overall infection rate
• Steroid-free regimen	
• Single-drug regimen	

(a)

Composite endpoints (various combinations including the following)
• BPAR
• CR
• Retransplantation
• Graft survival
• Patient survival

(b)

图3. 免疫抑制试验中报告的终点。（a）单一端点；（b）复合端点。

理想情况下，终点应该被整合到一项独立于赞助者、研究人员驱动的、包罗万象的研究中，遵循标准化的方案，在不同的研究分支中有条不紊地协调使用伴随的免疫抑制药物和其他药物。这种严格研究的一个例子是2002年的他克莫司和微乳化环孢素试验[31]，在该试验中，O'Grady和他的同事证实了基于他克莫司的免疫抑制在肝移植中的优势。标准化方案仔细协调了所有参与中心的两个治疗组的伴随药物，检查了一个复合终点，包括患者死亡、再次移植和治疗失败。《柳叶刀》的编辑们认为这样的设计可以作为后续免疫抑制研究的范例，从而建立了一个新的标准[138]。遗憾的是，在接下来的20年里，几乎没有关于免疫抑制的研究遵守这一标准。理想的试验应遵循Jadad和综合试验报告标准[6,139]。20年后，很少有实验遵循这些良好的做法。另外，应该考虑重组LT门诊部的请求。

对这些经常患有多种疾病的患者的护理，应该集中在围绕一些与免疫抑制处理有关的基本规则，进行集中管理（图3）。除了对肝胆疾病和肿瘤学有深入的了解外，迫切需要“普通移植内科和外科”这一专业来取代普通的“意大利腊肠护理”，在这种情况下，每个疾病都需要另一个专家会诊，并采用“全球护理”战略。这种集中的方法积极地使患者和她/他的周围参与进来，可以应对新生肿瘤发生的风险以及心血管、感染、肾脏和神经并发症。这些“普通”移植医生可能会优化伴随的内分泌、骨关节和自身免疫疾病的治疗，克服药物相互作用，最重要的是，促使更多长期接受LT的患者进入COT状态[29,87,119]。扩大肝移植治疗原发性和继发性肝肿瘤的选择标准就是这一建议的一个很好的例子。在不久的将来，将免疫抑制、化疗和免疫疗法结合起来，将为肝胆恶性肿瘤的肝移植结果提供至关重要的升级能力[140]。

8. 结论

在肝移植领域已经取得了重大进展。然而，长期结果仍然被许多与长期使用免疫抑制剂直接相关的副作用所掩盖。应根据有记录的组织病理学和免疫学长期随访，努力将免疫抑制降至最低。理想的免疫抑制方案和临床操作耐受性的开发将需要全面的、研究者驱动的、前瞻性的、双盲随机对照试验，以及由经验丰富的移植团队进行集中的、长期的、临床随访，旨在对肝脏受体进行“全面护理”。

Compliance with ethics guidelines

Jan Lerut and Samuele Iesari declare that they have no conflict of interest or financial conflicts to disclose.

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