Everolimus Personalized Therapy: Second Consensus Report by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology

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Abstract: The Immunosuppressive Drugs Scientific Committee of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology established the second consensus report to guide *Therapeutic Drug Monitoring* (TDM) of everolimus (EVR) and its optimal use in clinical practice 7 years after the first version was published in 2016. This version provides information focused on new developments that have arisen in the last 7 years. For the general

Received for publication February 8, 2024; accepted June 9, 2024.

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This work was supported in part by Grant-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Science, Culture, Sports and Technology of Japan (MEXT) (Grant numbers: 22K06736 to S Masuda).

The authors declare no conflict of interest.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.drug-monitoring.com).

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aspects of the pharmacology and TDM of EVR that have retained their relevance, readers can refer to the 2016 document. This edition includes new evidence from the literature, focusing on the topics updated during the last 7 years, including indirect pharmacological effects of EVR on the mammalian target of rapamycin complex 2 with the major mechanism of direct inhibition of the mammalian target of rapamycin complex 1. In addition, various concepts and technical options to monitor EVR concentrations, improve analytical performance, and increase the number of options available for immunochemical analytical methods have been included. Only limited new pharmacogenetic information regarding EVR has emerged; however, pharmacometrics and model-informed precision dosing have been constructed using physiological parameters as covariates, including pharmacogenetic information. In clinical settings, EVR is combined with a decreased dose of calcineurin inhibitors, such as tacrolimus and cyclosporine, instead of mycophenolic acid. The literature and recommendations for specific organ transplantations, such as that of the kidneys, liver, heart, and lungs, as well as for oncology and pediatrics have been updated. EVR TDM for pancreatic and islet transplantation has been added to this edition. The pharmacodynamic monitoring of EVR in organ transplantation has also been updated. These updates and additions, along with the previous version of this consensus document, will be helpful to clinicians and researchers treating patients receiving EVR.

Key Words: everolimus, mTOR inhibitor, therapeutic drug monitoring, transplantation, oncology

(*Ther Drug Monit* 2024;00:1–28)

INTRODUCTION

Therapeutic Drug Monitoring (TDM)–based immunosuppressive therapy following organ transplantation has been acknowledged as the cornerstone for rescuing patients with end-stage organ failure.[1–4] Two calcineurin inhibitors, cyclosporine (CsA) and tacrolimus (TAC), were introduced in 1980 and 1990, respectively, and both agents have been established as key players in preventing acute cellular rejection, considering HLA compatibility.[5–7] However, various adverse reactions with or without drug–drug interactions (DDIs) with pivotal cytochrome P450 3A inhibitors have been identified in patients undergoing organ transplantation, such as infections caused by overimmunosuppression, kidney injury, hyperlipidemia, and hyperkalemia.[8] More recently, mycophenolate and everolimus (EVR) were combined with calcineurin inhibitors to reduce the exposure to each drug, thereby reducing the risk of adverse drug reactions (ADRs) related to high exposure.[9–11]

The second-generation mammalian target of rapamycin (mTOR) inhibitor, EVR, has been approved for the prevention of transplanted organ rejection and the treatment of various types of cancer and tuberous sclerosis complex (TSC). Immunosuppressive therapy with EVR should consider its narrow therapeutic window, large intra- and interindividual pharmacokinetic (PK) variability, and well-established drug exposure–response relationships.[4] In patients undergoing organ transplantation, overexposure to EVR exacerbates certain adverse reactions and causes immunological compromise due to overimmunosuppression. By contrast, insufficient exposure to EVR increases the risk of transplant organ rejection. Therefore, TDM was recommended when EVR was registered for use to suppress rejection after organ transplantation. TDM-based EVR dose adjustment is supported by substantial clinical evidence, as described below. In addition to organ transplantation, the effectiveness of TDM-based EVR therapy as an anticancer strategy has been established in numerous clinical trials. Accurate and precise measurement of the EVR concentration in whole blood is a matter of course for the smooth implementation of EVR TDM; however, analytical methods, their characteristics, and the target concentration for each method are often overlooked. Furthermore, the lack of agreement regarding comparability and calibration between analytical methods strongly affects the interpretation of the results and the drug treatment itself.

In 2016, the Immunosuppressive Drugs Scientific Committee of the International Association of TDM and Clinical Toxicology published the first consensus report for the TDM of EVR. The aim of this study is to update the first report by providing information on new developments that arose during the last 7 years. For general aspects of the pharmacology and TDM of EVR that have retained their relevance, readers are referred to the 2016 report.[4] Most chapters include revisions in accordance with updated information. In this edition, EVR TDM for pancreatic and islet transplantation was added.

This study is intended for all professionals involved in the management of patients receiving pharmacotherapy with EVR in a variety of clinical settings, including organ transplantation and oncology, and the aim of this study is to improve both the standards of practice and patient care.

PHARMACEUTICS

Chemistry

EVR is a white to light yellow powder practically insoluble in water [<0.01% in water, 0.1 N hydrochloric acid, and citrate buffer (pH 2.0–10.0)]. EVR is soluble in organic solvents such as ethanol and methanol. EVR has a molecular weight of 958.25 g/mol and a molecular formula of $C_{53}H_{83}NO_{14}$.[12] EVR is a 40-*O*-(2-hydroxyethyl) derivative of the original mammalian (later adjusted to the "mechanistic") target of rapamycin (mTOR) inhibitor (mTORi), sirolimus (SRL), which makes the molecule more hydrophilic, and has different pharmacokinetic and pharmacodynamic properties. EVR is a signal transduction inhibitor targeting the mTOR.[12]

Formulations

EVR has been marketed by Novartis as Afinitor, Votubia, Certican, and Zortress.[12] It is available in different formulations and dosages: 0.25 mg, 0.5 mg, 0.75 mg, and 1 mg (and 2.5 mg, 5 mg, and 10 mg for Afinitor and Votubia) as oral tablets and tablets for suspension. The first generic was introduced by Teva Europe, then subsequently in Australia, the United States, and Canada. More recently, several other generics have been approved in the United States (Everolimus Hikma Pharms, Everolimus Par Pharm, and Everolimus BIOCON) and in Canada (Everolimus-PMS).

Preparations for the topical use of EVR are currently under development. The preparation of EVR micelles and nanosuspensions for ocular administration is expected to be

a new administration form for corneal transplantation, immunological rejection, and other ocular diseases. Owing to the presence of a blood–eye barrier, if a systemic administration is used, particularly for corneal transplantation, a large oral dose is required to be effective in the eye. Such a dose is likely to increase the risk of side effects such as thrombocytopenia and dyslipidemia, which occur in approximately 30% of the patients who take the drug. In contrast to systemic administration, topical preparations for the eye have the advantages of a small dosage and mild side effects and may be advantageous in corneal transplantation.[13]

PHARMACOLOGY

Mechanism of Action

Following its entry into the cytoplasm, EVR binds noncovalently to FK-binding protein-12 (FKBP12) (Fig. 1).[14,15] FKBP12 is a 12-kDa cytosolic protein that is abundantly expressed in all tissues and functions as a *cis/trans* peptidyl-prolyl isomerase (PPIase).[15–17] The EVR-FKBP12 complex specifically binds with high affinity to a region in the target mTOR protein near the C-terminus, called FKBP12-rapamycin binding (FRB), thereby inhibiting its kinase function.[14,15]

Immunosuppressive Agent

EVR functions as an immunosuppressant by blocking T-cell activation by cytokines, primarily interleukin-2 (IL-2), by interfering with some of the signals resulting from IL-2 binding to its receptor and ultimately blocking cell cycle progression from the G1 to S phase (Fig. 1).[18–21] The IL-2 receptor (IL-2R) signaling is initiated as IL-2 binds to the receptor at the cell surface, leading to signal transduction through the tyrosine kinases Janus kinase1 and Janus kinase3, resulting in the activation of mitogen-activated protein kinase

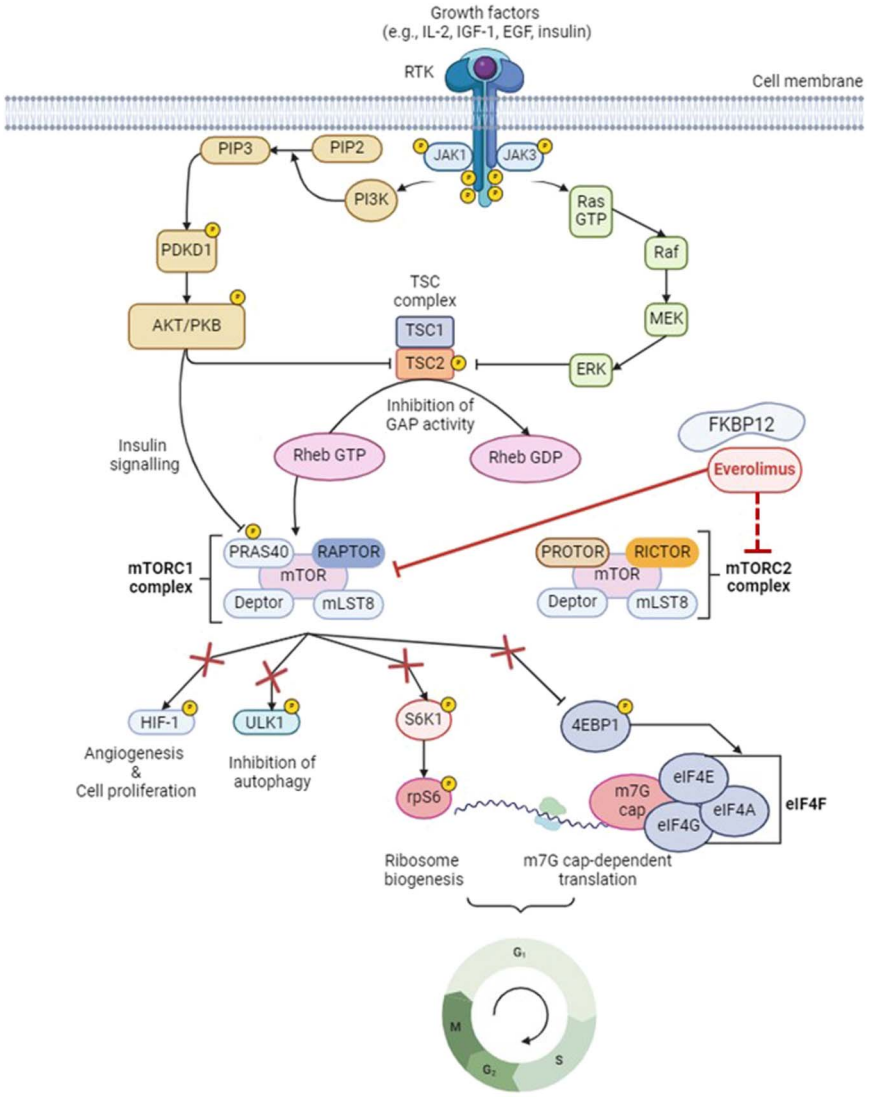

FIGURE 1. Cellular mechanism of action of everolimus. Circled P refers to phosphorylated protein; blunt arrows (⊥) indicate inhibition, while sharp arrows (→) indicate stimulation. EVR modifies cellular responses by associating with the intracellular protein FKBP12. The FKBP12-EVR complex binds directly to mTORC1 and inhibits its functions, resulting in the dephosphorylation and inactivation of hypoxia-inducible factor 1, leading to the inhibition of angiogenesis and cell proliferation, inactivation of ULK1, promotion of autophagy, and inactivation of S6K1 and 4EBP1, leading to G1 cell cycle arrest. The dashed line indicates inhibition of mTORC2 by prolonged exposure to everolimus. Deptor, DEP domain–containing mTOR-interacting protein; EGF, epidermal growth factor; eIF, eukaryotic translation initiation factor; ERK, extracellular signal-regulated kinase; FKBP12, FK506-binding protein-12; GTP, guanosine-5′-triphosphate; GDP, guanosine-5′-diphosphate; GAP, GTPase-activating proteins; IGF-1, insulin-like growth factor-1; JAK, Janus kinase; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PDKD1, phosphoinositide-dependent protein kinase-1; PI3K, phosphoinositide-3-kinase; PIP2, phosphatidylinositol 4,5 bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PRAS40, proline-rich AKT substrate 40; AKT/PKB, protein kinase B; Ras, rat sarcoma virus; Raf, rapidly accelerated fibrosarcoma; RTK, receptor tyrosine kinase; RHEB, Ras homolog enriched in brain; mLST8; mammalian lethal with Sec13 protein 8; RICTOR, rapamycin-insensitive companion of mTOR; PROTOR, protein observed with RICTOR; ULK1, unc-51-like autophagy-activating kinase 1; S6K1, ribosomal protein S6 kinase beta-1; rS6P, ribosomal S6 protein; m7G, 7-methylguanosine.

and phosphoinositide-3-kinase (PI3K).[22]PI3K phosphorylates the inositol ring of phosphatidylinositol 4,5-bisphosphate (PIP2) to produce phosphatidylinositol 3,4,5-trisphosphate (PIP3),[23] enabling the recruitment and activation of several proteins, including serine/threonine protein kinase B (PKB), also known as AKT.[18,23] AKT reduces the GTPase activity of RHEB, which activates mTOR complex 1 (mTORC1), and insulin-activated AKT phosphorylates mTORC1-associated proline-rich AKT substrate 40, causing it to dissociate from regulatory-associated protein of mTOR (RAPTOR), thereby activating mTORC1.[18,24,25]

Activated mTORC1 mediates downstream effects through the phosphorylation of multiple substrates and induces the activity of ribosomal protein S6 (rpS6) kinase beta-1 (S6K1, p70S6 kinase, p70S6K),[26–28] which exists in a complex with eukaryotic initiation factor 3, which in turn phosphorylates rpS6 to promote ribosome biogenesis. Simultaneously, mTORC1 phosphorylates and inactivates the translational repressor eukaryotic translation initiation factor 4E–binding protein 1 (4EBP1).[28–31] Phosphorylation of 4EBP1 by mTORC1 blocks the entry of cells into the S phase of the cell cycle and causes G1 cell cycle arrest.[15,18,23,32–36]

It is thought that mTORC2 (mTOR complex 2) is insensitive to rapalogs (rapamycin analogs). Structural studies have revealed that this insensitivity is due to the occlusion of the rapamycin/FKBP12-binding region in this protein complex; hence, mTORC2 is unable to directly associate with rapamycin.[37–40] However, it has recently been revealed that prolonged exposure to rapalogs can indirectly inhibit mTORC2 because this compound can target newly synthesized mTOR, thus preventing de novo mTORC2 assembly.[37,41] The growth-related functions of mTORC2 are conserved from yeast to humans, and the inhibition of mTORC2 by rapalogs may ultimately contribute to its proapoptotic effects.[37,41] Although the basal phosphorylation of AKT S473, a substrate of mTORC2, was found to correlate with the antiproliferative response to EVR, the increased AKT S473 phosphorylation induced by EVR did not. This suggests that the off-target effects of EVR on mTORC2 occur by mTORC1.[42]

Anticancer Agent

Overactivation of the PI3K/AKT/mTOR signaling axis is one of the most activated pathways in human cancers.[25] Detailed knowledge of the molecular targets of rapalogs has enabled the use of these drugs in cancer treatment. EVR is a rapalog that has been evaluated as monotherapy and combination therapy for the treatment of human cancer. The first indication for EVR was advanced renal cell carcinoma in patients whose disease progressed on or after treatment with vascular endothelial growth factor-targeted therapy.[43] This demonstrates the benefits of combination therapies, including drugs for specific targets relevant to such cancers.

Constitutively activated mTOR supplies oxygen and nutrients to carcinoma cells by increasing the translation of hypoxia-inducible factor-1 (HIF1) and supporting angiogenesis.[44] Studies in prostate cancer cell lines have shown that EVR downregulates HIF-1α expression.[45] mTORis have been shown to block HIF-1α function and have antiangiogenic effects, suggesting another possible mechanism for EVR anticancer activity.[46,47]

EVR, approved by the FDA and EMA, is now an option for combination therapies for advanced renal cell carcinoma, pancreatic neuroendocrine tumors, and progressive, nonfunctional gastrointestinal and lung neuroendocrine tumors. In addition, several hundred ongoing studies have investigated EVR and other drugs that inhibit mTOR complexes for cancer treatments.[48]

Another example where EVR specifically targets (noncancerous) disease is in patients with the TSC. This disease is caused by mutations in the above-mentioned TSC1 and 2 genes, leading to constitutive activation of the mTOR pathway. This dysregulation of mTOR is regulated by EVR, which has been shown to be effective for seizure control in patients with the disease.[49]

Antiviral Activity

The immunosuppressive regimen following solid-organ transplantation exposes graft recipients to an increased risk of opportunistic viral, bacterial, and fungal infections.[50,51] Interestingly, patients receiving mTORi-based immunosuppressive therapy have a lower incidence and severity of the most common opportunistic viral pathogens seen in transplant recipients [cytomegalovirus (CMV) and BK polyomavirus].[52,53]

The antiviral properties of mTORis are attributed to several mechanisms.[54] The activity of mTORis can improve memory T-cell quality, functionality, and efficacy in response to viral stimuli.[55,56] Moreover, the inhibition of mTOR can block the downstream effects related to viral replication and growth.[57,58] Experimental in vitro and in vivo as well as clinical studies have demonstrated the antiviral properties of EVR against CMV, Epstein–Barr virus, human herpes virus 8, hepatitis B virus, HIV, and human papillomavirus.[52]

The protective effect of CMV reactivation in patients treated with EVR is supported by its ability to boost the CMV-specific $CD8^+$ T-cell response.[56,59] Moreover, in vitro EVR treatment delayed and suppressed viral DNA synthesis and release from human embryonic lung cell culture supernatant.[60] Human CMV (HCMV), similar to multiple other viruses that rely on m7G-cap-dependent mRNA translation, requires mTOR complexes, mainly mTORC1, for efficient virus replication.[57,61,62] Therefore, HCMV strives to maintain mTOR kinase activation, as detected by the phosphorylation of 4EBP1 and S6K, through either RAPTOR or rapamycin-insensitive companion of mTOR (RICTO)R depletion in infected cells. Experimental depletion of RAPTOR and RICTOR also showed differing effects on the accumulation of viral proteins. HCMV alters the substrate specificity of both mTOR complexes in infected cells.[61,63–65] Translational control of viral protein kinases by mTOR signaling is involved in the production of all classes of HCMV proteins, mainly at the early stage of infection.[61]

PHARMACOGENETICS

EVR is primarily metabolized by CYP3A4, with minor contributions from CYP3A5 and CYP2C8. Metabolism

includes demethylation, hydroxylation, and ring degradation.[12,66,67] There is also evidence that EVR is a substrate for the drug transporter ABCB1, also known as P-glycoprotein 1 (permeability glycoprotein, P-gp), multidrug resistance protein 1, or cluster of differentiation 243, which is encoded by *ABCB1* gene.[68–70]

CYP3A4

Little information is available regarding the effects of CYP3A4 genetic variants on the PK of EVR. Two single nucleotide polymorphisms (SNP), CYP3A4*1.001 allele (rs2740574; c.-392G>A (previously called CYP3A4* 1 B[71]) and CYP3A4*22 (rs35599367; c.522-191C>T), have been evaluated to date.

However, the contribution of the CYP3A4*1.001 allelic variant to EVR PK variability remains controversial. In vitro studies have suggested that it is associated with increased CYP3A4 transcriptional activity,[72,73] whereas others have not.[74] It was initially hypothesized that this discrepancy was due to differences in the expression systems used in the studies.[72,74] It has also been suggested that CYP3A4 and CYP3A5 haplotypes are closely linked, and some effects originally thought to be due to the CYP3A4 allele are probably due to the CYP3A5 allele in linkage disequilibrium.[75,76] In lung transplant recipients, no association with EVR dose was found for CYP3A4*1.001,[77] suggesting that if any differences in CYP3A4 transcription exist, the clinical effect is minimal.

By contrast, the CYP3A4*22 variant allele has been associated with decreased hepatic activity in vitro[78] and in vivo, as assessed using CYP3A phenotyping probes in patients with cancer.[79] In a study evaluating the effect of this SNP in patients, of the 97 EVR-treated patients with renal transplants included in the study, 8 were heterozygous and 1 homozygous, which is in accordance with the reported minor allele frequency (~5%).[80] However, no significant influence on EVR PK was observed in relation to the variant, possibly due to the relatively small number of carriers of the defective allele in that study.[80]

CYP3A5

The CYP3A5*3 (rs776746; c.219-237G>A) allele results in splicing defects, leading to the production of a truncated protein with no enzymatic activity. Several studies have shown no association between the common CYP3A5*3 allele and EVR blood concentrations, dose requirement, or PK parameters estimated using population PK approaches.[67,77,80–85] Given that it plays little role in the overall metabolism of EVR,[12,66,67] this may not be a surprising finding.

One study has described a possible relationship between the CYP3A4*1.001–CYP3A5*3 haplotype, EVR, and SRL-related adverse effects.[81] In 184 kidney transplant recipients receiving either EVR or SRL, there was a significantly higher frequency of the CYP3A4*1–CYP3A5*1 (AA-GA) haplotype in patients with moderate (>0.5 g/L, ≤1.5 g/L) or significant (>1.5 g/L) proteinuria ($P = 0.008$ and $P = 0.003$, respectively). There were also significant differences in the EVR and SRL C0/dose/kg ratios between the haplotype groups. However, a confounding factor was that the patients were receiving calcineurin inhibitors (CNIs) either de novo (44.5%) or as rescue therapy (55.5%).

CYP2C8

The CYP2C8*3 variant allele is denoted by 2 highly linked variants: rs11572080 (c.416G>A; p.R139K) and rs10509681 (c.1196A>G; p.K399R). Other variants include CYP2C8*2 (rs11572103: c.805A>T; p.Ile199Phe) and CYP2C8*4 (rs1058930: c.792C>G; p.Ile264Met).[77] The effects of these alleles have been studied in a range of transplant types.[77,82,83] However, none of these studies found a significant relationship between EVR dosing or blood concentrations and the CYP2C8 genotype, which is consistent with its minor role in EVR metabolism.[67]

ABCB1

EVR is extensively eliminated in bile[20]; therefore, canalicular excretion of the drug or its metabolites, involving ABCB1, is likely to be important. In addition, ABCB1 acts as an absorptive barrier in the small intestine, reducing the oral bioavailability of EVR.[86] Several ABCB1 variants have been identified and described. Among the most studied ABCB1 SNPs are 3 SNP in strong linkage disequilibrium [(rs1128503 c.1236C>T, pGly412), (rs2032582, c.2677G>T/A, p. Ser893Ala/Thr), and (rs1045642, c.3435C>T, p. Ile1145Ile)][84]; however, the results reported thus far are inconsistent and, overall, the real functional impact of these SNPs remains controversial.[85] Other less frequent SNPs have been described for ABCB1 [(rs3213619 c.-129T>C) and (rs2229109 c.1199G>A, Ser400Asn)][84]; however, the results regarding their functionality are not conclusive either.

In a study including kidney transplant recipients who switched from triple therapy (CsA, mycophenolate mofetil [MMF], and prednisolone) to CNI-free dual therapy including EVR twice daily and prednisolone, no influence of ABCB1 c.1236C>T, 2677G>A/T, 3435C>T, or c.-129T>C was found for EVR apparent clearance (CL/F), Vd, or first-order absorption rate constant using a population pharmacokinetics approach.[82] Similarly, no effect of the ABCB1 SNP c.3435C > T on EVR PK was observed in a similar study in heart transplant recipients,[87] and no effect of the ABCB1 haplotype [c.1236C>T -, 2677G>A/T -, 3435C>T] on EVR steady-state dose-normalized C0 was observed in lung transplant recipients.[77] However, it is generally accepted that drug transporter activity has a greater influence on local drug distribution than on systemic exposure to substrates. Unfortunately, information is lacking regarding the impact of ABCB1 polymorphisms on EVR tissue distribution, and more specifically, on the uptake of the drug by T cells.

PG-PD Relationships

SNPs in genes encoding mTOR (particularly those in the FRB domain) or proteins of the EVR signaling pathway (FK-BP12, S6K1, raptor) may theoretically confer a resistance phenotype to the drug, as demonstrated in vitro in mammalian cell lines.[88] However, to our knowledge, no significant associations between any genetic variants of PD (Pharmacodynamics) genes and EVR effects have been reported.[89]

Recommendations

1. There is insufficient evidence to recommend prospective genotyping of *CYP3A5* and *CYP3A4* in solid-organ transplant recipients for an a priori dose adjustment of EVR.
2. There is some evidence to consider the evaluation of combined *CYP3A5*3* and *CYP3A4*22* genotyping to identify patients with high or low CYP3A total activity for the retrospective documentation of cases with unexpected EVR blood concentrations or adverse effects, combined with a comprehensive exploration of potential drug–drug or food interactions.
3. There is insufficient evidence to recommend genotyping for *ABCB1*, mTOR, or any other genes implicated in the immune signaling pathway targeted by EVR.

THERAPEUTIC DRUG MANAGEMENT

Pharmacokinetics

The average pharmacokinetic (PK) parameters of EVR in the subpopulations are summarized in Table 1. The relative bioavailabilities of different forms of EVR are similar.[12,90] The PK of EVR appears to be similar in patients with and without cystic fibrosis.[91] As mentioned above, EVR is a substrate for membrane transport by ABCB1 and metabolism by CYP3A4. Accordingly, hepatic dysfunction and CYP3A4 inhibition may increase systemic exposure, whereas the opposite occurs when combined with drugs that induce CYP3A4. DDIs are described in the following paragraphs.

It should be noted that most of the PK data on transplant recipients originated from early studies (Table 1). Data from newer studies on the use of EVR in cancer treatment are also included. The suggested target ranges for the TDM of EVR are, to a large degree, based on observational studies, which, in combination with a few prospective studies, provide a rationale for the suggested levels. Since the previous version of the EVR consensus document,[4] the recommended TDM target levels have been discussed in several comprehensive reviews and meta-analyses for solid-organ transplantation and cancer treatment, some of which are discussed in subsequent sections of this study.

Drug–Drug Interactions

DDIs and drug–food interactions with EVR are frequent and may involve both PD and PK interactions. DDIs are a major source of EVR PK variability and are dominated by the interactions between CYP3A and ABCB1. Potential effects must be identified early and controlled by careful drug monitoring. Quantitatively, the PK changes observed with EVR were higher than those with TAC, but less significant and more easily manageable than those observed with SRL.[122] Compared with TAC, the treatment of DDIs with EVR is simpler, mainly owing to its lower immunosuppressive potency and more favorable safety profile.

CsA inhibits EVR metabolism by approximately 50%.[123,124] EVR exposure in kidney transplant recipients is not influenced by TAC concentration,[125] and the EVR dose achieving equivalent exposure has been shown to be 1.5–2-fold higher with TAC than CsA.[126] EVR has been associated with DDIs with other immunosuppressants. Glucocorticoids (GCs) have a dual effect on EVR metabolism by CYP3A4, acting as strong inhibitors at acute high doses and moderate inducers at chronic low doses.[126]

Case examples of dose adjustments in patients treated with EVR involving enzyme induction of CYP3A4 and ABCB1 by rifamycins[127] and inhibition of CYP3A4 by antifungal azole drugs[128] also provide important additional information to the typical studies on the quantitative aspects of DDIs in healthy volunteers. Regarding concomitant antituberculosis treatment and immunosuppressive drugs, recent investigations have proposed the use of rifabutin instead of rifampicin because of fewer potential interactions. However, further studies are required to support these results.[129]

A recent study on peripheral blood mononuclear cells of healthy volunteers demonstrated the potential for antagonistic PD DDIs between EVR and TAC. A synergistic effect was observed at the therapeutic concentration range used for renal transplantation. However, these drugs antagonize each other to suppress the proliferation of activated PBMCs at concentrations higher than those clinically used.[130]

A recent review of CYP3A DDIs proposed guidelines for patients on anticancer drugs where EVR is included.[131–133] There is a high incidence of DDIs in cancer treatment, mainly due to poor screening for DDIs, and pharmacists should be actively involved in the identification of these interactions.[132] In addition, given the increased recreational use of marijuana among the population due to changes in legislation,[134] it is important to raise awareness of the potential PK interactions of tetrahydrocannabinol (THC) and cannabidiol (CBD). DDIs vary depending on the THC/CBD ratio, dose, and route of administration. Potential interactions with EVR PK are enabled by the inhibition of CYP3A4/5 enzymes.[12,86,134,135]

General EVR TDM Strategy

TDM is required for EVR therapy in organ and cell transplantation settings.[4] EVR TDM is recommended to limit the proportion of patients who receive subtherapeutic or supratherapeutic exposure early after the initiation of EVR therapy and to detect under- or overdosing due to DDIs, dosing errors, or poor adherence. The PK is linear, and the trough concentration (C_0) shows a good relationship with the overall exposure (area under the concentration–time curve, area under the curve (AUC)). Almost no EVR metabolite is correlated with the pharmacological effects, toxicological effects, and clinical outcomes of organ transplantation; therefore, ECR metabolites do not require monitoring for adjustment of the next dose of EVR in clinical settings.[4,20] Therefore, the predose C_0 of the unchanged form of EVR is a simple and reliable indicator for TDM.

Pharmacometrics and Model-Informed Precision Dosing

A population PK (POPPK) model describes how a drug behaves in patients and can be used as guidance for proposing

TABLE 1. Average Pharmacokinetic Parameters of EVR in Subpopulations for Organ Transplantation and Cancer Treatment

	Therapeutic Range (C0)	References	Tmax	Cmax (μg/L)	C0 (μg/L)	t1/2	AUC24	References
Healthy volunteers								
	NA		0.5–4 h	17.9 (±5.9)		31.5 (±6.4) h	122 (±52) μg × h/L	[92,93]
				Single dose 2 mg			Single dose 2 mg; fasting	
Renal tx								
With reduced CNI	3–8 μg/L	[53,94–100]	1–3 h	8–14 μg/L (/dose ≤1 mg)	2–7 μg/L	28 (±7) h	40–120 μg × h/L (dose normalized)	[92,93]
				21–55 μg/L (/dose ≥2.5 mg)			~450 μg × h/L (at steady state and 2.5 mg/d)	[94,100–102]
Without CNI	6–10 μg/L	[103–105]						
Lung/heart								
With reduced CNI	3–8 μg/L	[106–108]	1.7–2.4 h	10–20 μg/L	5–10 μg/L	25 h	80–160 μg × h/L	[108]
				Dose: 1.5–3 mg	Dose: 1.5–3 mg		Dose 1.5–3mg	
Liver								
With reduced CNI	≥3 μg/L	[109]	1–4 h	10–14 μg/L		25–40 h	107–137 μg × h/L (dose normalized)	[110]
				Dose ≤2.5 mg			~750 μg × h/L (at steady state and ≤7.5 mg/d)	[91]
				41–53 μg/L				
Without CNI	5–12 μg/L	[111]		Dose 7.5 mg				
Pediatric		[112]						
With CNI (CsA)	≥3 μg/L		1–2 h	10–20 μg/L	4.4 ± 1.7 μg/L	29.7 ± 11.1 h	66–220 μg × h/L	[90,113]
				dose: max 3 mg			Dose 0.8–1.6 mg/m^2; max 3 mg	[112]
Cancer								
10 mg × 1	12–20 μg/L	"Cut-off"[114] Lower:[115,118–120] Upper:[115,116,118,120] Summary[121]	1–6 h	62 (±18) μg/L	8–38 μg/L	26–38 h	435–565 μg × h/L	[114–117]

dosage regimens to achieve and maintain the therapeutic goals for each individual patient. The first EVR population PK model was developed based on data collected during early clinical trials when the drug was evaluated as a prophylactic agent against acute rejection episodes after kidney transplantation. Kovarik et al[136] used clinical trial data from 673 patients consisting of an approximately equal mix of predose troughs and 4 sample PK profiles collected over an eight-hour postdose interval. The structural model that best described the data was a one-compartment model with a first-order input, linear clearance, and dose-dependent bioavailability. Since then, multiple EVR population PK models have been published, covering different patient populations and therapeutic uses for different clinical indications. Supplementary Table 1 provides an overview of the published literature.

In addition to the EVR population PK models in renal transplant recipients,[80,82,137,138] other populations include heart transplant recipients,[87] patients with cancers such as thyroid cancer,[139] renal cell carcinoma,[140] and breast cancer,[137] and most recently, patients with TSC.[141] Depending on the richness of the collected concentration data, the models were parameterized as one- or two-compartment models, with a semiphysiological liver model with transit compartment absorption as the most recent iteration.[137,142] Most studies also included covariate analyses, identifying size parameters (body surface area, body mass index, or allometrically scaled weight) and hematocrit as the most frequently retained parameters in the final model. Other identified covariates included pharmacogenetic variants, such as the CYP3A5*1, ABCB1 haplotype, PPARA*42, PPARA*48, and POR*28.[78,139] Although several studies identified significant associations (or trends) between genetic variants and EVR clearance, it is unclear whether these studies were adequately powered to fully quantify such relationships (see **Supplementary Table 1**, **Supplemental Digital Content 1**, http://links.lww.com/TDM/A780).

More recently, complex EVR pharmacometric models have been developed (see **Supplementary Table 1**, **Supplemental Digital Content 1**, http://links.lww.com/TDM/A780). The first was a semimechanistic EVR model for patients with cancer described by van Erp et al,[142] which was subsequently used as a basis for the analysis of PK study data in patients with other conditions (renal transplants and other types of cancer).[138] The authors demonstrated that the population PK model accurately and precisely predicted future EVR exposure based on previous PK assessments. In addition, this study demonstrated the potential added value of hematocrit-normalized whole-blood concentration measurements as a promising strategy to further optimize EVR therapy in patients with extreme hematocrit values. Another pharmacometric approach using a nonparametric modeling platform in combination with a Bayesian estimator was reported by Limoges et al.[78] This approach was developed to simultaneously predict EVR exposure in whole blood and PBMCs and provides a basis for exploring the relationship between intracellular drug exposure and therapeutic and adverse effects. Finally, a promising new approach using machine learning (ML) algorithms to estimate EVR exposure built from data extracted from the Immunosuppressant Bayesian Dose Adjustment program was reported by the same research group.[143] This ML model was trained on simulated data from a previously published POPPK model and experimental data from the Immunosuppressant Bayesian Dose Adjustment program to predict EVR exposure ($AUC_{0–12h}$) based on a limited sampling strategy (predose, ~1 hour, and ~2 hours whole-blood concentrations). The ML algorithm slightly outperformed its current Bayesian estimator in terms of AUC predictive performance, based on the prediction error expressed as the root mean squared error. However, despite these findings and the increasing application of ML using big datasets, it is likely that the introduction of ML in precision dosing will not lead to the disappearance of population PK, quantitative systems pharmacology, or other pharmacometric methods.[144] Given that a major advantage of pharmacometric models is that their biological plausibility is considered, the addition of artificial intelligence and ML methodologies should be viewed as a welcome addition to the MIPD armamentarium.

EVR MEASUREMENT

Sample Handling

Primary Sample Matrix: Whole Blood

The appropriate collection and handling of samples intended for the determination of EVR concentrations were addressed in the first consensus report.[4] Owing to the high EVR incorporation and concentration-dependent binding to erythrocytes, whole blood is the recommended specimen for EVR quantification, preferably using ethylenediaminetetraacetic acid (EDTA) as the anticoagulant. This is further supported by the strong correlation between EVR concentrations in whole blood and PBMCs, which are the immunosuppressive sites of action of EVR.[145]

EVR in whole blood with EDTA is stable for 3 days at temperatures up to 37°C and 1 week at temperatures up to 30°C; however, for prolonged storage times, specimens should be stored at −20°C or below.[4]

Alternative Sampling Matrices

The use of alternative sample matrices for the TDM of EVR was addressed for the first time in the current update of the consensus report. In general, to use alternative matrices to facilitate the measurement of EVR concentrations in a limited sample volume, adequate sensitivity, selectivity, and robustness are required. Interest in these matrices is steadily growing, and many studies have reported the analytical performance of methods developed for application in clinical practice. The current focus is on dried blood spots (DBS), volumetric absorptive microsampling (VAMS) devices, oral fluid (OF), and PBMCs.

DBS and VAMS Devices

The use of DBS is a minimally invasive, cost- and time-effective sampling method that allows for multiple samplings, simplifies the determination of the AUC,[146,147] and can be performed by patients at home. Le et al[148] described a new liquid chromatography-tandem mass spectrometry (LC-MS/MS) method involving the incubation of DBS in water before adding acetonitrile and zinc sulfate as protein precipitators, which led to high dissociation efficiency and improved analytical results. Effective enrichment and purification of the analytes were achieved using cold-induced phase separation techniques. Deprez and Stove[149] implemented a method that combines fully automated DBS extraction with online coupled LC-MS/MS.

Factors such as blood spot volume, hematocrit, drying time, and analyte stability are also critical for the validation of DBS methods.[150,151] Among other immunosuppressive drugs, EVR and SRL are more susceptible to the effects of heat degradation, hematocrit, and drying time.[150] Knapen et al and Deprez and Stove observed a hematocrit-dependent relative recovery in their DBS method, the latter showing lower hematocrit values, yielding higher relative recoveries (and vice versa), and a difference of >15% at hematocrit of 0.18 and 0.60.[149,152]

The results assessing the interchangeability of DBS with whole-blood sampling remain contradictory and should be further explored.[153–155] In general, conventional venous whole-blood sampling has acceptable precision and bias. However, problems have been noticed with microsampling owing to variability in the fill volume of the tip caused by differences in sample viscosity and filling time.[156–158] Using the Mitra device (Trajan Scientific and Medical, Ringwood, Victoria, Australia), hematocrit-, blood sampling time-, and concentration-related recovery effects, as far as observed, were within the requirements of the purpose of the analytics in some evaluations.[157,159] However, Verheijen et al[160] reported considerable biases (from −20% to 31%) in their study and method over a 30%–50% hematocrit range.

In conclusion, although DBS and VAMS represent promising alternatives to conventional venous whole-blood sampling, further evidence with a higher number of samples

and broader patient populations is required to identify and overcome the possible pitfalls. Moreover, the results from a proficiency testing pilot for immunosuppressant microsampling assays demonstrated that these methods showed higher interlaboratory variation than whole-blood methods. As far as this variation can influence clinical decision making, the authors are calling for harmonization and standardization of the analytical methods, as well as for regular availability of proficiency testing for laboratories involved in patient care.[161]

OF

OF is considered an alternative noninvasive method to venipuncture blood sampling.[146,147] A recently published multidrug LC-MS/MS method for the determination of 5 immunosuppressants in OF was also validated for the determination of EVR concentrations.[162]

In addition, Molenaar-Kuijsten et al[163] reported the results of a small study in patients with cancer treated with EVR, in which they measured EVR concentrations in saliva by LC-MS/MS to investigate the possibility of using it for the prediction of stomatitis. Although salivary drug concentrations tended to be higher in patients with stomatitis, this relationship was not significant. Moreover, salivary EVR concentrations are poorly correlated with those in whole blood. The high variability of EVR PK in saliva (interindividual variability of 67.7%) was considered the most critical reason to not conclude the feasibility of this method in patients with stomatitis. This could be related to several factors, such as salivary gland function, water and electrolyte balance, protein binding, and pH differences. Based on the current knowledge, there is no evidence to support the use of OF as an alternative matrix to monitor EVR therapy.

Intracellular Concentration Monitoring

Intracellular concentrations appeared to be more closely related to drug efficacy than blood concentrations.[164] Intracellular quantification of EVR was described in peripheral blood mononuclear cells by different research groups[145,165,166] and was supposed to improve the prediction of rejection in transplantation.[167] However, significant variability in the results has been reported in published studies, underlining the critical importance of proper validation of preanalytical and analytical steps for intracellular concentration assays.

Currently, there are 2 articles published on EVR monitoring in PBMCs by LC-MS/MS, with controversial results regarding the correlation between intra-PBMCs and whole-blood concentrations.[145,165] Although Robertson et al[145] showed a high correlation between EVR whole-blood and PBMC concentrations, revealing a weak role of ATP-binding cassette subfamily B member 1 (ABCB1)–mediated efflux from PBMCs, this was not the case in the study by Roullet-Renoleau et al.[165] More recently, Pensi et al[166] developed and validated an LC-MS/MS method coupled with automated online solid-phase extraction (SPE) for the simultaneous quantification of TAC and EVR in PBMCs to study their potential pharmacokinetic interactions. The authors concluded that this method is suitable for use in a clinical setting to investigate drug exposure at the active site. The potential additive value of using this alternative sample material compared with the established whole blood remains to be demonstrated.

Sample Stability in Alternative Matrices

Stability data reported for EVR monitoring in whole blood have also been largely confirmed for analysis based on the use of dried blood. According to the study of Verheijen et al, VAMS EVR samples were stable for nearly a year at an ambient temperature.[168] However, the evidence available for the application of alternative sample matrices (dried blood, OF, or tissue material) is generally limited and potentially dependent on the sample collection materials used (eg, type of paper or tubes). Laboratories working with said matrices are advised to be aware of sample stability issues and to include stability evaluation in their method validation protocols.

Analytical Methods

Analytical methods to monitor EVR concentrations include chromatographic and immunochemical procedures.

Chromatographic Methods

Since the most fundamental clinical trials for the establishment of EVR TDM applied LC-MS/MS to the measurement of drug concentrations, this analytical technology has been decisive in the development of therapeutic targets in clinical practice. Accordingly, fully validated LC-MS/MS methods that fulfill the recommended analytical acceptance criteria are the preferred standards for EVR TDM.[4,169]

Chromatographic methods based on UV detection do not provide the required quality for this narrow therapeutic index drug and are therefore not recommended. The "Chromatographic Procedures" section described in the first consensus report[4] remains valid.

After the publication of the first consensus report, various concepts and technical options have been continuously explored in immunosuppressive drug analysis to improve the analytical performance parameters (range, precision, and accuracy) and facilitate the practical aspects of using the LC-MS/MS technique. One way to improve this method may be the use of a new type of column (eg, small-particle solid-core packing), which significantly shortens the runtime.[170] Replacing commonly used protein precipitation with semiautomated SPE on a 96-well hydrophilic–lipophilic balance microelution plate resulted in a chromatographically pure sample and a faint matrix effect. The advantage of the method is a wide analytical measuring range (0.1–50 ng/mL) with a low lower limit of quantification (LLOQ).[171] A new sample preparation technique for the analysis of 4 immunosuppressants including EVR was proposed by Kvamsøe et al.[172] Salting-out-assisted liquid–liquid extraction (SALLE) is a specialized type of LLE in which water-miscible solvents are used, and phase separation is achieved by adding salt first. With an almost complete extraction yield, a very low LLOQ level of 0.06 ng/mL was obtained for EVR. In the case of SALLE, it is possible to apply the sample directly without evaporating it, and the matrix effects are low; however, the analyst must consider the need for mass analysis of sodium $(M + Na)^+$ adducts.[172]

Interest in automating the various steps of routine LC-MS/MS analysis is steadily growing. Analysts use 96-well

microplates and pipetting robots,[171,172] but the real step forward seems to be the introduction of routine TDM of the assay available on a fully automated LC-MS/MS-based clinical analyzer. Hörber et al[173] evaluated the performance of the Thermo Scientific Cascadion SM Immunosuppressant Panel over 6 months in a 24/7 routine laboratory. The main practical advantages reported were the absence of manual sample handling and the possibility of using technical staff without MS experience after only 5 days of training. Further developments are ongoing, and time will show whether such MS analyzers with applications will be able to replace both classic LC-MS/MS methods and immunochemical methods, thus opening a new era in laboratory medicine, including the TDM of immunosuppressive drugs.

Bruns et al[174] presented a quantitative method for the determination of 4 immunosuppressants (including EVR) using high-resolution MS (HRMS). The ultra-performance liquid chromatography with time-of-flight mass spectrometry (UPLC-TOF-MS) was used, which, compared with the classic LC-MS/MS, provided equivalent quantitative results and similar operating parameters with the same sample preparation procedure. The advantages of using such equipment are the simpler development of analytical methods and the possibility of obtaining metabolite data.

Finally, the proposed reference method, LC-MS/MS (combined with SPE) for the determination of immunosuppressive drugs (including EVR) in blood should be noted. This methodology published in 2020 by Taibon et al[175] presents a refined analytical procedure, which is characterized by very good accuracy and precision, and therefore high reliability of measurements over wide concentration ranges (0.25–50 ng/mL for EVR). In the current absence of established reference materials for EVR, this methodology can serve as a reference procedure for other laboratories.

Immunochemical Methods

At the time of the development of the first consensus report, only 1 immunoassay was available to clinical laboratories to quantify EVR (the Quantitative Microsphere System (QMS) EVR immunoassay, Thermo Fisher Scientific); 2 further immunoassays were introduced recently. These include the Roche Elecsys EVR electrochemiluminescence immunoassay (ECLIA) and the Siemens Healthcare Diagnostics EVR affinity chrome-mediated immunoassay (ACMIA) for the Dimension Chemistry Systems. Although the immunoassays comply with most of the analytical requirements, such as quantification limits close to 1 ng/mL (except QMS; 1.5 ng/mL), interassay coefficient of variation of $<10\%$, and dynamic measurement ranges satisfactory for performing a predose concentration–based TDM,[169] they suffer from considerable cross-reactivity to both active and nonactive metabolites of EVR. This leads to inconsistent (biased) results both against the LC-MS/MS methods and between the immunoassays.[4,168,169,176–180] Table 2 summarizes the performance characteristics of different analytical methods.

The QMS applies value-assigned concentrations of calibrators using a factor set at approximately 70% of their gravimetric concentrations to compensate for the "average" bias against concentrations obtained by LC-MS/MS.[4] In addition to not considering interpatient variability of the bias around the "average" caused by individual differences and cross-reactivities with metabolites and other potential errors, 2 further problems of the concept have been reported recently: first, the underestimation of EVR concentrations in samples from heart transplant recipients (15.5% lower concentrations on average) probably related to insufficiency of the QMS value assignment that was developed by a training set of samples from patients undergoing liver and kidney transplantation only[177]; second, assay inconsistency over time. Six-year results of the Zotracker EVR study showed a significant shift in QMS results during the observation period; after initial results similar to that of LC-MS/MS, a significant positive bias emerged.[178] ECLIA and ACMIA do not use correction factors in calibration to align their results with those of LC-MS/MS. ECLIA has been found to overestimate EVR concentrations in transplant recipients. An estimated average relative bias of 36.5% and 26.5% was reported at the medical decision points of 3 and 8 ng/mL EVR, respectively, as evaluated using the Bland–Altman plot in a multicenter study.[176] This is in agreement with the bias observed in 2 other analytical trials.[177,179] Comparing ECLIA results with QMS revealed a mean positive bias of 32.6% and 37.9% in 2 single-center studies.[168,177]

ACMIA is the most recently launched immunoassay, but there is almost no practical experience regarding its performance. According to the manufacturer's instructions [https://content.doclib.siemens-healthineers.com/rest/v1/view?document-id=840406 (accessed July 2024)], comparison of ACMIA results with an LC-MS/MS method used as the reference showed a Deming regression equation with an intercept of 0.1 ng/mL and a slope of 1.06 ($n = 295$, correlation coefficient $r = 0.965$). The respective values for the comparison ACMIA versus QMS ($n = 125$) were reported to be 0.44 ng/mL (intercept), 1.09 (slope), and a correlation coefficient of 0.954 (Dimension Everolimus (EVRO) Assay). However, a recently published study that compared ACMIA with QMS with samples from patients who underwent kidney, liver, and lung transplantation by the same statistical method observed higher constant (intercept of 0.679 ng/mL) and proportional bias (slope of 1.326) between the 2 assays.[180] Correspondingly, there was a 39.9% mean positive bias in ACMIA versus QMS. These controversial results highlight the need for further independent studies to collect more evidence on the analytical performance of ACMIA.

In conclusion, based on currently available data, EVR concentrations generated using different analytical assays should not be used interchangeably. Laboratory reports should include information on the assays used. Switching between assays requires careful assessment of expected deviations and their therapeutic relevance using samples representative of the local patient population. Rebaselining of individual patients is recommended to ensure proper patient care.

Analytical Method Standardization

The urgent need for improvements in method standardization and harmonization of laboratory practices was stressed in the 2016 International Association of TDM and Clinical Toxicology consensus by Shipkova et al.[4] The analytical

TABLE 2. Analytical Performance of Currently Available EVR Methods

				Inaccuracy	Specificity for the Parent Drug	
Method	**Analytical Platform**	**Measurement Range***	**Total Method Imprecision**	**Method Comparison With a Validated LC-MS/MS method†**	**% Cross-Reactivity With Metabolites Relative to EVR**	**References**
LC-MS/MS‡	Various LC-MS/MS platforms	0.5–50 μg/L	≤10% Over the measurement range	Typically serves as the reference method	Specific for the parent drug	169
QMS	General chemistry analyzers	1.5–20 μg/L	<10%	Intercept: KTx: −0.005, HTx: −0.15, LTx: 0.98	At a concentration of 20 μg/L	
Thermo Scientific	Spectrophotometric detection		At concentrations ≥3.9 μg/L	Slope: KTx: 1.11, HTx: 1.00, LTx: 0.98 r = 0.93–0.96 Clinical samples§ KTx, n = 150; HTx, n = 41; LTx, n = 111	45,46-Hydroxy-: 2.0% 24-Hydroxy-: 5.0% 25-Hydroxy-: 22.0% RAD SA: 2.0% RAD PSA: 16.0% RAD PC: 59.0	181
ECLIA	Cobas platform: e modules	1.0–30 μg/L	<8.5%	Intercept: 0.478	At a concentration of 25 μg/L	168,176
Roche Diagnostics			At concentrations ≥2.8 μg/L	Slope: 1.21 r = 0.91 Clinical samples¶ n = 784 from KTx, HTx, and LTx, pooled data from 5 European laboratories	45,46-Hydrohy-: 6.0% 24-Hydroxy-: 21.3% 25-Hydroxy-: 15.4% RAD SA: 9.8% RAD PSA: 10.1% RAD PC: 109.3%	
ACMIA	Dimension and dimension Vista	1.0–25 μg/L	<7%		At a concentration of 5 μg/L	
Siemens Healthcare Diagnostics	Clinical chemistry systems		At concentrations ≥3.0 μg/L	n = 295, transplanted organ not reported Intercept: −0.10 Slope: 1.06 r = 0.965 Clinical samples‖	45,46-Hydrohy-: 14.0% 24-Hydroxy-: 26.0% 25-Hydroxy-: 74.0% RAD SA: 52% RAD PSA: 54% RAD PC: 64%–98% At a concentration of 20 μg/L RAD SA, RAD PSA, or 25-hydroxy-EVR test results are suppressed, RAD PC 96%	182

*The lower limit is defined either by the limit of quantification or by the functional sensitivity dependent on what is reported by the manufacturer.
†Appropriately validated LC-MS/MS method that serves as the reference; data shown are representative examples that may vary between patient populations, for example, with different types of transplantation.
‡Performance characteristic for state-of-the-art methods.
§Passing–Bablok regression.
¶Deming regression.
‖Not stated; CMIA, chemiluminescent microparticle immunoassay; LTx, liver transplantation; KTx, kidney transplantation; HTx, heart transplantation; RAD SA, seco acid of everolimus; RAD PSA, precursor of seco acid of everolimus; RAD PC, 40-phosphatidylcholine everolimus.

tools for achieving the clinically required performance have been discussed in detail.[4,122]

In addition to differences in assay design and actual measurement procedures, inconsistency in method calibration using different EVR calibration materials, which are not based on an appropriate reference, strongly contributes to the disagreement in interlaboratory results.[183]

Currently, for EVR, a single reference method has been described,[175] which can also be used to standardize CsA, TAC, and SRL. Said ISO 15193 compliant method is listed in the Joint Committee for Traceability in Laboratory Medicine database and has an expanded uncertainty (k = 2) of less than 2.7% for EVR concentrations greater than 1 ng/mL. Unfortunately, no reference materials for EVR certified by metrological institutions such as the Joint Research Centre of the European Commission[184] or the National Institute for Standardization[185] are available. Therefore, it is not currently possible to obtain metrological traceability for EVR.[186,187]

Recommendations

The analytical performance recommendations provided in the first consensus report remain valid without restrictions and are briefly summarized below.[4] New recommendations regarding the application of 2 recently approved immunoassays (ECLIA and ACMIA) are included below.

1. Whole blood is the recommended specimen for EVR quantification.
2. Analytical methods should be specific to EVR, and the methods should be validated. If available, cross-reactivity with metabolites should be reported along with a statement of clinical relevance. In the case of cross-reactivity with sirolimus, the EVR results are unreliable during the first week after switching from sirolimus.
3. A fully validated LC-MS/MS assay is preferred to measure EVR concentrations. Chromatographic methods based on UV detection are not recommended. The use of sirolimus-specific immunoassays for EVR determination is discouraged because none have been formally released or validated for EVR measurements.
4. For the reliable quantification of EVR, an LLOQ close to 1 ng/mL and imprecision of at least ≤10% with a total error of ≤15% (among other performance characteristics) are recommended.
5. Concentrations obtained using LC-MS/MS, QMS, ECLIA, and ACMIA should not be used interchangeably. The identification of the analytical assay used by the laboratory should be provided to users. Careful assessment of expected deviations and their therapeutic relevance with samples representative of the local patient population is required before switching assays. Rebaselining individual patients is a helpful tool for ensuring proper clinical care.
6. Continuous participation in an external QC program that includes the use of spiked samples, samples without EVR, and pooled samples from patients with different clinical indications for drug therapy is highly recommended.

EVR TDM IN Clinical Settings

Kidney Transplantation

The Therapeutic Window for EVR After Kidney Transplantation

In the 2016 version of the EVR consensus report, the therapeutic window for patients after kidney transplantation was defined as 3–8 ng/mL.[4] The lower concentration limit was based on indications of reduced efficacy below 3 ng/mL, which was largely based on an analysis of approximately 700 patients with kidney transplants simultaneously treated with CsA.[188] Freedom from acute rejection was significantly related to predose concentrations of EVR, with an incidence of 68% at 1.0–3.4 ng/mL, 81%–86% at 3.5–7.7 ng/mL, and only a small further improvement of efficacy to 91% at 7.8–15.0 ng/mL ($P = 0.03$). The importance of achieving EVR predose concentrations above 3 ng/mL was underscored by a Cox proportional hazards analysis that indicated a significantly higher risk of acute rejection (nearly 3-fold when levels were below 3 ng/mL, compared with levels above this cut-off value). The incidence of thrombocytopenia increased with increasing exposure to EVR. A subsequent study showed that maintaining EVR predose concentrations in the range of 3–8 ng/mL in the first posttransplant year with reduced exposure to CsA is associated with good efficacy and safety profiles.[94]

The multicenter US 92 study, in which EVR was combined with low-exposure TAC, showed a high incidence of acute rejection in patients with below-the-target EVR concentrations on days 3 (62.8%), 7 (55.8%), and 14 (33.5%) posttransplantation.[189] The incidence of treated biopsy-proven acute rejection (BPAR) was 64.7% among patients with EVR concentrations <3 ng/mL and strikingly lower at 14% among those patients with EVR concentrations >3 ng/mL.[190]

Development of Donor-Specific Antibodies

Although the studies mentioned above focused on acute (cellular) rejection episodes within the first 6 or 12 months after transplantation, in the last 10 years, several studies have been published with a focus on the development of de novo donor-specific antibodies (dnDSAs). One of the first publications on this topic was by Liefeldt et al.[191] The researchers randomized 127 patients to continue treatment with CsA or switch to EVR therapy 3 months after kidney transplantation.[191] Furthermore, 7 of 65 (10.8%) patients on CsA developed dnDSAs after a median of 991 days, whereas 14/61 patients (23.0%) randomized to EVR developed dnDSAs after 551 days (log-rank: $P = 0.048$).

In the ELEVATE study, 715 de novo kidney transplant recipients were randomized at 10–14 weeks to convert to EVR (n = 359) or remain on standard CNI therapy (n = 356; 231 TAC; 125 CsA), all with mycophenolic acid and steroids.[103] When dnDSA was assessed at months 12 and 24 in patients with no DSA at the time of transplantation, class II DSAs were more frequent in the EVR cohort at month 12 [18.4% (30/163) vs. 11.1% (22/199) in the CNI group] but showed a smaller difference at month 24 [13.1% (17/130) vs. 10.7% (18/169)]. However, no data have been reported on the association between EVR concentration and dnDSA development. It remains unclear whether EVR exposure at the upper end of the therapeutic window of 3–8 ng/mL or above 8 ng/mL reduces DSA development.

Concentration-Controlled Studies

The TRANSFORM study needs to be mentioned as it was the largest randomized controlled trial in the transplant

field.[53] In a multicenter noninferiority trial, 2037 de novo kidney transplant recipients were randomized to receive, in combination with induction therapy and corticosteroids, EVR with reduced-exposure CNI (EVR arm) or mycophenolic acid (MPA) with standard-exposure CNI (MPA arm).[192] The EVR dose was subsequently adjusted to a target EVR trough concentration of 3–8 ng/mL throughout the study. Treated biopsy-confirmed acute rejection (BPAR), graft loss, and death at posttransplant month 12 occurred in 14.9% and 12.5% of patients treated with EVR and MPA, respectively [difference, 2.3%; 95% confidence interval (CI), 21.7%–6.4%]. The de novo DSA incidence at 12 months and the antibody-mediated rejection rate did not differ between the arms.

In the ATHENA study, 655 patients who underwent de novo kidney transplantation were randomized to 1 of 3 treatment arms, EVR/TAC, EVR/CsA, or MPA/TAC, with similar TAC exposure in the EVR/TAC and MPA/TAC groups.[193] Again, the EVR target concentrations were 3–8 ng/mL, in both EVR groups. The primary end point of the study was eGFR at 12 months, and although noninferiority was aimed for, eGFR was numerically inferior in both EVR groups compared with MPA/TAC.[95] A concern was the high patient withdrawal rate, with only 52% of randomized patients reaching month 12 of the trial, on the study drug, and with an eGFR recorded.[194] Unfortunately, an analysis of the concentration–effect relationship between EVR and the incidence of adverse events has not been published.

In the pediatric arena, Ahlenstiel-Grunow et al[195] conducted a randomized controlled trial comparing a group of pediatric kidney transplant recipients (n = 31), in whom immunosuppression was guided using the viral load of virus-specific T cells in addition to TDM, with a group of patients (n = 33) with treatment adjusted only with TDM. From month 1, patients were treated with CsA with a target of 50–100 ng/mL and then 30–75 ng/mL from month 6 and with EVR with a target of 3–6 ng/mL and then 2–5 ng/mL from month 6. The primary outcome was the eGFR at month 24, and it did not differ between the groups. Coupling a pharmacodynamics monitoring approach to TDM allows decreasing the dosage (0.8 ± 0.3 vs. 1.2 ± 0.5 mg/m^2, $P = 0.004$) and trough levels of EVR (3.5 ± 0.7 vs. 4.5 ± 0.8 ng/mL, $P < 0.001$). There were also numerically fewer patients with $\geq$1 BPARs in the intervention arm. Although this was a small study, it underlines the potential interest in guiding EVR exposure using pharmacodynamic tests.

Immune Response After Vaccination

As a substudy of the OPTIMIZE study,[196] Boer et al[197] published data on the response to COVID-19 mRNA vaccinations. They showed that an immunosuppressive regimen without MMF/MPA, a lower CNI dose, and the use of EVR were associated with a higher humoral response rate against COVID-19 after vaccination in elderly patients after transplantation without any treatment against rejection. This confirmed the data from an earlier study that reported low seroconversion rates in patients receiving MMF/MPA maintenance treatment. Recent data have shown that within a group of patients treated with MMF/MPA, the MPA-AUC is predictive of the likelihood of seroconversion.[198] To the best of our knowledge, no data relating EVR concentrations to seroconversion rates are available.

Recommendations for Kidney Transplantation

The first recommendation is quoted mostly from the previous version, whereas the other 3 recommendations have been updated in this version.

1. The EVR target trough concentrations in kidney transplant recipients are recommended to be between 3 and 8 ng/mL if used in combination with CNI.[4]
2. If EVR is combined with CsA at a low-dose, low-CsA levels need to be targeted in view of a DDI that results in increased CsA-related nephrotoxicity.[53]
3. EVR with reduced-exposure CNI provides similar efficacy outcomes (incidence of rejection, renal function) as MPA with standard-exposure CNI.[53]
4. Despite the lower incidence of viral infections (both CMV and BK-virus), attention should be paid to the higher incidence of adverse events leading to a higher discontinuation rate in EVR with reduced-exposure CNI compared with MPA with reduced-exposure CNI.[53]

Liver Transplantation

As the clinical management of patients undergoing liver transplantation has improved over time, the goal in this type of transplantation has shifted from avoiding immunological complications (eg, graft rejection) to reducing the burden of pathological and treatment complications. Renal failure, new-onset diabetes mellitus, and hypertension now individually account for a 2-fold increase in patient mortality and are the primary focus of step-in liver transplant patient management.[199] The role of immunosuppressive drugs, particularly CNI, in the onset of such adverse events has been recognized; therefore, therapeutic strategies are required to address these ADRs.

In some countries, EVR is labeled as part of the immunosuppressive regimen indicated to prevent graft rejection in liver transplantation and may be a key player in decreasing the ADR rate in patients with liver transplants. Another advantage of EVR-based treatment is its ability to sharply decrease the incidence of cytomegalovirus infections compared with mycophenolic acid-based treatment.[200] The drug has been evaluated in clinical strategies to decrease CNI exposure or withdraw CNI from the patient's immunosuppressive regimen. Since the publication of the first version,[4] evidence in clinical settings has accumulated in the areas of introduction after liver transplantation, combined use with decreased exposure to CNI, use as a CNI-free regimen, prevention of acute rejection, treatment of chronic rejection, and liver transplantation in hepatocellular carcinoma.

The second version summarizes updated information and provides recommendations for each area.

EVR as an Agent to Decrease CNI Exposure

To reduce the long-term toxicity of immunosuppressive drug regimens, particularly nephrotoxicity, EVR can be added to the patient's treatment, thereby decreasing the CNI

dose and, most importantly, the CNI whole-blood exposure. Dose-ranging studies have shown that the efficacy of EVR is related to the whole-blood trough concentration (C_{min}), with patients with $C_{min} < 3$ ng/mL being at higher risk of three-year graft rejection (50% vs. 14% in patients with C_{min} between 3 and 6 ng/mL).[109] Most recent randomized control trials comparing immunosuppressive drug regimens composed of EVR with a target concentration of 3–8 ng/mL and a CNI with reduced exposure (TAC C_{min} between 3 and 5 ng/mL) reported better renal function in the experimental arm at month 12,[173] month 24,[174] and later.[111,201] Notably, when TAC minimization below 5 ng/mL was not achieved, the gain in preventing renal function deterioration was weaker.[202] EVR should be introduced on postoperative day 30 to avoid delayed wound healing. Real-life cohort data also confirm the benefit of the treatment in preventing renal function deterioration when EVR initiation is performed early,[203] whereas there is minimal benefit from the late introduction of the drug (eg, after 6 months).[204] It remains to be determined whether a de novo minimized TAC scheme (ie, with a TAC target $C_{min} < 6$ ng/mL from day 1) compares favorably with EVR with reduced TAC, as this first regimen might decrease the toxic pressure on the kidneys.[205] In addition to effects on wound healing, starting EVR before day 30 can be associated with a possible lower efficacy with numerically more graft rejection events, particularly subclinical rejection.[206] The safety profile of EVR in clinical trials has shown increased proteinuria, hematological disturbances, and hypercholesterolemia. Long-term use was associated with a higher likelihood of statin treatment, but no increase in cardiac events in patients treated with EVR.[207] Globally, the treatment discontinuation rate in recent randomized clinical trials is high with EVR and higher than in CNI/corticosteroids or CNI/MPA arms, with rates ranging from 18% to 37%, according to study protocols and follow-up durations.[208,209]

EVR as an Agent to Withdraw CNI From Immunosuppressive Treatment

Another option for the use of EVR in liver transplantation is to use the drug as a substitute for CNI agents. As the safety profile of CNI is expected to be less favorable than that of EVR, this strategy may lead to a decrease in the rate of CNI-specific ADRs such as chronic renal failure, diabetes, and hypertension. A large clinical trial was conducted in de novo liver transplant patients comparing 3 arms: EVR with TAC discontinuation, EVR with reduced TAC, and a control TAC arm.[210] In this study, EVR was administered to maintain a C_{min} concentration of 3–8 ng/mL from day 5 to the end of month 3 and then a target increase to 6–10 ng/mL was proposed. TAC was progressively eliminated by the end of month 4. Unfortunately, the EVR with the TAC elimination arm was prematurely terminated due to an excess in graft rejection. However, recent data obtained from randomized controlled trials and a prospective cohort showed that TAC elimination can be achieved in a selected population of liver transplant recipients by initially targeting a C_{min} of 8–12 ng/mL (or at least 6–10 ng/mL), then 6–10 ng/mL from month 6, and finally 6–8 ng/mL from year 1.[206,209,211] However, if such a strategy leads to reduced kidney function, it also exposes patients to less efficient immunosuppressive treatment, as suggested by the higher, although not always statistically significant, BPAR rate in these studies. A large dropout rate is also expected when EVR monotherapy is used, with approximately 20% and 50% of patients discontinuing their treatment after 6 months and 2 years, respectively.[206,209]

EVR as an Agent to Prevent Chronic Rejection

Some patients develop severe rejection that cannot be controlled with TAC or high-dose GCs. Acute cellular rejection following liver transplantation can be treated with high-dose GC pulse therapy. However, apart from retransplantation, there is no established treatment for chronic (ductopenic) rejection. The prevention of B-cell maturation by mTORis is anticipated to prevent chronic rejection after liver transplantation. The successful use of SRL[212] or EVR[213] in combination with reduced-exposure CNI to treat severe chronic (ductopenic) rejection after liver transplantation has been reported in a limited number of cases from Japan (introductory EVR C_0 10–12 ng/mL; CsA C_0 100–200 ng/mL; TAC C_0 5 ng/mL; maintenance EVR C_0 5–8 ng/mL); however, more evidence supporting the introduction of EVR to treat antibody-mediated rejection is needed in future studies.[214]

Prevention of Recurrence of Hepatocellular Carcinoma After Liver Transplantation

Hepatocellular carcinoma (HCC) is the main cause of liver cancer in the world[215] and the fourth leading cause of cancer-related death [estimated age-standardized mortality rates (World) in 2020, worldwide, both sexes, all ages (excl. NMSC)].[216]

Recurrence of HCC after liver transplantation in patients with cirrhosis (10%–20%)[217,218] has been a serious problem in the case of both deceased and living donors. HCC recurrent recipients have a lower median 1-year survival after diagnosis and 67% exhibit extrahepatic cancer.[218] CNIs are carcinogenic, and sustained higher levels of CNIs correlate with a higher rate of HCC recurrence, leading to a poor prognosis.[217,219,220] The Milan transplant criteria were originally established to select patients with cirrhosis and small HCC nodules for whom a good outcome is expected with lower rates of recurrence.[221] As many transplant centers may enlist patients beyond the Milan criteria, they show worse prognosis than HCC-free recipients.[202,222] mTOR signaling (cell growth, metabolism, proliferation, and apoptosis inhibition) is involved in several key stages of the neoplastic process (development, progression, and spreading).[220,223] Furthermore, upregulation of the mTOR pathway is a feature of different types of cancers, including HCC.[202,220] A recent meta-analysis revealed that patients with HCC treated with mTORis showed a lower rate of recurrence than those treated with CNI.[202] Consistent with the study by Cholongitas et al,[222] the recurrence of HCC was significantly lower compared with CNI (8.0% vs. 13.8%, $P < 0.001$). Despite the shorter follow-up for patients treated with EVR than for those receiving SRL or CNI (13, 30, and 43.2 months, respectively), the rate of HCC recurrence in recipients treated with EVR was significantly lower than in those treated with SRL and CNI (4.1% vs. 10.5% vs. 13.8%, respectively; $P < 0.05$).

A recent meta-analysis reported an increase in HCC recurrence-free survival 1 and 3 years after transplantation in patients taking mTORis compared with standard CNI therapy (risk ratio 1.09, 95% CI 1.01–1.18 vs. 1.1, 95% CI: 1.01–1.21). Furthermore, the recurrence rate was lower in the mTORi group (RR 0.67; 95% CI: 0.56–0.82).[224] By contrast, Kang et al[225] found that patients in the EVR cohort exhibited an increased number of tumors and microvascular invasion compared with those in the EVR-free arm. However, in patients with microvascular invasion who exceeded the Milan criteria, the time to recurrence was similar between the groups, whereas patients in the EVR arm had significantly longer overall survival than those in the non-EVR arm. In addition, increasing evidence suggests that EVR restricts HCC progression and recurrence.[217,226] Another important finding is that when EVR C_{min} is maintained at ≥ 6 ng/mL from month 6 to month 12, lower HCC recurrence rates are achieved.[227] Nitta et al[218] observed that patients treated with a synergistic combination of sorafenib and EVR, which blocks the activation of the PI3K/Akt and Ras-MAPK signaling pathways,[223] had prolonged survival. This emphasizes that one of the challenges in combination therapy is the management of adverse effects and comorbidities.[218] The current recommendation for avoiding HCC recurrence in liver transplant recipients is to reduce immunosuppression to the lowest effective dose to prevent graft.[228]

Currently, evidence on the use of EVR to prevent HCC recurrence is limited owing to the small number of studies and patients.[229] Several clinical trials are ongoing[220]; therefore, we expect these results to support better clinical decisions.

Recommendations for Liver Transplantation

The first recommendation is a quote from the previous version, whereas the latter 2 have been updated in this version.

1. In liver transplant recipients C_{min}, EVR targeted at 3–8 ng/mL can be used to reduce TAC C_{min} to 3–5 ng/mL from postoperative day 30.[4] It prevents the deterioration of renal function. Late introduction of the drug (6 months after transplantation and later) has little effect.
2. In the case of complete elimination of CNI from immunosuppressive treatment, an EVR C_{min} target of 8–12 ng/mL (or at least 6–10 ng/mL) until month 6, then 6–10 ng/mL from month 6, and finally 6–8 ng/mL from year 1 should be targeted; however, this strategy has been shown to be less efficient in preventing graft rejection.
3. Compared with CNI treatment, EVR administration at a relatively higher trough concentration (>6.0 ng/mL)[230] is expected to reduce the recurrence of HCC after liver transplantation because of its ability to inhibit cellular growth, proliferation, angiogenesis, and survival.[231]

Heart and Lung Transplantation

Heart Transplantation: EVR With CNI

Limited information has been published since the last consensus document. In a registration study, EVR was shown to be superior to azathioprine on a composite end point [death, graft loss, retransplantation, loss to follow-up, BPAR of grade 3A (International Society for Heart and Lung Transplantation) 1990 grading, or rejection with hemodynamic compromise], incidence of repeated rejection episodes, incidence of BPAR of grade <3A, and severity and incidence of cardiac allograft vasculopathy.[232] The EVR therapeutic target in combination with CNI was set to 3–8 ng/mL based on post hoc analysis and TDM simulation of PK results of the B2253 study, which observed freedom from rejection with EVR $C_0 > 3$ ng/mL ($P = 0.02$) and a lower incidence with this target range compared with an EVR fixed-dose regimen.[232,233] However, the upper limit of the therapeutic concentration range could not be defined because of the flat EVR concentration–safety parameter association (eg, for leukopenia, dyslipidemia, and renal insufficiency).[232] This therapeutic range allows for decreasing the CsA concentration in de novo heart transplant recipients without significant loss of efficacy[234] while ensuring similar efficacy and changes in renal function when compared with MMF.[235]

Forty stable heart transplant recipients were randomized at week 12 to either a low dose of EVR (mean 4.2 ± 1.7 ng/mL) and of TAC (mean 6.5 ± 3.5 ng/mL) or standard TAC (mean 8.6 ± 2.8 ng/mL), with both groups receiving MPA and prednisolone.[236] This study demonstrates that the combination of low-dose EVR and TAC compared with standard-dose TAC safely attenuated left ventricular hypertrophy in the first-year postcardiac transplantation; however, no significant difference was observed in the incidence of rejection or infection between groups.

The large multicenter, randomized, controlled NOCTET (Nordic Certican trial in heart and lung transplantation) study in Scandinavia explored the benefit of a quadruple regimen with EVR and a predefined CNI exposure reduction on renal function in maintenance thoracic transplant recipients (190 patients with heart transplants).[237] The quadruple regimen consisted of EVR (target C_0 of 3–6 ng/mL), CsA (C_0 of 75 ng/mL), TAC (C_0 of 4 ng/mL), MMF (1000 mg/d), or azathioprine, with or without GC therapy, according to local practice.

The mean change in measured glomerular filtration rate (mGFR) from baseline to month 12 was significantly in favor of the recipients receiving quadruple immunosuppression compared with controls receiving standard triple immunosuppression without EVR (5.8 mL/min vs. 20.1 mL/min, $P < 0.0001$) and continued at month 24[237] and up to month 60. Heart transplant recipients with preexisting moderate or severe renal failure (mGFR 30–59 or 20–29 mL/min^2/1.73 m^2, respectively) particularly benefited from being in the experimental arm.[238] The frequency of treated BPAR was similar between the quadruple and control group transplant recipients. A greater proportion of EVR-treated than control group patients experienced adverse events, including serious adverse events, from baseline to month 12 ($P < 0.05$).[237] Overall, this study showed that a quadruple regimen with EVR (C_0 3–6 ng/mL) and reduced CNI exposure has a beneficial long-term effect on renal function in heart transplant recipients and an acceptable safety profile.[106,237]

Heart Transplantation: EVR in CNI-Free Regimens

Over the last 10 years, EVR trials have focused on evaluating the potential of EVR in renal-sparing regimens, mainly CNI-free regimens, because CNIs are the most important contributors to end-stage renal disease in the long term.[239]

The SCHEDULE (The Scandinavian Heart Transplant Everolimus De Novo Study with Early Calcineurin Inhibitors Avoidance) trial and follow-up analyses at 36, 60, and 72 months were published and compared with the last EVR consensus report. Briefly, patients were assigned to receive low-dose EVR with reduced-dose CsA (n = 56) or standard-dose CsA (n = 59) with both MMF and GCs.[238] In the EVR group, CsA was discontinued between weeks 7 and 11 of the CNI regimen. At 12 months, both the incidence of BPAR without hemodynamic compromise and improvement in renal function were significantly higher in the EVR group than in the control group.

Follow-up analyses at 36 months and 60–72 months posttransplant confirmed significant improvement in renal function in the CNI-free arm.[240,241] There was no difference in the incidence of BPAR between the study groups at any time point. Overall, there was a similar incidence of adverse events and serious adverse events in the study groups, with a higher incidence of pneumonia and a substantially reduced risk of cytomegalovirus infection in the EVR arm. In addition, the incidence of cardiac allograft vasculopathy was reduced in patients treated with EVR compared with patients treated with CsA up to 7 years after heart transplantation.[241]

In Germany, the MANDELA study (NCT00862979) was the first multicenter, randomized, controlled, open-label study comparing 2 EVR-facilitated CNI-reduction strategies (reduction vs. withdrawal).[239] In addition, this study was used to investigate the combination of TAC and EVR in a randomized study for the first time. After randomization at 6 months posttransplant, a 2-month CNI-reduction (control group) or withdrawal (study group) phase was performed. This study compared renal function (primary end point) and composite efficacy at 18 months posttransplant in CNI-free patients treated with EVR (C_0 5–10 ng/mL) plus MPA and GCs with those in patients receiving EVR (C_0 5–10 ng/mL) plus reduced-exposure CNI (TAC C_0 3–8 ng/mL or CsA C_0 50–150 ng/mL) and GCs. Renal function improved in the first week after CNI reduction or withdrawal in both study groups compared with baseline, with continuation until the end of the study. However, the renal function was significantly better in the CNI-free group than in the CNI-reduction group at the end of the study period.

Although the incidence of BPAR was higher in the EVR-CNI-free group than in the control group, none of the rejections led to hemodynamic compromise. Interestingly, 40% of the BPAR episodes occurred in patients with an EVR C_0 target level of <5 ng/mL. This indicates that the incidence of BPAR in an EVR CNI-free regimen could potentially be reduced by careful regulation of EVR blood concentrations. As shown in earlier EVR studies, the risk of CMV infection was reduced in both groups, but with a more pronounced effect in the CNI-free group compared with the CNI-reduction group.

Recommendations for Heart Transplantation

Because little has been published since the last consensus document, all recommendations are quoted from the previous version.[4]

1. The use of EVR in de novo heart transplant recipients requires TDM to achieve and maintain a recommended whole-blood target C_0 of 3–8 ng/mL in combination with reduced CNI dosages.[4]
2. C_0 in patients receiving CNI-sparing regimens must be tightly controlled to achieve improved renal function.[4]
3. In a quadruple regimen with CNI, other cell cycle inhibitors, and steroids, EVR C_0 target levels should be 3–6 ng/mL to lower the risk of infections.
4. In a CNI-free regimen combined with MPA and GCs, the EVR target C_0 range should be 5–10 ng/mL,[4] and EVR C_0 levels below 5 ng/mL should be avoided to lower the risk of rejection.
5. Blood EVR C_0 concentrations >10 ng/mL are associated with an increased risk of adverse events and should be avoided.[4]

Lung Transplantation

Early EVR With CNI After Lung Transplantation

Limited information has been published on this topic since the first consensus report. In contrast to heart transplantation, EVR is mainly used after lung transplantation instead of the cell cycle inhibitor MPA in CNI-based regimens but only individually in CNI-free regimens.

Experience with EVR in de novo lung transplantation is limited, possibly due to the negative outcomes of the 2 initial de novo lung transplantation studies with SRL. Both studies reported significant wound dehiscence and airway complications, leading to death in some patients.[242,243] Therefore, it is recommended to start mTORi therapy after the anastomosis and airways are healed,[244] because of the drug's inhibitory effects on growth factors and fibroblast proliferation.[245] Consequently, all study protocols for the use of EVR in lung transplantation have avoided early administration of the drug.[246]

EVR combined with CsA and GCs at month 3 in lung transplant recipients (RAD001 B159) demonstrated a slowing in loss of pulmonary function compared with azathioprine with no difference at 24 months.[247] Conversion to mTORi and reduced CNI improvement in renal function after conversion in patients with an eGFR of $\leq$29 mL/min (median eGFR from 24 to 33 mL/min, n = 29, $P < 0.0001$), but not in patients with an eGFR of 30–44 mL/min (median eGFR from 36 to 42 mL/min, n = 26, $P = 0.1032$).[248] Furthermore, 1–3 months after EVR initiation with reduced CsA, the outcomes also compared favorably with enteric-coated mycophenolate sodium (with standard CsA) with similar renal function and less acute rejection.[249] However, high EVR dosing during the first 2 months after initiation could aggravate CNI-

related nephrotoxicity. The improvement in renal function was greater in patients without proteinuria at baseline.

More recently, in a single-center prospective study, an EVR-based regimen was investigated to prevent freedom from bronchiolitis obliterans syndrome (BOS). The patients (n = 190) received either EVR (6–8 ng/mL) and reduced CsA (C_0 150–200 ng/mL after month 6, 100–150 ng/mL after 12 months), or MMF in combination with standard CsA (C_0 200–250 ng/mL after month 6, 150–200 ng/mL after 12 months) and prednisolone in both groups.[250] Freedom from BOS was significantly higher in the EVR treatment arm than in the per protocol (PP) population at the 24-month follow-up. Interestingly, renal function decreased in both groups by approximately 50% within 6 months posttransplant, with comparable eGFR after 24 months between the study groups (EVR group: baseline eGFR 103 mL/min and 52 mL/min after 24 months vs. baseline eGFR 96 mL/min and 56 mL/min after 24 months in the MMF group). The incidence of BPAR was also lower in the EVR group than in the MMF group, demonstrating that the reduction in CsA exposure in the EVR arm did not lead to a higher incidence of rejection.

The randomized, multicenter 4EVERLUNG study in Germany explored a quadruple drug regimen in patients with mild-to-moderate renal insufficiency (eGFR of 50–90 mL/min/1.73 m^2). Patients in the study group (n = 67) received EVR (C_0 3–5 ng/mL) with reduced CNI (TAC 3–5 ng/mL or CsA 25–75 ng/mL) and a fixed dose of either MPA or AZA plus prednisone. In the control group (n = 63), patients received CNI (TAC C_0 >5 ng/mL or CsA >100 ng/mL) and a fixed dose of either MPA or AZA plus prednisone 3 to 18 months after lung transplantation.[107] The eGFR of patients in the quadruple regimen was significantly higher than the eGFR of patients in the control group (64.5 mL/min vs. 55 mL/min, respectively) after 12 months of follow-up. No differences were observed between the study groups regarding acute rejection and chronic lung allograft dysfunction or safety parameters.

Recommendations for the Early Phase After Lung Transplantation

Because limited new information has been published, the first 3 are mostly quoted from the previous version, whereas the rest are new recommendations added to this version.[4]

1. EVR should not be initiated until bronchial suture healing (endoscopic confirmation) or until at least 3 months after transplantation.[4]
2. When EVR is administered in combination with CNI, target EVR C_0 levels should be 3–8 ng/mL in the triple regimen[4] and 3–5 ng/mL in the quadruple reduced CNI regimen to lower the risk of infections.
3. There are insufficient clinical data to provide recommendations for appropriate therapeutic concentrations of EVR in CNI-free immunosuppressive regimens in lung transplant recipients.[4]
4. Early initiation of EVR (preferably in the first year) seems to be favorable to prevent the development of mild-to-moderate chronic kidney disease (CKD).
5. However, there is no clearly defined CKD stage for mTORi indication, nor does severe CKD preclude the improvement of kidney function under mTORis.
6. Baseline proteinuria may be a negative predictor of a favorable kidney response after the introduction of EVR.

Late EVR With CNI After Lung Transplantation

In the NOCTET study (see above), 92 lung transplant maintenance recipients were randomized to continue their current CNI-based immunosuppression or start a quadruple EVR regimen.[237] The mean change in mGFR from baseline to months 12 and 24 was significantly higher in the quadruple EVR regimen than in the standard control regimen. In the quadruple regimen, the between-group difference in the change of mGFR from months 0 to 12 was 2.3 mL/min ($P = 0.07$) and significant to month 24, with a mean difference of 6.0 mL/min ($P = 0.02$).[237] However, after 60 months, the benefit on renal function (mGFR) in EVR-treated recipients disappeared because of higher CNI levels in the EVR group than in the period until 24 months. No difference in efficacy was observed between the groups, with a significant decrease in forced vitality capacity from randomization to the last follow-up. Over the 60-month study period, one or more adverse events occurred more frequently in the EVR group than in the control group (eg, pneumonia).[251] A similar benefit on renal function of EVR-treated recipients with severely impaired renal function (eGFR of ≤29 mL/min/1.73 m^2) was replicated in the patients in a retrospective observational study.[252]

However, in the 4EVERLUNG five-year follow-up, no differences were observed in renal function between the study groups (123 randomized patients)[253,254] [intention to treat: eGFR, 56 (48–73) versus 58 (48–69) mL/min; $P = 0.951$; PP: eGFR, 59 (range: 50–73) versus 59 (range: 48–69) mL/min; $P = 0.946$]. In the quadruple EVR group, more patients were switched from their immunosuppressive regimen owing to a significantly higher incidence of dnDSA and thromboembolic events (ITT: 11% vs. 24%, $P = 0.048$; PP: 11% vs. 22%, $P = 0.162$) than in the triple control group. There was a trend toward higher CLAD-free survival in the quadruple EVR regimen in the PP population ($P = 0.082$).

The less effective renal-sparing regimens using EVR in lung transplantation compared with those using EVR in heart transplantation may be explained by the fact that the CsA C_0 level in the reduced regimen is generally higher than the reduced CsA C_0 levels studied in heart transplant recipients. This confirms that CNI reduction is the main driver for sparing the nephrons. Notably, the lung is a more immunocompetent organ than the heart and therefore requires higher immunosuppression, including CNI exposure.

Recommendations for the Late Phase After Lung Transplantation

The first recommendation is quoted mostly from the previous version, whereas the latter are new recommendations added to this version.[4]

1. Current data support the introduction of EVR in maintenance patients on quadruple immunosuppressive regi-

mens or with reduced CNI exposure to improve renal function, particularly in patients with severely decreased GFR (<29 mL/min/1.73 m^2). The time from the transplant to EVR initiation should be less than 5 years.[4]
2. At present, there are no conclusive data on the benefits of long-term EVR therapy in reducing BOS progression.

Pancreatic and Islet Transplantation

Approximately 80% of pancreatic transplants are classified as simultaneous pancreatic–kidney transplants in patients with end-stage renal failure associated with insulin secretion deficiency. In addition, approximately 15% of pancreatic transplant recipients undergo pancreatic transplantation after renal transplantation; therefore, approximately 95% of pancreatic transplant recipients have a history of severe diabetes with renal dysfunction.[255] Considering their background, pancreatic transplant recipients should be treated as compromised hosts who are at high risk of posttransplant infection.[256–259] Taken together, the target index of EVR in pancreatic or islet transplantation was set between 3 and 8 ng/mL, conforming to the therapeutic window for kidney transplantation.

In this context, the purpose of EVR for pancreatic transplantation is as follows.

1. As a first-line immunosuppressive agent with a CNI- or MMF-free protocol.
2. As a second-line agent in cases treated with low-dose CNI, improving transplanted renal function should be considered as an alternative to MMF to control infection and complement the reduction or discontinuation of medications to treat the side effects of CNI or MMF.

In a comprehensive review, evidence from various in vivo and in vitro studies has shown that mTORis, including EVR and SRL, impair the β-cell function, induce β-cell apoptosis, and suppress β-cell proliferation.[260] Although these basic scientific findings have been presented, in the clinical setting, an analysis of pancreatic transplants in the UNOS (United Network for Organ Sharing) database from 1987 to 2016 showed that the use of mTORis was associated with a 7% reduction in the risk of allograft failure and significantly higher patient survival up to 10 years after transplantation compared with immunosuppression without the use of mTORis.[261]

The First World Consensus Conference on Pancreas Transplantation convened in 2019 to complete comprehensive evidence-based guidelines for the practice of pancreas transplantation, which collected expert opinions on pancreas transplantation.[262] According to these guidelines, data on the benefits (as immunosuppressants) and side effects of mTORis, including EVR, compared with CNI or MMF, are scarce; therefore, further research is required on the role of mTORis in pancreatic transplantation.[263]

In a randomized trial that compared the effects of immunosuppression with those of SRL, another mTORi, and TAC,[264] despite the noninferiority of SRL to TAC with respect to graft survival, SRL treatment was associated with a high discontinuation rate in simultaneous pancreatic and kidney transplantation owing to intolerable adverse events. However, in a single-center study with an observation period of >10 years, Ciancio et al[265] reported that SRL in combination with TAC was better tolerated and more effective than MMF.

In the absence of data on the first-line use of EVR in pancreatic transplantation, Sageshima et al[266] conducted a single-center study comparing EVR with a low-dose TAC protocol and the use of enteric-coated mycophenolate sodium in pancreatic transplantation. They reported that no rejection was observed in patients with EVR and that there were no significant differences between the groups in serum creatinine, HbA1c, surgical complications, or medical complications.

There have been several reports[104,105,267–270] on the early conversion of EVR after kidney transplantation. These studies reported that EVR and low-dose CNI are associated with improved posttransplant renal function. Consequently, the use of EVR as a second-line agent should bring the same benefit in pancreatic transplant recipients because >90% of pancreatic transplant recipients have a kidney graft. Marcella-Neto et al[271] found that among 535 pancreatic transplant recipients, 13 patients had complications associated with CNI or MMF that forced them to switch to mTORis. Although the pancreatic graft was lost after conversion in approximately 20% of the patients, the kidney and pancreas function of the graft was well maintained in the remaining patients.

By contrast, in islet transplantation, mTORi has long been the mainstay of immunosuppressants because of the short-term success of the Edmonton protocol[272]; however, SRL is often selected. However, owing to the high incidence of side effects of high-dose SRL and the consideration of impairment of β-cell regeneration, the use of mTORis in islet transplantation decreased from 83.6% in 1999–2002 to 46.8% in 2011–2014.

Most islet transplantation data are based on SRL and evidence for EVR from clinical trials is sparse. Nevertheless, smaller studies on EVR have reported successful insulin independence in at least 50% of the patients with type 1 diabetes mellitus.[273] In pancreatic/islet transplantation, since the late 1990s, mTORis, including EVR, have transitioned from first-line immunosuppressants to second-line agents, which are now mainly used in patients with CNI or MMF intolerance, CNI-related nephrotoxicity, or malignancy.

Recommendation for Pancreatic/Islet Transplantation

1. More than 90% of pancreatic transplant recipients also received renal allografts. These transplantations primarily rely on a CNI-based regimen for the maintenance of immunosuppression, and EVR is used in combination with CNI. Based on robust evidence for EVR use in kidney transplantation, the target trough level of EVR in pancreatic transplantation should be 3–8 ng/mL.
2. The use of an mTORi is recommended for islet transplantation. However, evidence supporting the use of EVR in

clinical studies is lacking. Nevertheless, a target trough concentration of 3–8 ng/mL could still be considered as recommended in kidney transplantation.

3. A higher trough level of EVR may be associated with an increased risk of adverse effects such as interstitial pneumonia, stomatitis, hyperglycemia, and hyperlipidemia.

Oncology

Within the field of solid-organ transplantation, EVR is considered a drug with a narrow therapeutic index for which TDM is routinely applied. The situation is significantly different in the field of oncology. Standard dosing aimed at the maximum tolerated dose within a population is the norm. Therefore, TDM of the EVR has not been considered in patient management for most oncological indications. EVR is currently used in metastatic renal cell carcinoma (mRCC) with or without lenvatinib, metastatic neuroendocrine tumors, advanced hormone-sensitive human epidermal growth factor receptor 2 (HER-2) negative breast cancer, subependymal giant cell astrocytoma, and renal angiomyolipoma associated with TSC.[274] For the latter 2, dose titration to attain a trough concentration of 5–15 ng/mL is recommended.[12]

However, dose interruptions and dose reductions due to severe adverse events occur in up to 60% of patients when standard dosing is used. Because toxicity is related to high EVR blood concentrations, this offers an opportunity to implement dose reductions based on drug exposure before the occurrence of toxicity. One example is pulmonary adverse events related to EVR, which have clearly been shown to be related to higher EVR concentrations.[275] The efficacy may also improve if patients with low exposure have higher rates of progression. The first consensus report concluded that "further studies are required to determine the clinical utility of TDM in non-transplantation settings."[4] In this section, we present an updated version.

Oncologic PK and TDM data on EVR are largely from studies on mRCC and breast cancer. In a series of 40 patients with mRCC, EVR exposure (AUC_{0-24h}) was measured within the first 2 weeks of treatment.[139] Patients who required a dose reduction (n = 18) due to toxicity at any time during treatment had significantly higher EVR exposures [mean AUC = 600 versus 395 ng·h/mL ($P = 0.008$)] than patients without a dose reduction (n = 22). This study confirmed the data obtained in an earlier pooled analysis of 5 phase 2/3 studies in which higher EVR exposure was linked to the incidence of grade 3 or 4 pneumonitis, stomatitis, and metabolic events.[118] The latter study also showed that the likelihood of tumor size reduction increased with increasing EVR trough concentrations. Moreover, a retrospective analysis based on data from large randomized controlled trials showed superior median progression-free survival in the C-through window of 10–30 ng/mL compared with <10 ng/mL and >30 ng/mL in a number of different populations treated for various tumor types, including carcinoid, non-small cell lung cancer, renal cell carcinoma, and pancreatic neuroendocrine tumors.[118]

More support for a concentration–effect relationship comes from a study that reported a 4-fold increased risk of toxicity [HR = 4.12, IC 95% = (1.48–11.5), $P = 0.0067$] if EVR trough concentrations were above 26.3 ng/mL, whereas troughs below 11.9 ng/mL were associated with a 3-fold increased risk of progression [HR = 3.2, IC 95% = (1.33–7.81), $P = 0.001$] in patients with breast, renal, and neuroendocrine tumors.[115] Fukudo et al showed that, in 19 patients with metastatic breast cancer, the median progression-free survival in patients who maintained a steady-state C-trough below the threshold of 17.3 ng/mL was numerically longer than in those who did not {[(327 days (95% CI 103–355 days) vs. 194 days (95% CI 45 days–not estimable); $P = 0.35$) estimable]; $P = 0.35$}.[114] In this study, the cumulative incidence of dose-limiting toxicity was significantly higher in patients with C-trough ≥17.3 ng/mL than in other patients (subhazard ratio 4.87, 95% CI 1.53–15.5; $P = 0.007$).[114]

A strong impact of drug exposure was also observed in a study of patients with mRCC that compared patients with EVR exposure either below or above the median.[119] The median EVR trough concentration in the 42 patients was 14.1 ng/mL. Fourteen patients (67%) with EVR concentrations above the median were free from progression at 6 months compared with 8 (38%) patients with EVR concentrations below 14.1 ng/mL ($P = 0.06$).

Several studies have reported associations between drug exposure and toxicity without any effect on efficacy. In a small Japanese study, the median EVR concentration on day 8 was substantially higher in patients for whom EVR had to be discontinued or reduced compared with patients in whom the EVR dose could be maintained (median, 18.0 vs. 8.2 ng/mL; $P = 0.0139$).[276] Among 44 patients with breast cancer, the geometric mean EVR trough concentration was higher in patients with toxicity than in patients without (17.4 vs. 12.3 ng/mL ($P = 0.02$).[116] The optimal cut-off value to predict toxicity was a trough of 19.2 ng/mL. In this study, the trough concentrations in patients with and without progressive disease within 3 months were not significantly different (12.0 vs. 15.2 ng/mL ($P = 0.118$)). A post hoc analysis of the phase 3 EVEREST study also identified significant associations between EVR exposure and the probability of toxicity.[277]

Ideally, randomized trials comparing standard dosing with TDM-based dosing should be performed to demonstrate reduced incidence of toxicity. Feasibility is a challenge because pharmaceutical companies currently do not want to invest in such trials. Considering the high frequency of toxicity-based dose reductions, the benefits of TDM can be observed in relatively small study populations (less than 100 patients). For improvements in efficacy, it is likely that the sample size will need to be larger, but not more than 300–400 patients. Novel microsampling techniques such as dried blood spot sampling or volumetric absorptive microsampling can facilitate blood collection at multiple time points and allow the availability of TDM results before the patient's visit to the outpatient clinic.[119] We encourage oncologists to conduct such a study to improve patient outcomes.

Recommendation for Use of EVR in Oncology

1. Given the narrow therapeutic index, highly variable inter-individual PK, and positive exposure–efficacy relation-

ship, there is a strong rationale for the TDM of EVR in oncology. Based on the data presented above, a therapeutic window for trough concentrations between 12 and 20 ng/mL was defined.
2. However, there may be a difference in the optimal target range between the EVR monotherapy and combined treatment regimens.[260121]

Pediatrics

The PK of EVR has been evaluated in several pediatric populations, including in patients with neurofibromatosis type 1, TSC, solid tumors, and kidney and liver transplant recipients.[278–282] All these pediatric studies showed large between-patient variability in predose trough concentration and exposure, with a substantial number of patients below or above the target range, even with the relatively wide ranges of 5–15 ng/mL used in some studies. Earlier trials in patients with TSC (EXIST-3 trial) studied once-daily oral EVR titrated to achieve a target trough level of 3–7 ng/mL (low target) or 9–15 ng/mL (high target).[283] Recent efforts by the originator company have focused on modeling and simulation approaches that incorporate population PK, pharmacodynamics, and physiologically based pharmacokinetic (PBPK) modeling to predict EVR exposure in infants and children. TSC-associated seizures affect newborns and infants; therefore, a pediatric EVR PBPK model was developed to predict a clinically relevant reduction in disease symptoms, including seizures, in a population of very young infants.[284] Since EVR is both a CYP3A4 and a P-gp substrate, the model included a combination of changes resulting from both physiology and maturation (ontogenetic factors) as a function of the child's age. Importantly, despite existing treatments, infants with TSC are at a very high risk of developing epilepsy and have a poor prognosis. Clinical trials are ongoing to evaluate whether early intervention by initiating preventive therapy with SRL or EVR using an MIPD approach would alter the natural course of TSC before seizures begin (ClinicalTrials.gov Identifier: NCT04595513[285]).

Recommendation for EVR Use in Pediatrics

Although updated information is accumulating, the recommendations for EVR TDM have been quoted from the previous version.[4] The pharmacokinetics of EVR in children are variable and related to age, weight, and body surface area,[20] and in the light of data obtained from adult transplant recipients, the requirement of TDM for EVR therapy in children is valid.

PHARMACODYNAMICS

PD Monitoring

Immunosuppression can be achieved by depleting lymphocytes, diverting lymphocyte trafficking, or blocking response pathways. EVR is an immunosuppressive drug intended to reduce lymphocyte proliferation. Activation of mTORC1 promotes cell growth by activating the synthesis of proteins, lipids, and nucleotides and the biogenesis of miRNAs. The mTORC2 complex regulates cell survival and metabolism. SRL mainly inhibits the mTORC1 complex, and in the case of chronic exposure, it can inhibit mTORC2 in certain cell types.[286] Treatment with SRL or EVR decreases the translational activity, cell cycle progression, and cell proliferation (see section on the mechanism of action).

As stated in the first consensus report, PD monitoring aims to individualize drug therapy to complement TDM. It focuses on the effects of drugs on target cells or molecules. In 2016, limited data were available regarding PD monitoring of EVR in transplantation medicine and oncology. At that time, there was no evidence of an association between EVR PD markers and clinical outcomes. We performed a literature search from January 2015 to August 2022 to update the available information.

Nonspecific PD Monitoring of EVR

Approaches used for nonspecific PD monitoring of EVR include cell proliferation assays with lymphocytes or tumor cells; cytokine production in lymphocytes and T cells; intracellular production of ATP in $CD4^+$ T cells; cell-free DNA, surface activation markers on T cells, miRNA expression, and changes in the proportions of lymphocyte subsets.[4,287]

Donor-Derived Cell-Free DNA

Donor-derived cell-free DNA (dd-cfDNA) is a noninvasive biomarker that has been shown to be useful for the early detection or exclusion of allograft injury from multiple causes, including rejection, in all transplanted solid organs.[287–290] A literature search failed to identify published studies that systematically investigated the association between EVR and dd-cfDNA. However, in a few reports that measured dd-cfDNA levels, EVR was administered in combination with other immunosuppressants. For example, the recipient of a marginal liver graft monitored using serial dd-cfDNA was switched from TAC to EVR.[291] At the time of the switch, substantial underimmunosuppression resulted in acute rejection associated with a major increase in dd-cfDNA. A subsequent positive clinical response to a steroid bolus was confirmed by a rapid decrease in dd-cfDNA, and changes in dd-cfDNA were monitored as EVR treatment was optimized. In another report, in a heart transplant patient treated with cyclosporine and EVR undergoing attempted immunosuppression minimization between days 280 and 366, a highly significant increase in dd-cfDNA was observed, suggesting underimmunosuppression. A biopsy demonstrated acute rejection.[292] The results of this patient suggest that minimization of immunosuppression could have been better guided by serial dd-cfDNA monitoring. The successful use of dd-cfDNA monitoring has also been reported in a study on cardiac allograft rejection.[293] EVR has also been used in some patients (12/189) in a prospective study on the absolute quantification of dd-cfDNA.[291] In conclusion, dd-cfDNA appears to have the potential when combined with TDM to better guide EVR dose adjustments and achieve more effective, personalized immunosuppression. Assessment of its clinical utility in large prospective outcome studies in patients treated with EVR is warranted. Drug-specific PD biomarkers have

been studied in the fields of transplantation and oncology (Supplementary Table 2).

Specific PD Monitoring of EVR

As elaborated in the first consensus report, drug-specific PD monitoring of EVR can be performed through measurements of mTOR activity through phosphorylation of its downstream targets.[4] In the field of solid-organ transplantation, no new evidence was generated to support specific PD monitoring of EVR; therefore, the reader is referred to the first consensus document.[4] However, most drug-specific PD biomarkers are used in oncology for diverse clinical conditions. Drug-specific biomarkers have been used for PD monitoring in EVR therapy in oncology (summarized in Supplementary Table 3).

mTOR Activation

The activation of the mTOR pathway may be used to select patients who would benefit from EVR therapy. Fukudo et al[114] investigated the PD effect by measuring mTOR activity in isolated PBMNCs from patients with metastatic breast cancer. A correlation was observed between blood EVR concentration and mTOR activity; however, EVR did not completely inhibit mTOR activity at therapeutic concentrations.

In a study on primary RCC, elevated mTOR levels were associated with aggressive pathological features, impaired overall survival, and cancer-specific survival.[294] However, in a study by Li et al,[295] in patients suffering from anti–vascular endothelial growth factor-refractory mRCC, the expression levels of phosphorylated mTOR and phosphorylated S6RP (rpS6) were identified as potential predictive biomarkers for EVR efficacy.

In a trial on non-small cell lung cancer, the effect of EVR on changes in the expression of active phosphorylated forms of mTOR, AKT, eIF4e, S6K1, and 4EBP1 and the metabolic response or anatomic tumor shrinkage were dose-dependent.[296]

The ability to monitor intratumor S6K1 was suggested by Benselama et al.[297] Gagliano et al[298] showed that both the total and phosphorylated mTOR and S6K1 activity were higher in tumor tissue in a group of patients that were EVR-sensitive compared with patients that were considered resistant.

Summary: PD Monitoring

PD monitoring to guide EVR therapy in solid-organ transplantation and oncology has not yet been established in clinical routine. Drug-nonspecific PD biomarkers, such as graft-derived cell-free DNA in transplantation, have emerged; whereas, drug-specific biomarkers, such as those involved in mTOR activation (p-mTOR, mTOR activity, or p-rpS6), have been used to monitor EVR efficacy or toxicity.

CONCLUSIONS

mTORis, such as EVR, remain an option for immunosuppressive therapy in solid-organ transplantation and present a breakthrough in cancer treatment. Their proper therapeutic use requires appropriate TDM, which is best performed using laboratory-developed LC-MS/MS methods for quantification. However, strict control of the mitigation of PK variability factors, such as drug–drug and food–drug interactions, and the utilization of MIPD approaches can contribute to improved outcomes. Reaching appropriate whole-blood target concentrations in patients treated with EVR may maximize their efficacy and safety, notably in the treatment of special populations such as patients with solid-organ transplants with minimized CNI. These agents may also have virtues, coupled with TDM, in cases such as the need for calcineurin inhibitor withdrawal, viral infections, and rejections, and to provide better immunization in patients benefiting from the COVID-19 vaccine. In the oncology era, TDM-guided strategies can improve the safety profile of EVR. New monitoring approaches, including microsampling or PD biomarkers, may help further optimize EVR therapy but require additional validation to be implemented together with TDM in clinical settings.

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