Hepatocellular cancer and recurrence after liver transplantation: what about the impact of immunosuppression?

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Abstract: Liver transplantation (LT) has originally been designed to treat hepatobiliary malignancies. The initial results of LT for hepatocellular cancer (HCC) were, however, dismal this mainly due to the poor patient selection procedure. Better surgical and perioperative care and, especially, the refinement of selection criteria led to a major improvement of results, making HCC nowadays (again!) one of the leading indications for LT. This evolution is clearly shown by the innumerable reports aiming to further extend inclusion criteria for LT in HCC patients. Nonetheless, the vast majority of papers only deals with morphologic (tumour diameter and number) and (only recently) biologic (tumour markers and response to locoregional treatment) parameters to do so. Curiously enough, the role of both the immune competent state of the recipient as well as the impact of both immunosuppression (IS) type and load has been very poorly addressed in this context, even if it has been shown for a long time, based on both basic and clinical research, that they all play a key role in the outcome of any oncologic treatment and in the development of de novo as well as recurrent tumours. This chapter aims to give, after a short introductive note about the currently used inclusion criteria of HCC patients for LT and about the role of IS in carcinogenesis, a comprehensive overview of the actual literature related to the impact of different immunosuppressive drugs and schemes on outcome of LT in HCC recipients. Unfortunately, up to now solid conclusions cannot be drawn due to the lack of high-level evidence studies caused by the heterogeneity of the studied patient cohorts and the lack of prospectively designed and randomized studies. Based on long-term personal experience with immunosuppressive handling in LT some proposals for further clinical research and practice are put forward. The strategy of curtailing and minimising IS should be explored in the growing field of transplant oncology taking thereby into account the immunological privilege of the liver allograft. These strategies will become more and more compelling when further extending the indications in which adjuvant chemotherapy will probably become an inherent part of the therapeutic scheme of HCC liver recipients.

Keywords: Liver transplantation (LT); immunosuppression (IS); tumour recurrence; steroid avoidance; IS minimization; steroids; antimetabolites; azathioprine (AZA); mycophenolic acid (MPA); calcineurin inhibitor (CNI); cyclosporine A (CyA); tacrolimus (TAC); mTOR inhibitor; sirolimus (SRL); everolimus (EVL); liver cancer; hepatocellular cancer (HCC)

Introduction

In 1963 medicine and surgery have been revolutionized by the introduction in clinical practice of liver transplantation (LT) (1). After a difficult, 20-year long, period of "trial and error", two major events made its breakthrough happen. The first one was the introduction of a selective immunosuppressant, the cyclosporine A (CyA), a calcineurin inhibitor (CNI); the second one the "1983 National Institutes of Health Consensus Conference", which valued LT as "*a promising alternative to current therapy in the management of the late phase of several forms of serious—benign and malignant—liver disease*". This conclusion was based on the, at that time existing, worldwide experience comprising only 540 transplants done in four medical centres, Denver, Cambridge, Groningen and Hannover during the period 1963–1983 (2). Since then the LT experience literally exploded. So far more than 300,000 procedures have been performed.

The cumulated experience, gathered during half a century, enabled to answer almost all the questions related to benign end-stage liver and liver-based metabolic diseases. Conversely, the transplant community still struggles with many answers to medical and, even more so, ethical questions related to the best treatment of primary malignant liver diseases, around 90% of them consisting of HCC.

Innumerable studies have been and are being conducted in order to improve selection/inclusion criteria for LT as well as liver allocation algorithms in order to balance principles of utility (or good outcome without recurrence) and justice (or good access without interference with the "transplant chance" of patients with benign liver diseases) in the ever increasing population of HCC patients. Almost all studies focused initially on morphologic and, more recently, biologic tumour characteristics. The combination of several features made it possible to build different scores, criteria… and opinions! Today different organ allocation organisms handle somewhat different scores all aiming to distribute in the best possible way scarce liver allografts to the individual HCC patient looking thereby in particular at disease-free survival (DFS) (3-7). It is curious that one of the most important confounders related to DFS of whatever oncologic treatment, notably, the immunosuppressive state, and thus the use of IS, has been sparsely addressed in this context. Logically one should seek for a balance between immunologic (graft rejection) and oncologic risk (development of tumour recurrence and/or also *de novo* tumour formation) in order to boost outcomes in HCC liver recipients. Yet much confusion persists as exemplified by one of "2010 Zurich Consensus Conference on HCC and LT" recommendations: "*there is currently insufficient evidence from clinical trials to base a recommendation for choosing the type or dose of IS therapy to influence the incidence of HCC recurrence or its prognosis*" (8). So the debate about the optimal IS after LT in HCC patients is still open.

This article aims to review the available literature concerning the relationship between IS and outcomes of HCC after LT. In view of a deeper understanding, two short updates are first made about selection criteria of HCC patients for LT and about some basic knowledge about IS and oncogenesis. Based on these data as well as on a large experience in both transplant oncology and immunosuppressive management, some personal recommendations will also be provided.

Hepatocellular cancer (HCC) and LT: selection

The concept of LT was originally developed by T. E. Starzl in Denver in order to treat (unresectable) liver tumours. Indeed eight of the worldwide ten first LT attempts were done because of liver malignancies. The 10th patient was the first long-term liver recipient. She died due to tumour recurrence 400 days after LT (9). Five patients presented a HCC, one a cholangiocellular cancer and two colorectal liver metastases. All these indications are today hot topics in almost every hepatology or transplantation meeting.

The idea of total hepatectomy as "the" treatment of liver cancer was further explored during the "adolescent phase" of LT (covering the period 1963–1983). The late R. Pichlmayr greatly fostered the upcoming field of "transplant oncology" (10). However, the enthusiasm of the pioneering centres rapidly declined due to poor long-term outcomes and very high recurrence rates, all explained by the lack of adequate selection criteria. Progressive improvements in surgical technique, postoperative care and selection reversed the tide. The 5-years overall survival (OS) rates improved over the last decades from the low 12% before 1985 to the high 70% long-term survival during the last decade (ELTR data). The major determinant for this success was the introduction of selection criteria, which led to move away from "transplantation of the unresectable HCC" towards "transplantation of the resectable HCC". The introduction of the Milan Criteria (MC) in 1996 best translated this shift. These are static and morphologic criteria based on both tumour number (up to 3) and diameter (up to 5 cm). Adoption of these inclusion criteria resulted in up to 96%

5 and 10 years DFS, results to be considered as one of the best in the whole field of oncology (11). Therefore, these criteria were rapidly (in total or in part) integrated in almost all algorithms for liver allocation. However, recent experiences questioned the adherence to the restrictive MC as this attitude may unjustifiably exclude at least 20% (and some Eastern experiences even raise this proportion to 60%) of HCC patients from a potentially curative treatment (6,12). In contrast, several papers convincingly showed that the MC can be widened by complementing "static and morphologic" tumour criteria with "dynamic and biologic" ones. Tumour-released molecules, such as α-fetoprotein (AFP) and des-γ-carboxyprothrombin (DCP), also known as protein induced by vitamin K absence-II (PIVKA-II), and inflammatory markers, such as neutrophil-to-lymphocyte ratio (NLR) or platelet-to-lymphocyte ratio (PLR), as well as tumour response to neo-adjuvant locoregional treatments, such as transarterial chemo/radio-embolization and radiofrequency or alcohol ablation, are all independent predictors of dropout before and tumour recurrence after LT (13-18). This "dynamic" approach proved to be valuable enabling to transplant more patients without compromising their outcomes (4-6,18,19).

Combination of both "morphologic and static" and "biologic and dynamic" tumour features has been warmly welcomed in Asian countries due to the explosive development of living donor liver transplantation (LDLT). The LDLT constellation has the major advantage of transforming LT for cancer into an elective, scheduled procedure. Accordingly both "factor tumour" and "factor time" can be controlled and both neo-adjuvant and radical treatment can be intertwined. This opportunity pushed some centres to (re)consider LT in patients presenting macrovascular tumour invasion (20).

The aforementioned static and dynamic tumour characteristics are linked to tumour differentiation, inflammation, molecular features and vascular invasion (21-23).

Immunosuppression (IS) and carcinogenesis

The integrity of the immune system is the mainstay in cancer and infection control. When analysing the 21st-century literature in relation to the incidence of tumour recurrence after LT, the lack of attention paid to the impact of IS on the outcome of liver cancer recipients is surprising. The link between IS and oncogenesis has been unequivocally proven both in laboratory and clinical settings (24-30). The increase in cancer risk has already

been ascertained in patients receiving IS as well as in patients with an end-stage organ failure. Nevertheless, only virus-related cancers have a higher incidence after post-transplant IS. Oncogenic viruses can immortalise infected cells by disrupting the cell-cycle control and, in a setting of induced lowered immune surveillance, this phenomenon is likely to cause tumorigenesis (29,30). Basic research is important for further progress in transplantation oncology as demonstrated by the following two examples. Yokoyama showed in 1991 that IS drugs stimulate cancer cell growth (24). In contrast, Guba showed that mammalian target of rapamycin inhibitors (mTORis), including sirolimus or rapamycine (SRL) and everolimus (EVL), interfere with carcinogenesis by inhibiting the PI3K/Akt/mTOR pathway, the key regulator of cellular proliferation and angiogenesis (27). In vivo, the use of mTORis proved to reduce the risk of post-transplant *de novo* cancers, in particular of non-melanoma skin cancers, in kidney transplanted populations (31,32).

Around 1850 Virchow identified first the relationship between inflammation and cancer. Unravelling this relationship during the 21st century fostered a deeper insight in the process of carcinogenesis (33). Inflammatory parameters such as C-reactive protein, erythrocyte sedimentation rate, NLR, PLR and inflammatory cytokines (such as IL1b, 6, 7 and 17) have all been linked to tumour aggressiveness and recurrence after LT because of their role in promotion of neoangiogenesis and tumour growth. Similarly studies on the type of lymphocytic infiltration in tumour and peritumoral liver tissues also showed that a reduced immune status, as expressed by a disturbed balance between T-regulatory and CD8 lymphocytes, favours tumour aggressiveness and recurrence after transplantation. In summary, tumour-induced inflammation and reduced immune defence against cancer are responsible for increased recurrence (34).

Basic science findings together with many (observational) clinical experiences indicate that not only the type of immunosuppressive drug(s) or scheme(s) but, even more decisively, the total immunosuppressive load plays a role in cancer recurrence. This interaction will gain an ever growing relevance because inclusion criteria for LT are wider and wider and (re-)implementing LT as a treatment for secondary neuro-endocrine and colorectal liver tumours is around the corner.

Beside the issue of HCC recurrence, long-term results in all fields of organ transplantation are seriously compromised not only by the occurrence of cardiovascular and metabolic

(in 30% to 40% of recipients), renal (18% of recipients) and infectious complications but also by *de novo* tumour formation (more than 10% of recipients). These are the major causes of patient loss in presence of a functioning graft (35,36).

HCC, LT and IS

Despite compelling data, very few studies have addressed the impact of IS on outcomes in HCC recipients and, when done, they lack high level of evidence due to the heterogeneity of the studied patient cohorts and the lack of prospective design and randomization (37,38). Moreover, this key field of transplant oncology surely suffered from the tsunami of industry-driven IS trials in which "minimization" approach is not addressed at all or limited to the dose reduction or (timely) elimination of one specific drug in favour of another one. The best example of such design is the recent study centred on the T-cell co-stimulation blocker belatacept, examining the benefit of a quadruple IS scheme in LT (39).

Till 2016 we found in the literature 21 articles dealing with LT and IS, of which only one is prospective and randomized and two are SRL-related meta-analyses (*Table 1*) (37). Six ones studied the impact of CNI use or load, eleven the impact of SRL, two the steroid use or withdrawal and one the impact of ATG/OKT3 and steroid-based IS. Overall recurrence rate ranged from 12% to 54%.

From these observational studies and two meta-analyses including 474 and 2,950 HCC recipients experiences two conclusions can be drawn: (I) the higher the exposure to CNIs, either CyA or tacrolimus (TAC), the higher is the recurrence risk and (II) mTOR inhibitors reduce the recurrence risk (49,56,57,62).

The Rodríguez-Perálvarez' retrospective minimization study offers strong evidence for a significantly lower recurrence rate (14.7% *vs.* 27.7%) in case of low CNI exposure during the first post-LT month (57).

Unfortunately the protective effect of mTORi has been seriously weakened by the results of the, so far only, large, transcontinental, multicentre, prospective, randomized SILVER study (60). This trial was based on SRL and merely showed a lower recurrence rate in early HCC at 3 years. This effect was erased at 5 years. Two major remarks must be made in relation to these findings. First the results obtained in T2 HCC lesions (this means MC-in lesions) at 3 years are inferior to those obtained in most transplant centres in the absence of mTORi-based IS (11,63). Second

the results of this large patients' cohort suffer from high heterogeneity: the only constant IS factor was the addition of SRL to very different IS schemes. Each centre had his own "IS cocktail" with or without steroids, with or without steroid withdrawal, early or late introduction of CNI, presence or absence of induction therapies, using different kinds of anti-lymphocytic sera or antibodies.

The role of mycophenolic acid (MPA), an antimetabolite, is contradictory but its impact *in vivo* seems negligible (64-66). The use of azathioprine (AZA), another antimetabolite known to induce non-skin malignancy in LT, has not been analysed in respect of HCC recurrence (67). The same can be stated for the use of corticosteroids, anti-lymphocytic sera and anti-interleukin-2 receptor α antibodies (46,58,68-73).

Few solid conclusions can be made from this literature review about the true impact of any specific immunosuppressant on the real incidence of HCC recurrence. Nevertheless, IS load seems to play a determinant role in cancer recurrence (49,57). Basic clinical research and many observational studies favour this hypothesis. The most relevant data about the several categories of IS drugs in relation to the risk of malignancy are listed in *Table 2* (34). The minimization approach is of special value in an era where transplant oncology is slowly but steadily becoming the first indication in adult LT. More and more patients are undergoing transplantation with MC-out or advanced HCC based on the "modern" oncologic selection process, which integrates both tumour morphology and biology (18,19,74,75). Moreover, minimization strategies are justified by the intrinsic immunosuppressed status oncologic patients usually display and by the immunologic privilege of the liver, which allows a substantial reduction in IS load without compromising both patient and graft survival (38,76,77). Further broadening of indications for LT in case of primary and secondary liver tumours will forcibly require adjuvant chemotherapy (78), a reason more to opt for immunosuppressive minimization protocols.

Conclusions

Today we are on the brink of a real (r)evolution both in LT and oncology. The more widespread use of machine perfusion for severely compromised deceased-donor livers (79) and of living-donor LT, together with the possible eradication of viral diseases, will lead to an ever growing number of transplants for not only primary but also secondary liver tumours.

Sound oncologic principles from all other fields taught us that outcomes optimization entails a combination of

Table 1 Overview of publications investigating the effects of immunosuppression on HCC recurrence after liver transplantation

Reference	Year	Centre	Evidence level (40)	Patients (n)	Immunosuppressive drug	Recurrence rate (%)	Results	P
Vivarelli et al. (41)	2002	Policlinico Sant'Orsola-Malpighi, Bologna University, IT	4	82	CyA, STER, AZA (n=34); CyA, STER (n=35); TAC, STER, AZA (n=4); CyA switch to TAC (n=3)	12.2	5-yr OS: 64%; 70% of recurrences within first year after LT; CyA dose-dependent 5-yr DFS: 93% low dose vs. 76% high dose	0.01
Kneteman et al. (42)	2004	Walter C. Mackenzie Centre, Alberta University, Edmonton, CA	3b/4	40	SRL de novo CNI (n=40); CNI tapered to 0 (3 to 6 mo)	12.5	MC in, n=19: 1- and 4-yr OS 94% and 87%; MC out, n=21: 1- and 4-yr OS 91% and 83%; MC in vs. MC out	0.68
Vivarelli et al. (43)	2005	Policlinico Sant'Orsola-Malpighi, Bologna University, IT	4	70	CyA, STER, AZA (n=44); CyA, STER (n=26)	10.0	RR: 0% vs. RR: 33.3%; DFS related to CyA exposure independent risk factor	<0.001
Decaens et al. (44)	2006	Hôpital Henri Mondor, Créteil, FR	4	412	CyA based (n=284); TAC based (n=128)	31.8	5-yr DFS and OS: 57.1% and 57.9%; 5-yr DFS CyA vs. TAC: 52.5% vs. 70.8%	0.003
Decaens et al. (44)	2006	Hôpital Henri Mondor, Créteil, FR	4	412	ATG/OKT3 induction (n=55); no induction (n=357)	31.8	5-yr DFS: 58.8% vs. 5-yr DFS: 45.4%	0.02
Zhou et al. (45)	2006	Zhongshan Hospital, Fudan University, Shanghai, CN	4	36	All switched to SRL: SRL, TAC (n=26); SRL, TAC, STER (n=10)	27.4	Group A (early switch for advanced HCC, n=11): 1-yr OS 68%; Group B (switch after HCC recurrence, n=18): 1-yr OS 67%; Group C (switch for CNI nephrotoxicity, n=7): no recurrence	NA
Chen et al. (46)	2007	Tongji Hospital, Huazhong University, Wuhan, CN	3	44	STER withdrawal at 3 mo (n=28); STER maintenance (n=26)	53.7	6-mo recurrence rate: 25.0% vs. 42.3%; 1-yr survival rate: 64.2% vs. 46.1%; 1-year tumour recurrence rate: 39.2% vs. 69.2%	>0.05; >0.05; <0.05
Toso et al. (47)	2007	Walter C. Mackenzie Centre, Alberta University, Edmonton, CA	3b/4	70	SRL, de novo CNI (n=70)	11.4	1- and 4-yr OS: 85% and 75%; 1- and 4-yr DFS (MC in): 85% and 73%; 1- and 4-yr DFS (MC out): 84% and 74%	0.9
Zimmerman et al. (48)	2007	UCH, Colorado University, Denver, US	3b	130	CNI based (n=81); SRL based (n=49)	12.4	1-, 3-, and 5-yr DFS: 84%, 74%, and 67%	>0.05
Vivarelli et al. (49)	2008	Policlinico Sant'Orsola-Malpighi, Bologna University, IT	3b	139	CyA based (n=79); TAC based (n=60)	15.1	3-yr DFS CyA vs. Tac: 89% vs. 79%; dosage-dependent DFS survival for TAC and CyA patients	0.047

Table 1 (*continued*)

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Reference	Year	Centre	Evidence level (40)	Patients (n)	Immunosuppressive drug	Recurrence rate (%)	Results	P
Nocera et al. (50)	2008	Ospedale San Martino, Genoa University, IT	4	18	SRL de novo CNI (n=11); CNI based, later SRL mono (n=7)	5.6	1 of 18 died within 12 mo because of pneumonitis; 1 of 18 experienced HCC recurrence at 14 mo post-LT	NA
Zimmerman et al. (51)	2008	UCH, Colorado University, Denver, US	3b	97	SRL based (n=45); CNI based (n=52)	12.4	1- and 5-yr OS: SRL vs. CNI: 96% vs. 83% and 80% vs. 62%; 1- and 5-yr DFS: SRL vs. CNI: 93% vs. 75% and 79% vs. 54%	0.087; 0.03
Chinnakotla et al. (52)	2009	Baylor University Medical Center, Dallas, US	3b	227	SRL based (n=121); TAC based (n=106)	11.0	1-, 2-, and 5-yr probability for recurrence for SRL based: 5.2%, 9.2% and 11% vs. TAC 5-yr OS: SRL vs. Tac: 80% vs. 59%	0.0001; 0.0001
Vivarelli et al. (53)	2010	Policlinico Sant'Orsola-Malpighi, Bologna University, IT	3b	62	SRL containing (n=31); TAC based (n=31)	25.8	3-yr DFS: SRL vs. Tac: 86% vs. 56%	0.04
Toso et al. (54)	2010	Walter C. Mackenzie Centre, Alberta University, Edmonton, CA	3b/4	2,491	SRL: (n=109); no SRL: (n=2,382)	–	3- and 5-yr OS: on SRL vs. off SRL: 86% vs. 79% and 83% vs. 69%	≤0.05
Vivarelli et al. (55)	2010	Policlinico Sant'Orsola-Malpighi, Bologna University, IT	4	86	SRL based (n=86)	16.0	Only detection of risk factors for recurrence	NA
Liang et al. (56)	2011	First Affiliated Hospital, Sun Yat-sen University, Guangzhou, CN	2	2,950	SRL: (n=332); no SRL: (n=2,618)	–	1-yr OS OR 4.53, 95% CI = 2.31–8.89; 3-yr OS OR 1.97, 95% CI =1.29–3.00; 5-yr OS OR 2.47, 95% CI =1.72–3.55; recurrence OR 0.42, 95% CI =0.21–0.83, SRL vs. no SRL	<0.05
Rodríguez-Perálvarez et al. (57)	2013	Reina Sofía University Hospital, Córdoba, ES	4	219	High CNI exposure (n=48); reduced CNI exposure (n=171)	17.6	1-yr DFS 90.6 vs. 95.7; 5-yr DFS 72.3 vs. 85.3	0.007
Xing et al. (58)	2013	First People's Hospital, Shanghai Jiao Tong University, CN	4	178	Basiliximab, TAC, MPA (n=78); STER, TAC, MPA (n=100)	41.0	5-yr OS 42.5% vs. 50.5%; 5-yr DFS 38.9% vs. 39.2%	0.734; 0.913
Cholongitas et al. (59)	2014	Thessaloniki Hippokration General Hospital, Aristotle University, GR	4	43	EVL, CyA or MPA (n=21); CyA (n=22)	9.3	Recurrence 0% vs. 18.5% after a mean of 49 mo	>0.05

Table 1 (*continued*)

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Reference	Year	Centre	Evidence level (40)	Patients (n)	Immunosuppressive drug	Recurrence rate (%)	Results	P
Geissler et al. (60)	2016	Multicentric, University Hospital, Regensburg, DE	2	525	Mix IS without SRL (n=264); mix IS with SRL (n=261)	32.7	DFS 64.5% vs. 70.2% at 8 yrs (end-point); OS 68.4% vs. 74.6% at 8 yrs (end-point); DFS 72.3% vs. 80.6% at 3 yrs; OS 70.3% vs. 79.4% at 5 yrs	0.28; 0.21; <0.05; <0.05
Yanik et al. (61)	2016	Multicentric, National Cancer Institute, Rockville, US		3,936	SRL (n=234); no SRL (n=3,702)	6.1	All-cause mortality, HCC recurrence and cancer-specific mortality without differences; among recipients ≤55 years old, worse outcomes for SRL: all-cause mortality HR =1.76, 95% CI =1.12–2.75; recurrence HR =1.49, 95% CI =0.62–3.61; cancer-specific mortality HR =1.54, 95% CI =0.71–3.32	>0.05; <0.05

95% CI, 95% confidence interval; AZA, azathioprine; CNI, calcineurin inhibitor; CyA, cyclosporine A; DFS, disease-free survival; LT, liver transplantation; MC, Milan criteria; MPA, mycophenolic acid; OR, odds ratio; OS, overall survival; SRL, sirolimus; STER, steroid; TAC, tacrolimus, mo, month; yr, year.

Table 2 Influence of immunosuppressive drugs on (*de novo*) tumour development

Property	Anti-oncogenic	Pro-oncogenic
CNI	–	+
mTORi	+	–
MPA	+ (in vitro)	–
AZA	–	+
STER	–	±
Anti-IL-2Rα antibodies	–	–
rATG	–	+
rATLG	–	+

AZA, azathioprine; CNI, calcineurin inhibitor; MPA, mycophenolic acid; mTORi, mammalian target of rapamycin inhibitor; rATG, rabbit anti-thymocyte globulins (Thymoglobulin®); rATLG, rabbit anti-T-acute lymphoblastic leukaemia cell line Jurkat globulins (Grafalon®); STER, steroid; IL2Rα, interleukin-2 receptor α.

tumour biology criteria, neo- and adjuvant (even including systemic chemotherapy) treatments and, last but not least, adapted, minimized and individualized IS. In the absence of well-designed studies, no firm conclusions can be drawn about the impact of a given immunosuppressive drug on recurrence or *de novo* development of malignancies. In order to significantly influence HCC recurrence after LT it will be necessary to reduce as much as possible the immunosuppressive load. mTOR inhibitors may possibly play a favourable role in this context, especially in case of advanced tumour burden. The advent of immunotherapy will be an additional tool in the race against liver cancer (80).

The final step in the optimization of patients' selection for a (potential curative) transplant procedure will be the integration of the "static-to-dynamic" paradigm switch into the intention-to-treat benefit concept, which looks at the difference between pre- and post-transplant outcomes (81). Outweighing transplant utility (post-LT outcome read recurrence) and urgency (pre-LT outcome) will be the best guarantee to assure a transplant to the highest possible number of patients with the best results. Adapted immunosuppressive therapy will be an important player in this scenario.

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Footnote

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